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Commonwealth Environmental Water Office Monitoring, Evaluation and Research Project: Edward/Kolety-Wakool River System Selected Area Technical Report 2019-20

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Cover photo: *Confluence of Yallakool-Wakool during Commonwealth environmental watering action on 27 August 2019. Photo by Damian McRae, CEWO.*

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EXECUTIVE SUMMARY

Introduction

The Commonwealth Environmental Water Office (CEWO) Monitoring, Evaluation and Research (MER) Program (2019 to 2022) is an extension of the Long-Term Intervention Monitoring (LTIM) and Murray-Darling Basin Environmental Water Knowledge and Research Project (EWKR) projects, with monitoring, evaluation and research activities undertaken within a single integrated program.

This report describes the monitoring, evaluation and research activities that were undertaken in the Edward/Kolety-Wakool system as part of the CEWO MER Program in 2019-20. This project was undertaken as a collaboration between Charles Sturt University, NSW DPI (Fisheries), NSW Department of Planning, Industry and Environment, La Trobe University and Streamology. The fish spawning research was undertaken in partnership with the Edward-Wakool Angling Association.

This report has thirteen sections. This introduction (section 1) is followed by a description of the Commonwealth environmental water use objectives and watering actions for this system for 2019-20 (section 2). An overview of the monitoring, evaluation and research undertaken in this system for the MER project and its relationship to LTIM monitoring is described in section 3. Summaries of the evaluation of responses of each indicator to Commonwealth environmental watering and unregulated flow events are presented in sections four to eight; hydrology (section 4), water quality and carbon (section 5), stream metabolism (section 6), riverbank and aquatic vegetation (section 7), and fish movement, fish spawning, fish recruitment and fish community (section 8). Sections nine to twelve report on the outcomes of several components of an integrated research program focused on the Edward/Kolety River. Section 9 reports on physical habitat research, section 10 on primary productivity research, section 11 on fish spawning research and section 12 on eDNA biodiversity research. Recommendations to inform adaptive management of environmental water in the Edward/Kolety-Wakool system in the future is presented in section 13. A summary report (Watts et al. 2020) provides an overview of the monitoring and key findings of the ecosystem responses to environmental watering actions in the Edward/Kolety-Wakool system in 2019-20 including findings across the six years of the combined LTIM/MER program.

Monitoring and evaluation

The monitoring and research described in this report is undertaken using methods and approaches described in the Edward/Kolety-Wakool MER Plan (Watts et al 2019a). As the MER project is a continuation of the LTIM Project, for some of the monitoring indicators we will evaluate long-term trends across the six years of the LTIM/MER project.

The MER project includes monitoring in the following hydrological zones:

- Monitoring sites established during the LTIM project that focussed on the upper and mid reaches of the Wakool-Yallakool system (zones 1, 2, 3 and 4) were maintained for the MER project.

- Twenty sites that were established for fish community surveys in 2010 and were monitored in year one (2015) and year five (2019) of the LTIM project were maintained for the MER project and will be surveyed in year three of MER (2022).
- Additional sites were added to the existing network of water quality monitoring sites established during LTIM project. For the MER project there are 17 water quality monitoring sites throughout the whole system.

An evaluation of the outcomes of Commonwealth environmental watering undertaken in 2019-20 was undertaken for the following indicators: Hydrology, Water quality and carbon, Stream metabolism, Aquatic and riverbank vegetation, and fish movement, reproduction, recruitment, and community.

Responses to Commonwealth environmental water were evaluated in two ways:

i) Indicators that respond quickly to flow (e.g. hydrology, water quality and carbon, stream metabolism, fish movement, fish spawning) were evaluated for their response to specific watering actions. Hydrological indicators were calculated on the discharge data with and without the environmental water.

ii) Indicators that respond over longer time frames (e.g. riverbank and aquatic vegetation, fish recruitment, fish community) were evaluated for their response to the longer-term environmental watering regimes. This was undertaken by comparing responses over multiple years, and/or comparing responses in reaches that have received environmental water to zones (e.g. upper Wakool River zone 2) that has received none or minimal environmental water.

Research

The focus of the research is the Edward/Kolety River downstream of Stevens Weir. The Edward/Kolety River was not included in LTIM program, so there are considerable knowledge gaps that need to be addressed to inform the future delivery of environmental water to the Edward/Kolety River and the management of environmental water in relation to the Werai Forest, which is part of the NSW Central Murray Forests Ramsar site (NSW Office of Environment and Heritage 2018).

The Edward/Kolety integrated research project includes physical, ecological, and social research that will address questions relating to how managed flows in the Edward/Kolety River and the operation of Stevens Weir. The research examines physical aspects (e.g. lateral connectivity and physical form) as well as ecological processes, such as river productivity, wetland plant emergence and survival, turtle movement and condition, and fish spawning. In addition, a project on e-DNA approach was undertaken to determine the presence and spatial distribution of threatened, uncommon and iconic or rare taxa that have not been the target of the LTIM/MER monitoring and evaluation. Integrated with these biophysical research themes, social research will be undertaken in 2020-21 to examine stakeholder attitudes to, and acceptance of, the concept and use of Commonwealth environmental water. Some of the research components have different reporting timelines. The research implementation will be undertaken throughout the MER program (2019-2022), and the research outcomes will be integrated in the final MER report in 2022.

Environmental watering in the Edward/Kolety-Wakool system 2019-20

This report reports on responses to Commonwealth environmental watering actions in the Edward/Kolety-Wakool Selected Area from 1 May 2019 to 30 June 2020. This reporting period commences in May 2019 to enable an evaluation of the winter watering action that commenced in May 2019.

Three watering actions were planned by the Commonwealth Environmental Water Office for the 2019-20 water year in the Wakool-Yallakool system and the Colligen-Niemur system (Table i). Some of the water during these actions was sourced as return flows from the Southern Connected Flow in the Murray River. This influenced flows in the Edward/Kolety-Wakool system from 28 August to 9 September 2019, and 23 September to 1 October 2019. The return flows from Millewa Forest may have affected the water quality in the Edward/Kolety-Wakool system on these dates, and on later dates at sites further downstream. Actions 1 and 3a specifically targeted hydrology outcomes of connectivity.

Watering action number 1 was a winter base flow. Watering action 2 was for a short period of time in August 2019 when there was no operational demand, so CEW was used to prevent water levels reducing to low levels for a short period between action 1 and action 3. Watering action 3 was partitioned into 5 components to describe a series of flows commencing on 28 August and ending on 22 December 2019 (Table i)

Table i Planned Commonwealth environmental watering actions in the Edward/Kolety-Wakool system in 2019-20 in the Edward/Kolety Wakool River system. This report focusses on watering actions 1 and 3.

Watering Action No	Name	Objectives (from CEWO)	Dates
Action 1	Winter base flow	For native fish condition and movement, vegetation in-channel, **longitudinal connectivity;** refuge habitat during irrigation shut-down period	15/05/19 - 9/08/2019
Action 2	Winter to spring transition flow	At this time, there was no operational demand so CEW was used to prevent water levels reducing to low levels for a short period between action 1 and action 3.	10/08/19 - 27/08/19
Action 3a	Winter/spring early fresh	To provide early season **rise in river level to contribute to connectivity**, water quality, stimulating early growth of in-stream aquatic vegetation, pre-spawning condition of native fish and/or spawning in early spawning native fish.	28/08/19 - 4/09/19
Action 3b	Early spring elevated base flow	To maintain nesting habitat for Murray Cod, and inundation for aquatic vegetation growth.	5/09/19 - 22/09/19
Action 3c	Late spring fresh	To promote silver perch spawning, influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit. May also assist with dispersal of larvae and juveniles of a number of fish species.	23/09/19 - 11/10/19
Action 3d	Late spring elevated base flow	To influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit. May also assist with dispersal of larvae and juveniles of a number of fish species.	12/10/19 - 30/11/19
Action 3e	Recession	Slow recessions for instream water plants	1/12/19 - 22/12/19

Outcomes of monitoring and evaluation of environmental watering

Key results from environmental watering actions in 2019-20 1 are presented in Table ii.

Table ii Results for each indicator in response to environmental watering actions in the Edward/Kolety-Wakool system in 2019-20.

Theme	Indicator	Key result
Hydrology	Maximum and minimum discharge	The winter watering action (action 1) maintained base flows in Yallakool Creek, the mid and lower Wakool River, and the Colligen-Niemur system. In the absence of environmental water there would have been an extended period of cease to flow in these rivers. Watering action 3 increased the maximum discharge in all zones compared to operational flows.
	Flow variability	Watering action 3 increased the coefficient of variation of discharge compared to operational flows. In the absence of this watering action there would have been extended period of low variability of flows.
	Longitudinal connectivity	Watering action 1 (winter watering action) maintained longitudinal connectivity in Yallakool Creek, the mid and lower Wakool River, and the Colligen-Niemur system.
	Lateral connectivity	Watering action 3 increased lateral connectivity compared to the modelled connectivity under operational flows.
Water quality and carbon	Dissolved oxygen concentration	Dissolved oxygen concentration was consistently higher during late summer and early autumn in zones 1, 3 and 4 than zone 2 that received minimal environmental water than zone 2. Concentrations of dissolved oxygen in the Edward/Kolety River, Wakool River and the Colligen-Niemur River were above the range of concern to fish populations (4 mg/L). The expected seasonal variations were observed, with higher concentrations in winter and lower concentrations during periods of higher water temperature.
	Nutrient concentrations	Total Phosphorus and Total Nitrogen were slightly elevated, likely due to high turbidity, but bioavailable nutrient remained low. The absence of overbank flows meant that substantial nutrient inputs were not expected.
	Temperature regimes	None of the watering actions targeted temperature. Water temperatures in the system were primarily controlled by the prevailing weather conditions.
	Dissolved organic matter	There was no detectable effect of environmental watering actions on dissolved organic matter and no adverse water quality outcomes.
Stream metabolism	Gross Primary Production (GPP)	Watering actions did not substantially affect areal rates of gross primary productivity (GPP)(mg O_2/m^2/day), which largely followed seasonal trends. However, when GPP was calculated as the amount of organic carbon produced per day (kg C/day) the watering actions were shown to have a beneficial effect (more 'food' is better). The size of the beneficial impact was related to the proportion of total flow that came from the watering action, with greater proportional effects of environmental water in winter low-flow periods. Carbon production was enhanced by between 15% and 278% during watering actions, with a median across all sites and watering actions of 50% more carbon produced during Commonwealth environmental watering actions compared to no environmental water.
	Ecosystem Respiration (ER)	As with GPP, areal rates of ecosystem respiration (ER)(mg O_2/m^2/day) were largely driven by seasonal trends. However, when ER was calculated as the amount of organic carbon consumed per day (kg C/day), then watering actions had a beneficial effect. A higher amount of organic carbon consumed means more nutrient recycling and hence greater nutrient supply to fuel GPP. Carbon consumption was enhanced by between 18% and 263% during the watering actions, with a median across all sites and watering actions of 51% more carbon consumed during Commonwealth environmental watering actions compared to no environmental water.

Table ii (continued) Key results for each indicator in response to environmental watering action 1 (the 800 ML/day flow trial) in the Edward/Kolety-Wakool system in 2018-19.

Theme	Indicator	Key result
Riverbank and aquatic vegetation	Total species richness	There was an increase in the mean total species richness in each of the five monitored zones since the flood in 2016. The mean species richness has not yet recovered to the same levels as prior to the flood. The mean total number of taxa was consistently lower in zone 2, which has received minimal or no environmental water compared to the other zones. The exception was in 2018-19 when zone 2 received environmental water.
	Richness of functional groups	Since 2017-18 there has been a gradual recovery of submerged taxa in all zones, but the total richness has not yet reached levels observed prior to the 2016 flood. In 2019-20 the maximum mean precent cover of submerged taxa increased (zones 1 and 8) or was maintained (zones 3 and 4) in zones that received environmental water, but reduced in zone 2 (upper Wakool River) that did not receive environmental water in 2019-20. Since the flood the number of amphibious taxa has increased in all zones. The mean total richness was higher in zones that received environmental water (zones 1, 3, 4 and 8) than in zone 2 that received no or minimal environmental water. However, zones 3 and 4 have not yet recovered to the same total richness observed prior to the flood.
	Percent cover of functional groups	In 2019-20 there was a significant increase in the cover of *Chara* (submerged macro algae) in the monitored hydrological zones that received environmental water (zones 1, 3, 4, 8), and the cover has returned to pre-flood levels in these zones. The response in cover of amphibious taxa since the 2016 flood has not been consistent among zones because there were different dominant taxa in different zones. Spiny mud grass (*Pseudoraphis spinescens)* was the most abundant taxa in zone 4 and has increased in percent cover in zone 4 such that it currently has a higher percent cover than was recorded prior to the flood. The common spikerush (*Eleocharis sp.)* was the dominant taxa in zone 8 (Colligen Creek) prior to the flood, but tolerated the flooding and has maintained similar mean percent cover across all years. In contrast, floating pondweed (*Potamogeton tricarinatus)* was the dominant amphibious taxa in zone 3 prior to the flood and significantly reduced in cover or was killed by the flood in 2016. It was recorded again for the first time in 2019-20 in zone 3 at low percent cover. Similarly, milfoil (*Myriophyllum spp)* was abundant in zones 1, 3 and 4 prior to the flood but was recorded at low percent cover in zones 1 and 3 in 2019-20.
Fish movement	Movement of golden perch and silver perch	No discernible differences were observed in the scale of the movements of golden perch or silver perch during the 2019 winter watering event, although we note that sample sizes were low. Modelling based on previous water delivery years (2017 and 2018) indicates that CEW deliveries in winter result in an increase in the frequency of movement of golden perch, silver perch and Murray cod, but that it is most pronounced in silver perch. Occupation of Yallakool Creek zone 1 by golden perch was enabled during winter watering in 2019 in comparison to winter 2018 (no watering), indicating that increased habitat was both available and utilised during the watering event.
	Larval abundance of periodic species	Significantly more bony herring larvae were found in some study rivers that received environmental water compared to the Upper Wakool River, which did not receive environmental water. Despite the Southern Connected Flow in the Murray River that influenced the Edward/Kolety-Wakool system, there was no evidence of golden or silver perch spawning recorded.

	Larval abundance of opportunistic species	Significantly more Australian smelt larvae were found in all study rivers that received environmental water compared to the Upper Wakool River which did not receive environmental water.
Fish recruitment	Murray cod, silver perch and golden perch recruitment	Murray cod YOY recruits were detected in zones 3 and 4 for first time since 2015-16. Murray cod 1+ recruits were at their highest relative abundance since surveys began, although slower growth rates were observed compared to the previous year. Silver perch 1+ recruits were present at a low relative abundance and YOY recruits were not detected. No golden perch 1+ or YOY recruits were detected.
Fish populations	Adult fish populations	Eight native species and two alien fish species were captured during fish community sampling. Both flathead gudgeon and Eastern gambusia were absent, although these were in low and/or variable abundance in previous years. Almost-no carp recruitment were observed in 2020, and the adult population exhibited decreased relative abundance and biomass. The golden perch population continues to exhibit no recruitment, and is predominantly comprised of large adults. The population is ageing but stable. Murray cod relative abundance and biomass continue to increase following fish kills in 2016. Bony herring were present at the highest relative abundance observed in the program, reflecting a strong spawning and recruitment year. Typical annual fluctuations were observed in small bodied generalist species

Outcomes of research projects

Edward/Kolety River physical habitat

We investigated the impacts of flow events on physical habitat on the riverbank in two reaches of the Edward/Kolety River and one reach in Colligen Creek. Unmanned Aerial Vehicle (UAV) technology was used to capture high resolution aerial imagery to process with photogrammetry methods to produce; Detailed digital elevation models (DEMs), DEMs of Difference (DEMODs) and quantifying bank condition changes, and riparian vegetation maps displaying spatial and temporal differences associated with flow events, quantification of the percentage loss of riparian vegetation, and identify areas of most/least impact.

Questions addressed were:

- What are the features of the flow regime and river operations that drive erosion and deposition?
- What are the features of flow regime and river operations that affect riverbank vegetation and aquatic vegetation cover?

Operational flows that produce prolonged invariable periods of inundation to riverbanks within a defined zone of the river bank appear to be the main driver of notching on riverbanks in the Edward/Kolety River downstream of Stevens Weir. The position of the notch relative to the water level of subsequent flow delivery was a critical variable which implicated the scale and pattern of the erosion response. Commonwealth environmental water (CEW), operational flows and unregulated flows that result in periods of inundation of the riverbank above the notch, can result in large quantities of unstable sediment and upon draw-down. It is the combination of the following processes in sequence that are the driving force behind extensive areas of channel widening resulting from mass-failure events; a) *Summer operational flows:* create a deep notch and drying of the upper bank, and b) *Environmental or unregulated flows:* saturation of upper bank and drawdown of the water level.

The influence of CEW actions or unregulated flows on bank condition cannot be measured in isolation. Preparation of the bank during operational flows plays a critical role in driving erosion events throughout the entire year and in years following. The erosion volumes at the Edward/Kolety River site downstream of Stevens Weir are linked to the historic pattern of operational flows.

Flows which resulted in the most deposition relative to erosion were unregulated flows during the winter months. This was due to the following combination of factors; a) the source of the water delivered during this period (high tributary %), b) the range of these flows (between 500 - 3,000 ML/day), and c) the lack of erosion evident in response to these events. CEW actions that are delivered with a gradual draw-down of the receding limb are likely to result in less erosion due to mass-failure events, and higher levels of deposition as a result of mud-draping. However, this will have a limited impact if summer operational flows are not re-designed.

Prolonged inundation (+30 days) during spring appeared to reduce riparian vegetation cover above the bank zone relating to 3,000 ML/day in the Edward/Kolety River downstream of Stevens Weir. This was evident in Colligen Creek and the Edward/Kolety River, however some of this vegetation grew back over the summer and autumn months, highlighting the ability of vegetation to recolonise under favourable conditions.

This study highlights the important role that historic flow patterns play on influencing future erosion events. In systems like the Edward/Kolety-Wakool, where historic flow patterns have led to excessive notching within channels, bank responses to flow events cannot be looked at in isolation. Thus, to be able to correctly assess the outcome of CEW on riverbanks, the impacts of operational flow strategies must be considered and addressed. If the management of operational flows does not change, then the potential benefits to bank condition as a result of CEW actions-will not materialise. If this is not possible to change operational flow delivery then environmental flow deliveries need to be designed with the position of the existing notch considered and with close attention to the rate of fall to minimise responding mass-failure events.

Edward/Kolety River primary productivity research

The stream metabolism monitoring for LTIM/MER project to date has focussed on in-channel flow in the Wakool-Yallakool system. The aim of this new research was to advance understanding of Gross Primary Production (GPP) and Ecosystem Respiration (ER) in the Edward/Kolety River downstream of Stevens Weir, due to potential for flows to inundate parts of Werai Forest, connecting low-lying floodplains, anabranches and floodplain wetlands and runners that sometimes return discharge back into the river. The main research question was ‘How does variation in the flow regime downstream of Steven’s Weir drive changes in rates of GPP, ER and net ecosystem production (NEP, GPP – ER) in the Edward/Kolety River?’.

GPP, ER and NEP were calculated for one site upstream of Werai Forest and one site downstream of the forest. The downstream site integrates a reach of the Edward/Kolety River bordering Werai Forest as well as outflows from Werai Forest. Unfortunately there was not enough useable data in 2019-20 to answer the research question. However, the most notable trends in the available data were a greater occurrence of high GPP and ER events downstream of Steven’s Weir when compared with the site downstream of Werai Forest, and a seasonal progression from higher to lower GPP/ER ratios from summer to winter 2020. There was little correlation between other potential indicators of inundation within the forest (% inundation, daily rainfall) and changes in GPP or ER rates.

Examination of Sentinel satellite imagery suggests that low-lying areas prone to inundation are more likely to occur in the centre of Werai Forest than along the Edward/Kolety River. These inundated floodplain wetlands within Werai Forest are likely to act as a strong "sink" for nutrients and carbon (i.e. retained within the system and either incorporated into organism biomass or deposited) rather than exported downstream to support in-channel respiration. Thus, much of the carbon cycled during inundation of the forest may be both produced and consumed within shallow, slow-flowing anabranches and inundated floodplains, and may not be reflected in oxygen cycles within the river.

A more comprehensive understanding of how inundation events from anabranch and connection flows influences whole-river metabolism will require monitoring of several sites within Werai Forest. We recommend that a campaign/intervention monitoring type of study be undertaken during a flow event >2700 ML/day that inundates low lying parts of Werai forest and is likely to return flows to Colligen Creek or the Edward/Kolety River. The evaluation of primary productivity associated with the event would be enhanced by the installation of temporary gauges to collect data on the inflows to the forest. Analysis of Sentinel images would also quantify extent of inundation within Werai Forest. This research is focussed on the Werai Forest, and the lessons learned from this project may be transferrable to other low lying forested areas within the Edward/Kolety-Wakool system, such as Koondrook Perricoota Forest.

Fish spawning in the Edward/Kolety River

Throughout the LTIM/MER project there has been no evidence of golden perch spawning in Wakool-Yallakool system and only a very small number of silver perch larvae recorded in that system. Local fishers had previously observed golden perch congregating in the Edward/Kolety River downstream of Stevens Weir during late spring, prompting the establishment of this research project. The aim of the research was to determine if golden perch and silver perch spawn in the Edward/Kolety River downstream of Steven's Weir. The project was undertaken as a citizen science project through collaboration between Charles Sturt University and the Edward-Wakool Angling Association. The MER program funded the field work and employment of EWAA members on the project. Members of EWAA from Deniliquin undertook drift net sampling at three sites in the Edward/Kolety River once per week over a period of twenty-two weeks in 2019-20, and samples were analysed at Charles Sturt University.

In 2019-20 there was no indication of golden or silver perch spawning at the three study sites in the Edward/Kolety River as evidenced by the lack of eggs or larvae of golden perch and silver perch. Regardless of this result, further monitoring over a longer period of time is warranted as these are long-lived species that may not spawn every year. The project demonstrated that collaboration between researchers and community groups is an effective way to undertake research and engage the local community, draw on local expert knowledge, provide local employment and training, and make cost savings and reduced carbon emissions due to reduced travel.

Targeted eDNA research to identify the presence and spatial distribution of threatened, uncommon and iconic species

The aim of this research was to use a targeted, single species eDNA to identify the presence and spatial distribution of threatened, uncommon and iconic species in the Edward/Kolety-Wakool system. This approach allows flexibility in the choice of the target gene to maximise the chance that the target species can be detected and differentiated from congeneric species.

Water samples were collected from 10 sites; six MER fish monitoring sites, and four additional sites in the Edward/Kolety River to link with other components of the integrated research project. PCR assay design was already available for platypus. The research successfully developed designs for six additional species; Murray cod, trout cod, silver perch, dwarf flathead gudgeon, freshwater catfish and Murray crayfish. The assays were tested for specificity by comparing DNA from the target species and closely related species. Redfin perch were not a target species, this invasive species was included in the study because the assay was already available.

Murray cod were detected at 8 of 10 sites and trout cod were detected at 4 of 10 sites, with the highest proportion of positive detections in the upper Edward/Kolety River at Four Posts. Silver perch were detected in 7 of 10 sites, however there was a lower proportion of positive replicates of silver perch per sample per site than Murray cod. Redfin perch were detected at a single site. Platypus were not detected at any sites, however two samples were determined to be false positives and this will be sequenced to check if the qPCR product is platypus. Dwarf flathead gudgeon, freshwater catfish and Murray crayfish were not detected at any of the 10 sites.

Developing targeted eDNA assays is time consuming and expensive, particularly if there are complications in primer development, such as a lack of variability in the target gene between closely related species. However, we concluded that once assays have been developed and tested, targeted eDNA is an effective method to detect the presence of rare and threatened species. It is particularly suitable to document the distribution of species inefficiently sampled by other methods. We recommend future work explores occupancy modelling to enable detection probabilities to be estimated. This eDNA approach could potentially be used in the future to identify population expansion as a result of targeted environmental watering.

Recommendations for future management of environmental water

A summary of recommendations from previous Edward/Kolety-Wakool LTIM annual reports (Watts et al. 2015, 2016, 2017b, 2018, 2019) and the extent to which they have been implemented to improve the planning and delivery of Commonwealth environmental water are summarised in Table iii. Details of CEWO adaptive management response and actions undertaken to implement these recommendations are outlined in previous reports.

Table iii Summary of recommendations from Edward/Kolety-Wakool LTIM annual reports 2014-15, 2015-16, 2016-17, 2017-18 and 2018-19 showing year implemented. R = recommendation number.

Recommendation	Year(s) recommended	Year(s) implemented
1. Consider a trial to increase the delivery of environmental water to the upper Wakool River	2014-15 (R3) 2015-16 (R6) 2016-17 (R5)	2018-19
2. Consider the implementation of an environmental watering action in the Edward/Kolety River to target golden perch and silver perch spawning.	2014-15 (R8) 2015-16 (R4) 2016-17 (R4) 2017-18 (R3)	Not yet implemented
3. In collaboration with stakeholders explore options to implement a short duration environmental flow trial in late winter/spring 2016 at a higher discharge than the current constraint of 600 ML/d at the Wakool-Yallakool confluence. This would facilitate a test of the hypothesis that larger in-channel environmental watering action will result in increased river productivity. Implement a second flow trial in-channel fresh in late winter or early spring that exceeds the current normal operating rules, to increase the lateral connection of in-channel habitats and increase river productivity. The earlier timing of flows would help to prime the system and thus increase the outcomes of subsequent watering actions delivered later in spring or early summer.	2014-15 (R7) 2015-16 (R3) 2017-18 (R4) 2018-19 (R3)	2018-19

4. Each year plan to deliver at least one flow event with higher than normal operating discharge to the upper Wakool River. This may include delivery of water through the Wakool offtake regulator or via the Wakool escape	2018-19 (R1)	2018-19
5. Increase the duration of the recession of environmental watering actions relative to the Yallakool Creek environmental watering actions in 2012-13 and 2013-14	2014-15 (R1) 2015-16 (R8)	2015-16 2016-17 2017-18
6. Consider the delivery of continuous base environmental flows during autumn and winter to promote the temporal availability and continuity of instream habitat	2014-15 (R4) 2015-16 (R2) 2016-17 (R3)	Winter 2017
7. Implement a second trial of continuous base winter environmental flow (no winter cease to flow) in tributaries of the Edward/Kolety-Wakool system to promote the temporal availability and continuity of instream habitat to benefit fish and other aquatic animals and assist recovery of submerged aquatic plants.	2017-18 (R2)	Winter 2019
8. Avoid long periods of constant flows by introducing flow variability into environmental watering actions. Include variation in the timing of environmental watering actions among water years to promote the temporal availability and continuity of instream habitat to benefit fish and other aquatic animals and assist the recovery of submerged aquatic plants in the system.	2014-15 (R2) 2015-16 (R5) 2018-19 (R2)	2015-16 2016-17 2018-19
9. Implement environmental watering actions for freshes in spring and early summer (October to December) that include flow variability up to a magnitude of + 125 to 150 ML/d. Undertake trials to improve understanding of the magnitude of variability that provides beneficial ecosystem outcomes.	2017-18 (R1)	
10. Explore options to implement in-channel pulses at any time of the year to connect additional in-channel habitats and increase river productivity.	2018-19 (R4)	Not yet implemented
11. Continue to include a water use option in water planning that enables environmental water to be used to mitigate adverse water quality events	2014-15 (R5) 2015-16 (R7)	2014-15 2015-16 2016-17 2017-18 2018-19
12. If there is an imminent hypoxic blackwater event during an unregulated flow and the quality of source water is suitable, water managers in partnership with local landholder and community representatives should take action to facilitate the earlier release of environmental water on the rising limb of the flood event to create local refuges prior to DO concentrations falling below 2 mgL^{-1}.	2016-17 (R1)	Not yet implemented
13. Trial a carefully managed environmental watering action through Koondrook-Perricoota Forest via Barbers Creek to improve the productivity of the mid and lower Wakool River system.	2017-18 (R5)	Not yet implemented via Barbers Ck
14. Explore and develop a range of options for the delivery of environmental water during times of drought to ensure connectivity of habitat and avoid damage to key environmental assets. Inform the community of the factors limiting water delivery in extreme drought.	2018-19 (R5)	Not yet implemented
15. Set watering action objectives that identify the temporal and spatial scale at which the response is expected and are realistic given the magnitude of watering actions proposed	2014-15 (R6)	ongoing
16. Undertake a comprehensive flows assessment for the tributaries of the Edward/Kolety-Wakool system to better inform future decisions on environmental watering in this system.	2014-15 (R9) 2015-16 (R1)	Partly undertaken
17. Collaborate with other management agencies and the community to maximise the benefits of Commonwealth environmental watering actions	2014-15 (R10)	ongoing
18. The installation of a DO logger on a gauge downstream of Yarrawonga and upstream of Barmah-Millewa Forest should be considered a priority. Consideration should also be given to installing DO loggers, both upstream and downstream of other forested areas that influence water quality in the Edward/Kolety-Wakool system	2016-17 (R2)	Not yet implemented
19. Undertake in-channel habitat mapping for key reaches of the Edward/Kolety-Wakool system, which could then be combined with existing hydraulic modelling to facilitate learning about this system	2016-17 (R6)	Implemented in part by NSW DPI
20. The CEWO and other relevant agencies undertake a review of the 2016 flood and subsequent hypoxic blackwater event in the Murray system and support further research into understanding these events	2016-17 (R7)	2017

Recommendations from 2019-20 watering actions

We continue to endorse the recommendations from previous LTIM reports as summarised in Table iii. In addition, we outline the following 15 recommendations to improve the planning and delivery of Commonwealth environmental water in the Edward/Kolety-Wakool system.

Recommendations for small in-channel freshes

Recommendation 1: Although small watering actions have provided a beneficial outcome for the riverine ecosystem productivity, it is highly probable that reconnecting backwaters and the floodplain to the river channel would result in much larger positive outcomes. It is recommended that, when possible, consideration be given to providing a more variable flow regime in the Edward/Kolety-Wakool system in future years.

Recommendation 2: Deliver a series of freshes to all rivers in all major tributaries of the Edward/Kolety-Wakool system to increase the wetted area of the bank. Late winter/early spring freshes that inundate slackwater areas, in-channel benches or low lying areas of riverbank within the channel will trigger emergence of river bank vegetation. Following the recession of flows, these damp banks provide ideal conditions for plants to establish and grow prior to the onset of hotter weather in summer that can quickly dry out the river banks.

Recommendation 3: In years with high water availability, consider a late spring/early summer pulse, immediately after Murray cod larvae have left the nest, to support food resources for Murray cod larvae while at the same time providing opportunities for spawning to occur in silver perch and golden perch.

Recommendation 4: Consider adaptive use of water to coincide with high Murray River flows to maximise attraction/immigration of upstream migrating juvenile golden perch and silver perch in late summer. The probability of silver perch moving into and then staying in other more upstream tributaries of the Murray River (Goulburn and Campaspe rivers) is elevated in March-May (Koster et al. 2020), so delivering attraction flows in the Edward/Kolety-Wakool river system at this time or before (e.g. January-March) may be optimal for this more downstream tributary.

CEWO Adaptive Management Response: The CEWO agrees that late winter/early spring pulses are important for a range of outcomes, including vegetation, native fish and connectivity. When flows in the Murray River may focus on late spring/early summer pulses, the CEWO will examine the delivery of two pulses into the Edward/Kolety system – one in late winter/early spring and another synchronised with Murray River flows in late spring/early summer.

Recommendations for flows to mitigate poor water quality events

Recommendation 5: In watering years where risk of hypoxic blackwater events is probable, consider how CEW watering actions could be used to mitigate effects on fish populations. One option to explore could be use of flows to encourage movement out of high risk reaches.

Recommendations for winter flows

Recommendation 6: Delivery of environmental water had the greatest proportional effect during winter low-flow periods. We recommend that discharge and wetted area are maintained during low flow periods to maintain zooplankton and other invertebrates that feed on phytoplankton and periphyton, and in turn increasesfood availability for fish and other higher order consumers during periods in which food availability might otherwise be low.

Recommendation 7: Prevent negative impacts of a-seasonal cease-to-flow events by delivering winter base flows to promote temporal availability and continuity of instream habitat for aquatic vegetation. This will have minimise damage from damage from frost and livestock if the system is shut down during the winter, and result in positive benefits for the survival and maintenance of aquatic and riverbank vegetation.

Recommendation 8: Prevent negative impacts of a-seasonal cease-to-flow events by delivering winter base flows to promote temporal availability and continuity of instream habitat for fish. Evidence from 2019-20 monitoring indicates this has positive benefits for the survival and local retention of juvenile fish.

CEWO Adaptive Management Response: The ability to prevent winter cease-to-flow conditions in the Yallakool-Wakool and Colligen-Niemur systems is not controlled by the CEWO. The opportunity to provide winter base flows is determined by the need to undertake annual maintenance on Stevens Weir. The CEWO will continue to work with WaterNSW to identify opportunities to deliver winter base flows in the Edward/Kolety River system.

Flow recommendations for the upper Wakool River

Recommendation 9: Undertake watering actions to improve the aquatic and riverbank vegetation outcomes in the Upper Wakool River. Deliver larger freshes with increased variability to enable riverbank vegetation to establish and be maintained.

Recommendation 10: Deliver elevated base flows to the Upper Wakool River from September-December to maximise nesting and spawning opportunities for Murray cod. Record catches of larvae have been recorded when this type of watering action is delivered. This type of flow delivery should be supported with subsequent winter base flows throughout the Selected Area to maximise retention and survival of YOY in the region.

CEWO Adaptive Management Response: The CEWO increased flows into the upper Wakool River system during summer and autumn 2021, primarily to improve water quality in this reach. The CEWO is interested to see if the monitoring also shows any change in vegetation and fish outcomes as a result of these increased flows.

CEWO Adaptive Management Response: A number of the recommendations above are linked to recommendations for aquatic and riverbank vegetation outcomes. The CEWO will seek to implement these recommendations via multi-objective watering actions, as it has done so in the past.

Flow recommendations for Edward/Kolety River downstream of Stevens Weir

Recommendation 11: We recommend that options for a high flow event downstream of Stevens Weir (>2700 ML/day) that inundates low lying part of Werai forest and is likely to return flows to either Colligen Creek or the Edward/Kolety River are explored.

CEWO Adaptive Management Response: Options for delivering environmental water to Werai Forest are being explored. There are issues around delivery and gauging of water that need to be resolved.

Recommendations for monitoring and research

Recommendation 12: We recommend that a campaign/intervention monitoring type of study be undertaken during a high flow event (>2700 ML/day) that inundates low lying part of Werai forest and is likely to return flows to either Colligen Creek or the Edward/Kolety River. The evaluation of primary productivity associated with the event would be enhanced by the installation of temporary gauges to collect data on the inflows to the forest. Analysis of Sentinel images would also quantify extent of inundation within Werai Forest.

Recommendation 13: Targeted eDNA methods are most suitable when the objective is to document the distribution of species inefficiently sampled by other methods. This research has shown that eDNA is an effective method to detect the presence of rare and threatened species in the Edward/Kolety-Wakool system. We recommend future work explores occupancy modelling to enable detection probabilities to be estimated.

Recommendation 14: Although there were no golden or silver perch eggs or larvae detected in the Edward/Kolety River in 2019-20, further monitoring over a longer period of time is warranted. The growing appreciation of large spatial scales at which these species operate highlights the need for continued monitoring of spawning and recruitment indicators across key main channel and off-channel environments in both the southern and northern Murray-Darling Basin. Ongoing monitoring and analysis of the pattern of flow delivery and water velocities across multiple years will be able to better inform a discussion about spawning of silver perch and golden perch in the Edward/Kolety River.

Recommendation for communication and engagement

Recommendation 15: Consider developing communication products and contribute to engagement programs in collaboration with other agencies (e.g. Local Land Services) to support projects that reduce risks to recovery and maintenance of aquatic and riverbank plants by carp, pigs and livestock. Disturbance of the riverbank caused by carp, pigs and livestock has a high potential to undo the positive outcomes of environmental watering actions.

1 INTRODUCTION

1.1 Purpose of this report

The Commonwealth Environmental Water Office (CEWO) Monitoring, Evaluation and Research (MER) Program (2019 to 2022) is an extension of the Long-Term Intervention Monitoring (LTIM) and Murray-Darling Basin Environmental Water Knowledge and Research Project (EWKR) projects, with monitoring, evaluation and research activities undertaken within a single integrated program.

The LTIM Project was implemented over five years from 2014-15 to 2018-19 to deliver five outcomes:

- Evaluate the contribution of Commonwealth environmental watering to the objectives of the Murray-Darling Basin Authorities (MDBA) Environmental Watering Plan.
- Evaluate the ecological outcomes of Commonwealth environmental watering in each of the seven Selected Areas.
- Infer ecological outcomes of Commonwealth environmental watering in areas of the MDB that are not monitored.
- Support the adaptive management of Commonwealth environmental water; and
- Monitor the ecological response to Commonwealth environmental watering at each of the seven Selected Areas.

The MER Program consists of evaluation, research and engagement at a Basin-scale and on ground monitoring, evaluation, research and engagement across seven Selected Areas, one of which is the Edward/Kolety-Wakool system. The MER Program aims to provide the critical evidence that is needed to understand how water for the environment is helping maintain, protect, and restore the ecosystems and native species across the Murray–Darling Basin. The program will demonstrate outcomes of environmental watering actions, inform management of Commonwealth water for the environment and will help meet the CEWO's legislative reporting requirements through to June 2022.

This report describes the monitoring, evaluation and research activities that were undertaken in the Edward/Kolety-Wakool system as part of the CEWO MER Program from July 2019 to June 2020. This project was undertaken as a collaboration between Charles Sturt University, NSW DPI (Fisheries), NSW Department of Planning, Industry and Environment, La Trobe University and Streamology. The fish spawning research was undertaken in partnership with the Edward-Wakool Angling Association. The monitoring and research described in this report is undertaken using methods and approaches described in the Edward/Kolety-Wakool MER Plan (Watts et al 2019a). As the MER project is a continuation of the LTIM Project, for some of the monitoring indicators we will evaluate long-term trends across the six years of the LTIM/MER project.

This report has thirteen sections. This introduction (section 1) is followed by a description of the Commonwealth environmental water use objectives and watering actions for this system for 2019-20 (section 2). An overview of the monitoring, evaluation and research undertaken in this system for the MER project and its relationship to LTIM monitoring is described in section 3. Summaries of the evaluation of responses of each indicator to Commonwealth environmental watering and unregulated flow events are presented in sections four to eight; hydrology (section 4), water quality and carbon (section 5), stream metabolism (section 6), riverbank and aquatic vegetation (section 7), and fish movement, fish spawning, fish recruitment and fish community (section 8). Sections nine to twelve report on the outcomes of several components of an integrated research program focused on the Edward/Kolety River. Section 9 reports on physical habitat research, section 10 on primary productivity research, section 11 on fish spawning research and section 12 on eDNA biodiversity research. Recommendations to help inform adaptive management of environmental water in the Edward/Kolety-Wakool system in the future is presented in section 13. A summary report (Watts et al. 2020) provides an overview of the monitoring and key findings of the ecosystem responses to environmental watering actions in the Edward/Kolety-Wakool system in 2019-20 including findings across the six years of the combined LTIM/MER program.

1.2 Edward/Kolety-Wakool Selected Area

The Edward/Kolety-Wakool system is a large anabranch system of the Murray River in the southern MDB, Australia. The system begins in the Millewa Forest and travels north and then northwest before discharging back into the Murray River (Figure 1.1). It is a complex network of interconnected streams, ephemeral creeks, flood-runners and wetlands including the Edward/Kolety River, Wakool River, Yallakool Creek, Colligen-Niemur Creek and Merran Creek. There are also several small intermittent and ephemeral creeks of ecological significance. Under regulated conditions flows in the Edward/Kolety River and tributaries remain within the channel, whereas during high flows there is connectivity between the river channels, floodplains and several large forests including the Barmah-Millewa Forest, Koondrook-Perricoota Forest and Werai Forest (Figure 1). These three forests make up the NSW Central Murray Forests Ramsar site (NSW Office of Environment and Heritage 2018), being one of the matters of national environmental significance to which the EPBC Act applies.

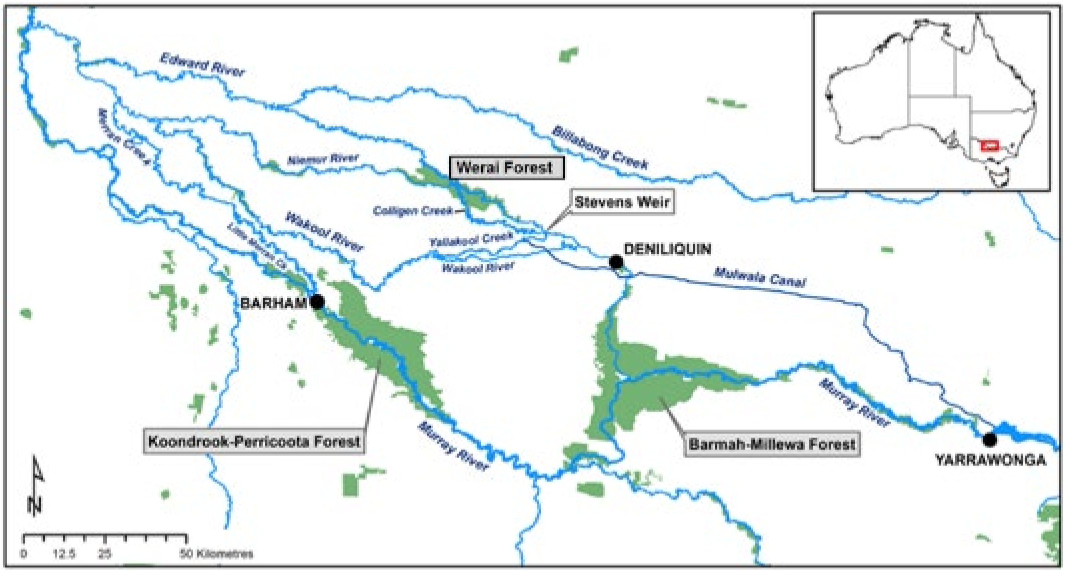


Figure 1.1 Map showing the main rivers in the Edward/Kolety-Wakool system. (Source: Watts et al. 2013)

The Edward/Kolety-Wakool Selected Area can be broadly divided into three aquatic ecosystem types: 1) The main semi-permanent flowing rivers including Yallakool and Colligen creeks and the Wakool, Niemur and Edward/Kolety rivers, 2) The floodplain forests and woodlands including the Niemur and Werai Forests, and 3) Several small intermittent and ephemeral creeks of ecological significance including Tuppal, Jimaringle, Cockran and Gwynne's Creeks.

Edward/Kolety River, Colligen- Niemur, Yallakool Creek and Wakool River

These rivers and creeks support high regional biodiversity values and have significant value as drought refugia for native fish and other biota. The dominant vegetation is river red gum (*Eucalyptus camaldulensis*) with areas providing habitat for a number of threatened species.

Floodplain – Werai and Niemur Forest

Werai Forest is of special significance to the Aboriginal community. The Werai Forest is a culturally significant area of land identified as a potential future Indigenous Protected Area, the first in the Murray region of NSW. The higher floodplain areas are dominated by river red gum with lower lying areas typically dominated by giant rush. The low lying areas, floodrunners and backwaters in Werai Forest may be important habitat for larval and juvenile fish and is a potential source of carbon to feed the lower Edward/Kolety River and Niemur River systems. The Werai Forest supports significant breeding colonies of several species of cormorants, whilst the Niemur Forest supports egrets and nankeen knight heron breeding colonies. Both forests support a number of listed species and migratory species. Werai Forest is part of the Ramsar listed NSW Central Murray State Forests (NSW OEH 2018) and Niemur Forest is located in a National Park (CEWO 2012c).

Ephemeral and intermittent creeks - Tuppal, Jimaringle, Cockran and Gwynnes

Tuppal Creek is an intermittent flood runner connecting the Murray River to the Edward/Kolety River and has a largely continuous riparian corridor which provides habitat connectivity for over 120 terrestrial native species and supports a number of state listed threatened and vulnerable species (Brownbill and Warne 2010; CEWO 2012c). Jimaringle, Cockran and Gwynnes Creeks are all ephemeral creeks and considered a biodiversity hotspot of significant regional value.

The Edward/Kolety-Wakool system is considered to be important for its high native species richness and diversity including threatened and endangered fish, frogs, mammals, and riparian plants. It is listed as an endangered ecosystem, as part of the 'aquatic ecological community in the natural drainage system of the lower Murray River catchment' in New South Wales (NSW Fisheries Management Act 1994). This system has abundant areas of fish habitat, and historically had diverse fish communities which supported both commercial and recreational fisheries. Threatened species include the Trout Cod, Murray Hardyhead, Murray Cod, Australian Bittern, Australian Painted Snipe, Superb Parrot, and Swamp Wallaby Grass (Department of Environment and Energy 2019).

The area supports a productive agricultural community, has a rich and diverse Indigenous history, and supports both active and passive recreational uses such as fishing, bird-watching and bush-walking. Many Aboriginal nations maintain strong connections to the country, including the Wamba Wamba or Wemba Wemba, Perrepa Perrepa or Barapa Barapa, and Yorta Yorta. The Werai Forest is in the process of conversion to an Indigenous Protected Area.

The Edward/Kolety-Wakool system plays a key role in the operations and ecosystem function of the Murray River and the southern MDB, connecting upstream and downstream ecosystems in the mid-Murray River. The multiple streams and creeks in this system provide important refuge and nursery areas for fish and other aquatic organisms, and adult fish regularly move between this system and the Murray River. As some of the rivers in the Edward/Kolety-Wakool system have low discharge (compared to the Murray River) there is a risk of poor water quality developing in this system, particularly during warm periods or from floodplain return flows. Maintaining good water quality is crucial for both the river ecosystem, the communities that rely on water from this system, and downstream communities along the Murray River that are influenced by the water quality in this system.

2 ENVIRONMENTAL WATER USE OBJECTIVES AND WATERING ACTIONS IN 2019-20

2.1 Expected outcomes from Basin-wide Environmental Watering Strategy relevant to the Mid-Murray Region

Expected outcomes from the Basin-wide Environmental Watering Strategy (MDBA 2014) that are relevant to the Mid Murray Region are listed below and in Table 2.1 and Table 2.2.

River flows and connectivity

- Base flows are at least 60 per cent of the natural level
- Contributing to a 30 per cent overall increase in flows in the River Murray
- A 30 to 60 per cent increase in the frequency of freshes, bankfull and lowland floodplain flows

Vegetation

- Maintain the current extent of water-dependent vegetation near river channels and on low-lying areas of the floodplain
- Improve condition of black box, river red gum and lignum shrublands
- Improve recruitment of trees within black box and river red gum communities
- Increased periods of growth for non-woody vegetation communities that closely fringe or occur within the river and creek channels, and those that form extensive stands within wetlands and low-lying floodplains including Moira grasslands in Barmah–Millewa Forest

Fish

- No loss of native species
- Improved population structure of key species through regular recruitment, including:
  - Short-lived species with distribution and abundance at pre-2007 levels and breeding success every 1–2 years
  - Moderate to long-lived with a spread of age classes and annual recruitment in at least 80% of years
- Increased movements of key species
- Expanded distribution of key species and populations

Table 2.1 Important Basin environmental assets for native fish in the Mid Murray (from MDBA 2014)

Environmental asset	Key movement corridors	High Biodiversity	Site of other Significance	Key site of hydrodynamic diversity	Threatened species	Dry period / drought refuge	In-scope for Commonwealth water
Koondrook–Perricoota	*	*	*	*	*		Yes
Gunbower	*	*	*	*	*		Yes
Barmah–Millewa	*	*	*	*	*	*	Yes
Edward–Wakool system	*		*	*	*	*	Yes
Werai Forest			*	*			Yes
Billabong–Yanco–Columbo Creeks		*	*	*	*	*	Yes
Lake Mulwala	*		*	*	*	*	Yes

Table 2.2 Key species for the Mid Murray (Source: MDBA 2014)

Species	Specific outcomes	In-scope for Commonwealth water in the Mid Murray?
Flathead galaxias (*Galaxias rostratus*)	Expand the core range in the wetlands of the River Murray	Yes
Freshwater catfish (*Tandanus tandanus*)	Expand the core range in Columbo-Billabong Creek and Wakool system	Yes
Golden perch (*Macquaria ambigua*)	A 10–15% increase of mature fish (of legal take size) in key populations	Yes
Murray cod (*Maccullochella peelii peelii*)	A 10–15% increase of mature fish (of legal take size) in key populations	Yes
Murray hardyhead (*Craterocephalus fluviatilis*)	Expand the range of at least two current populations. Establish 3–4 additional populations, with at least one in the Mid Murray conservation unit.	Yes
Olive perchlet (*Ambassis agassizii*)	Olive perchlet are considered extinct in the southern Basin. Reintroduction using northern populations is the main option for recovery. Candidate sites may result from improved flow that reinstates suitable habitat in the River Murray.	Restoration of flow to Murray River could support future reintroduction of the species
River blackfish (*Gadopsis marmoratus*)	Expand the range of current populations from the Mulwala canal	Yes
Silver perch (*Bidyanus bidyanus*)	Expand the core range within the River Murray (Yarrawonga–Euston)	Yes
Southern purple-spotted gudgeon (*Mogurnda adspersa*)		Yes
Southern pygmy perch (*Nannoperca australis*)	Expand the range of current populations at Barmah-Millewa and other Mid Murray wetlands	Yes
Trout cod (*Maccullochella macquariensis*)	Expand the range of trout cod up the Murray upstream of Lake Mulwala and into the Kiewa River. For the connected population of the Murrumbidgee–Murray–Edward: continue downstream expansion.	Yes
Two-spined blackfish (*Gadopsis bispinosus*)	Establish additional populations (no specific locations identified)	Yes

2.2 Water Quality targets

The water quality targets of the Basin Plan (2012) are outlined in Chapter 9, Part 4, sub-section 9.14(5) of the Plan. The targets for recreational water quality in Section 9.18 contains Guidelines for Managing Risks in Recreational Water. The target for DO in the Plan is to maintain DO at a value of at least 50% saturation and suggests this be determined at 25°C and 1 atmosphere of pressure (sea level). This equates to a DO concentration of approximately 4 mg/L. The CEWO has used a trigger of 4.0 mg/L for the potential provision of refuge flows into catchments like the Edward/Kolety-Wakool River system. The Guidelines for Managing Risks in Recreational Water also guide the green, amber and red alert levels issued by relevant state management agencies (e.g. in NSW – the Regional Algal Coordinating Committees) who are responsible for the catchment scale management of algal blooms. The CEWO has access to the alert advice issued by these state agencies and can adjust the use of Commonwealth environmental water accordingly.

2.5 Commonwealth environmental watering actions 2009-2019

Commonwealth environmental watering actions have occurred in the Edward/Kolety-Wakool system since 2009 (Table 2.3). Between July 2009 and June 2019 Commonwealth environmental watering actions delivered base flows and freshes, contributed to the recession of flow events, delivered water from irrigation canal escapes to create local refuges during hypoxic blackwater events, and contributed to flows in ephemeral watercourses (Table 2.3). Many of the watering actions in ephemeral creeks were undertaken jointly with NSW DPIE. One Commonwealth watering action in 2009-10 for Werai State Forest (DEE 2017) was undertaken to deliver environmental water to Edward/Kolety-Wakool forests (Table 2.3).

The winter of 2017 was the first time in which a watering action was undertaken to maintain winter base flows during the period when the regulators to some of the smaller streams are usually shutdown in winter (Table 2.1).

It has not been possible to deliver large within channel freshes or overbank flows due to operational constraints in this system (e.g. operational constraint of 600 ML/d at confluence of the Wakool River and Yallakool Creek). However, in 2018-19 a flow trial was undertaken to deliver 800 ML/day at the confluence of the Wakool River and Yallakool Creek.

In addition to watering actions specifically targeted for the Edward/Kolety-Wakool system, water from upstream Commonwealth environmental watering actions and actions that are targeted for downstream watering actions transit through the Edward/Kolety-Wakool system in some years. For example, in 2015-16 environmental water returning from Barmah-Millewa Forest influenced the hydrograph in the Edward/Kolety-Wakool system (Watts et al. 2016).

Table 2.3 Summary of Commonwealth environmental watering actions and unregulated overbank flows in the Edward/Kolety-Wakool system from July 2010 to June 2019. More detailed information about environmental watering in the mid-Murray catchment is available from the CEWO website (Department of the Environment and Energy 2017)

	In-channel environmental watering actions				Environmental watering actions using irrigation infrastructure			Unregulated overbank flows
Water Year	Base flows and small freshes	Contribute to flow recession	Maintain winter base flows	Larger within channel freshes[1]	Flows from canal escapes during hypoxic events	Flows in ephemeral streams[2]	Watering forests	Flooding forests and/or floodplains
2009-10							✓	
2010-11					✓	✓		✓
2011-12	✓					✓		
2012-13	✓				✓	✓		
2013-14	✓	✓				✓		
2014-15	✓	✓				✓		
2015-16	✓	✓				✓		
2016-17	✓	✓			✓	✓		✓
2017-18	✓	✓	✓			✓		
2018-19	✓	✓				✓		

[1] Delivery of larger within channel freshes to the Wakool River and Yallakool Creek is not possible under current operational constraints (e.g. constrained to 600 ML/d at the confluence of the Wakool River and Yallakool Creek).
[2] Some of the watering actions in ephemeral creeks done jointly with NSW Office of Environment and Heritage

2.3 Environmental Watering Priorities for 2019-20

CEWO Portfolio management Plan for the Mid-Murray in 2019–20

The antecedent and catchment conditions and the demand for environmental water in 2019–20 in the Edward/Kolety-Wakool system was described as being a moderate to high demand for environmental water in the Edward/Kolety-Wakool system (CEWO 2019). It was described that flows would seek to support the recovery of large bodied native fish and instream aquatic plants after the 2016 flood and hypoxic blackwater event. Where possible, this includes providing winter base flows and preventing cease-to-flow conditions in the Yallakool-Wakool and Colligen-Niemur systems, and also the maintenance of breeding habitat and unobstructed movement pathways between interconnected streams and channels.

The Commonwealth environmental water portfolio management plan (CEWO 2019) outlines the environmental water objectives for the mid-Murray region for 2019-20 (Table 2.4).

Table 2.4 Summary of objectives being targeted by environmental watering in the Mid-Murray Region. Source CEWO (2019).

BASIN-WIDE MATTERS (Matters in red link to the Basin-wide Environmental Watering Strategy)	OBJECTIVES FOR MID-MURRAY ASSETS						
	IN-CHANNEL ASSETS			OFF-CHANNEL ASSETS			
	River Murray (Hume Dam to Euston)	Edward-Wakool River System	Gunbower Creek	Barmah-Millewa Forest	Gunbower-Koondrook-Perricoota Forest	Edward-Wakool Forests (e.g. Werai, Neimur)	Off-channel wetlands and ephemeral creeks
VEGETATION	Maintain riparian and in-channel vegetation condition. Increase periods of growth for non-woody vegetation communities that closely fringe or occur within river corridors.			Maintain the current extent of floodplain vegetation near river channels and on low-lying areas of the floodplain. Improve condition of black box, river red gum and lignum shrublands. Improve recruitment of black box and river red gum communities. Increased periods of growth for non-woody vegetation communities that closely fringe or occur within the creek channels, and those that form extensive stands within wetlands and low-lying floodplains including moira grasslands in Barmah-Millewa Forest.			Maintain and improve condition of wetland vegetation.
WATERBIRDS	Provide habitat and food sources to support waterbird survival and recruitment, and maintain condition and current species diversity.						
				Support naturally triggered colonial bird breeding events.			
FISH	Provide flows to support habitat (including longitudinal connectivity and bench inundation) and food sources and promote increased movement/dispersal, recruitment and survival/condition of native fish.			Provide flows to support habitat and cues for increased movement, recruitment and survival/condition of native fish (particularly for floodplain specialists).			
INVERTEBRATES	Provide habitat to support increased microinvertebrate and macroinvertebrate survival, diversity, abundance and condition.						
OTHER VERTEBRATES	Provide habitat to support survival, maintain condition and provide recruitment opportunities for frogs and turtles.						
CONNECTIVITY	Maintain lateral connectivity by contributing to an increase in the frequency of freshes, bankfull and lowland floodplain flows.						
	Maintain baseflows and increase overall flows in the River Murray. Maintain longitudinal connectivity along the River Murray to fulfil important environmental functions such as nutrient and sediment transport, organism dispersal and water quality.			Maintain connectivity through creeks and anabranches, thereby enhancing connectivity and functioning through the length of the River Murray.			
PROCESSES	Increase primary productivity, nutrient and carbon cycling, biotic dispersal and movement. Increase transport of organic matter, salt and nutrients downstream.						
WATER QUALITY	Maintain water quality and provide refuge habitat from adverse water quality events. Increase mobilisation and export of salt from the River Murray system.						
RESILIENCE	Provide drought refuge habitat and maintenance/condition of native biota.						

Information sourced from: MDBA (2014); Department of the Environment (2014); Department of the Environment (2011a-d); MDBA (2012a-f); DELWP (2015).

Considerations for water delivery in 2019-20 were described by CEWO (2019) as follows:

- Permanent Waterways: Environmental water will contribute to year-round variable base flows and freshes to support the recovery of in-stream habitat, particularly aquatic vegetation and areas supporting the various life stages of native fish. Watering actions will be scalable depending on catchment conditions and water availability during the year. Environmental water use may also provide a more gradual recession following periods of high flow (e.g. rain rejection flows) and improve water quality to provide refuges for aquatic plants and animals if required and where feasible to do so.
- Ephemeral waterways and wetlands: The purpose of these annual watering events would be to maintain ephemeral instream and wetland habitat, particularly water quality, aquatic vegetation and areas supporting the various life stages of native frogs, birds and aquatic invertebrates. 11
- Edward/Kolety-Wakool forests: The purpose of watering events may include the protection or maintenance of floodplain vegetation health, the provision of localised habitat for aquatic native

plants and animals, contributing to hydrological connectivity and nutrient/carbon cycling processes. Environmental flows, including pumping, could be considered subject to stakeholder support, operational delivery infrastructure, third party impacts and accounting being addressed.

Standard operational considerations in 2019-20 were described by CEWO (2019) as follows:

- WaterNSW is responsible for managing flows in the Edward/Kolety-Wakool River system, which is highly regulated. Depending on the location and purpose of the action, water may also be sourced from either Murray Irrigation and/or private landholder irrigation infrastructure. Commonwealth environmental water may be delivered in combination with natural, consumptive or other held or planned environment water.
- Forest and ephemeral waterway actions will be timed for winter/spring and late autumn to minimise the risk of hypoxic blackwater impacts. Operational considerations for Werai Forest are being reviewed to improve the potential for watering to be undertaken, particularly syncronising flows into the Werai with flows targetting Millewa Forest.
- Contingency flows may be made available, if required, to provide critical refuge habitat for aquatic species such as large bodied native fish during hypoxic events.
- Planning for actions will take into consideration the potential impacts of inundating areas that have acid sulphate soils and/or deep pools that may result in the movement of salt.
- Maintenance work will be undertaken on Stevens Weir and other delivery infrastructure in the Edward/Kolety-Wakool River system during May, June and July 2018. This will prevent the delivery of flows into the Yallakool-Wakool and Colligen-Niemur systems during the winter 2018 period. The recommencement of delivering winter flows will be sought for winter 2019.
- Flows will be delivered within constraints, unless otherwise agreed with potentially impacted landholders and state government agencies. During August 2018, following discussions with the landholder representatives and relevant NSW agencies, an 800 ML/d flow trial in the Yallakool-Wakool is planned, targeting productivity, native fish, and instream aquatic vegetation outcomes.

CEWO planned watering actions for the Edward/Kolety-Wakool in 2019–20

As per Water Use Minute WUM 10083, the proposed watering actions sought to achieve the following expected outcomes:

Primary expected outcomes

- support the recovery of instream aquatic vegetation and large bodied native fish for three years following the 2016 hypoxic blackwater event.
- maintain the diversity and condition of native fish and other native species through maintaining suitable habitat and providing/supporting opportunities to move, breed and recruit
- maintain health of riparian and in-channel aquatic native vegetation communities
- maintain/improve water quality within the system, particularly dissolved oxygen, salinity and pH
- maintain ecosystem and population resilience through supporting ecological recovery and maintaining aquatic habitat.
- support inundation of low-lying wetlands/floodplains habitats within the system

Secondary expected outcomes

- maintain habitat quality in ephemeral watercourses
- support mobilisation, transport and dispersal of biotic and abiotic material (e.g. sediment, nutrients and organic matter) through longitudinal and lateral hydrological connectivity

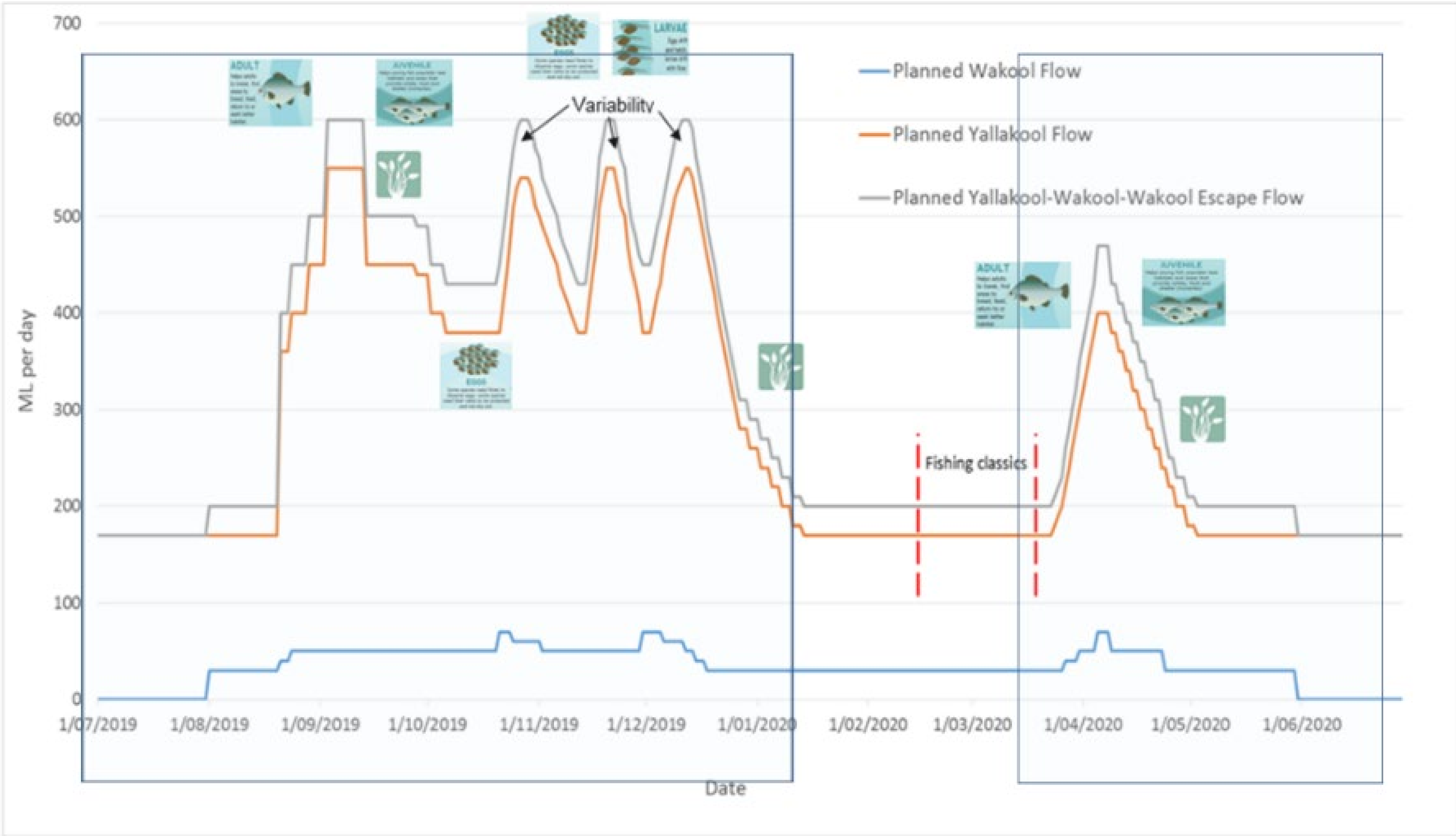


Figure 2.1. Annual hydrograph planned for Yallakool-Wakool at start of 2019-20 action. Shaded area shows period of potential use of CEW. Operational base flows are usually around 170 ML/day except in winter when they are zero unless provided via unregulated flows. (Source: CEWO 2019)

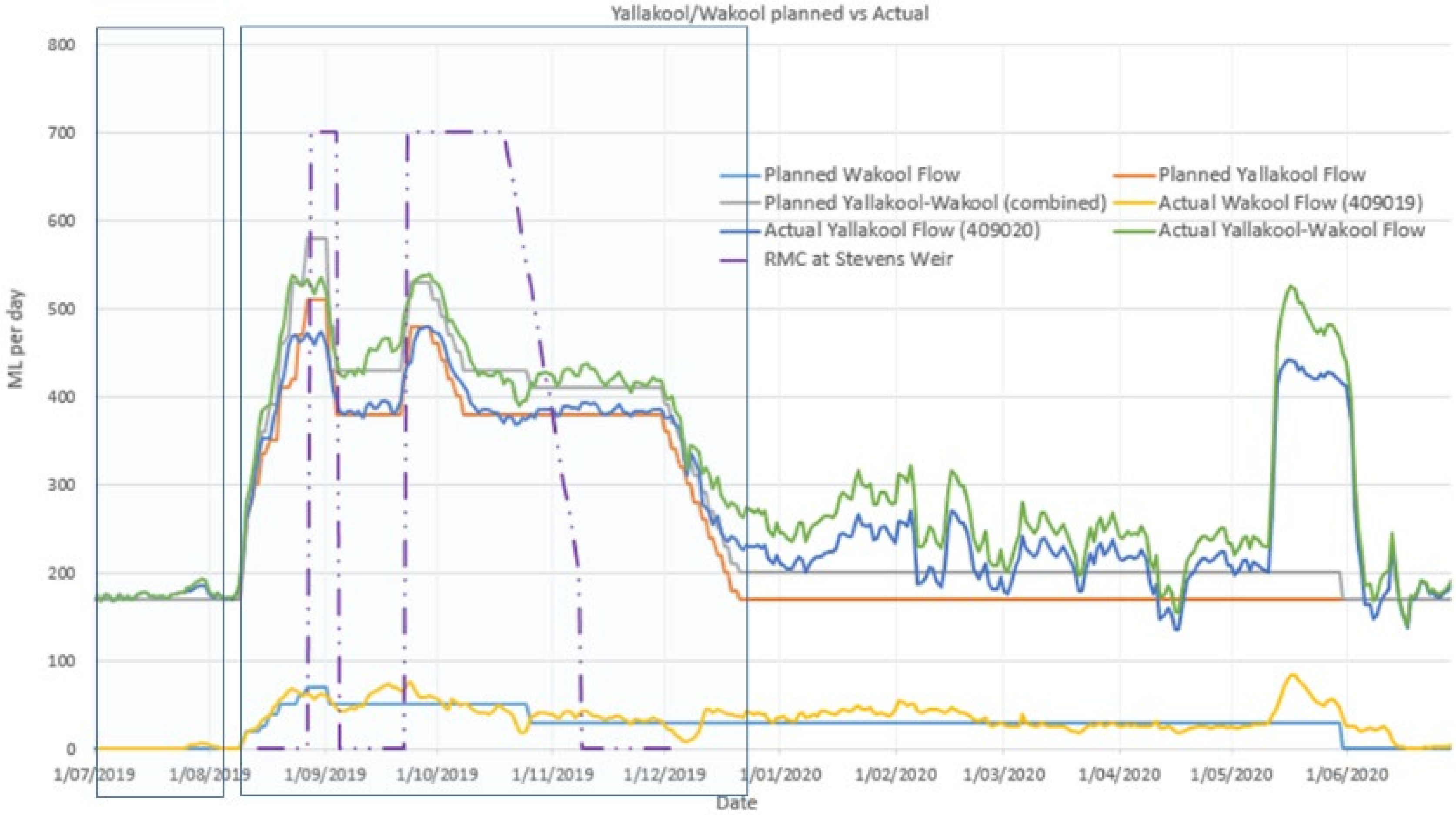


Figure 2.2. Annual hydrograph for Yallakool-Wakool at end of 2019-20 action, showing the change made to the planned flows due to deceasing water availability in the Murray system. Operational flows were provided from January - May. Unregulated flows events provided flows during May-June 2020. Shaded area shows period of CEW use. (Source: CEWO 2019)

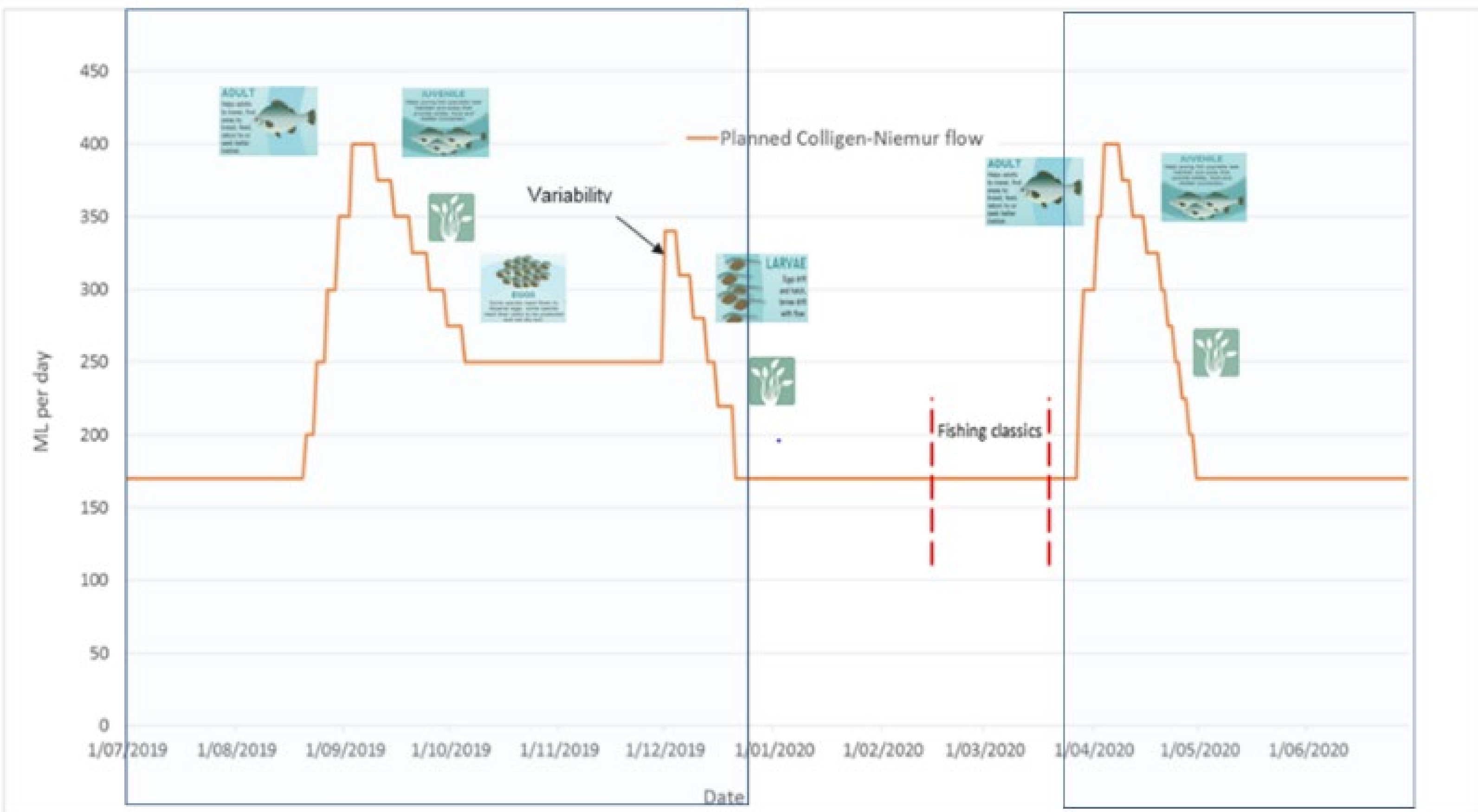


Figure 2.3. Annual hydrograph planned for Colligen-Niemur at start of 2019-20 action. Shaded area shows period of potential use of CEW. Operational base flows are usually around 170 ML/day except in winter when they are zero unless provided via unregulated flows. (Source: CEWO 2019)

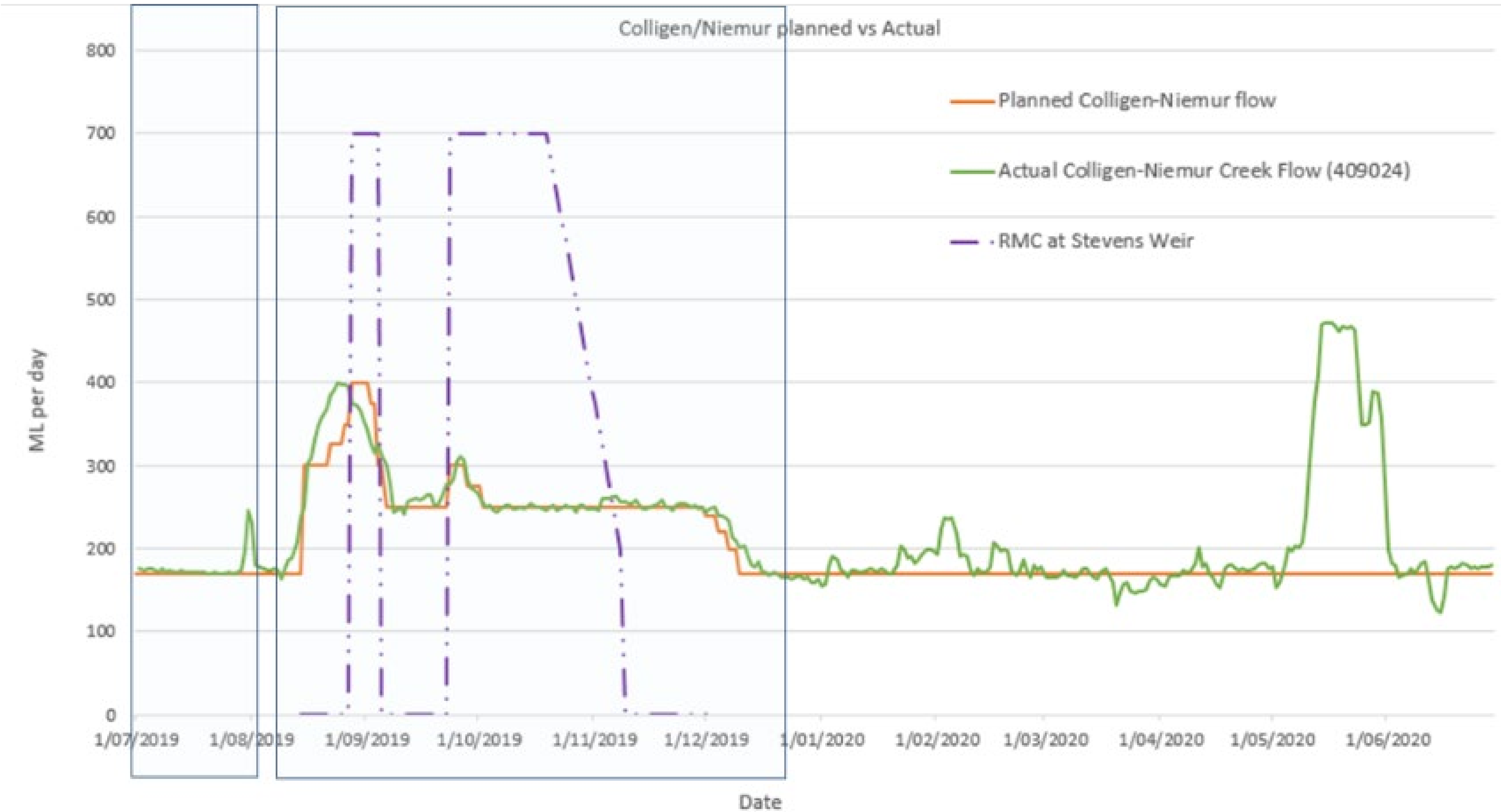


Figure 2.4. Annual hydrograph for Colligen-Niemur at end of 2019-20 action, showing the change made to the planned flows due to deceasing water availability in the Murray system. Operational flows were provided from January - May. Unregulated flows events provided flows during May-June 2020. Shaded area shows period of CEW use. (Source: CEWO 2019)

2.4 Practicalities of environmental watering in the Edward/Kolety-Wakool system

The main source of Commonwealth environmental water for the Edward/Kolety-Wakool system is from the Murray River through the Edward/Kolety River and Gulpa Creek. The main flow regulating structures within the Edward/Kolety-Wakool system are the Gulpa Creek Offtake, Edward/Kolety River Offtake (both located on the Murray River), and Stevens Weir, located on the Edward/Kolety River downstream of Colligen Creek (Figure 1.1). This structure creates a weir pool that allows Commonwealth environmental water to be delivered to Colligen Creek-Niemur River system, Yallakool Creek, the Wakool River, the Edward/Kolety River and Werai Forest.

Water diverted into the Mulwala Canal from Lake Mulwala can also be delivered into the Edward/Kolety-Wakool system through 'escapes' or outfalls managed by the irrigator-owned company Murray Irrigation Limited (MIL). During a hypoxic blackwater event in 2010, environmental water was released from the Mulwala Canal escapes to lessen the impact of hypoxia and create localised refugia with higher DO and lower DOC (Watts et al. 2017a). There are numerous smaller escapes throughout the MIL network that can also be used to deliver small flows to the river system. Escapes were also used to deliver environmental water as refuge flows in response to the 2016 hypoxic blackwater event (Watts et al. 2017b).

The ability to deliver environmental water to the Edward/Kolety-Wakool system depends on water availability and circumstances in the river at any given time. Environmental water delivery in this system involves various considerations as outlined by Gawne et al. (2013), including:

- the capacity of the off takes / regulators and irrigation escapes
- channel constraints (e.g. to avoid third party impacts)
- the availability of third party infrastructure to assist in delivering water into the system
- existing flows and other demands on the system.

Delivery of instream flows to the Edward/Kolety River, Wakool River, Yallakool Creek, Colligen-Niemur system and Merran River system are managed within regular operating ranges as advised by river operators to avoid third party impacts. For example, in the Wakool-Yallakool system the operational constraint is 600 ML/d at the confluence of the Wakool River and Yallakool Creek. Thus, the types of flow components that can be achieved under current operating ranges are in-channel baseflows and freshes. Environmental watering may also be constrained due to limitations on how much water can be delivered under regulated conditions. At times of high irrigation demand channel capacity will be shared among water users. If the system is receiving higher unregulated flows, there may not be enough capacity to deliver environmental water (Gawne et al. 2013). Environmental water may be delivered to contribute to the slower recession of freshes, delivered during low flow periods to provide refuge habitat, or delivered to manage water quality issues, such as hypoxic events (Gawne et al. 2013; Watts et al. 2017a).

2.5 Commonwealth watering actions in Edward/Kolety-Wakool system 2019-20

Three watering actions were planned by the Commonwealth Environmental Water Office for the 2019-20 water year in the Wakool-Yallakool system (Table 2.5) and the Colligen-Niemur system (Figure 2.1 and 2.4). Some of the water during these actions was sourced as return flows from the Southern Connected Flow in the Murray River. This influenced flows in the Edward/Kolety-Wakool system from 28 August to 9 September 2019, and 23 September to 1 October 2019. The return flows from Millewa Forest may have affected the water quality in the Edward/Kolety-Wakool system on these dates, and on later dates at sites further downstream.

Table 2.5 Planned Commonwealth environmental watering actions in 2019-20 in the Edward/Kolety-Wakool River system.

Watering Action No	Name	Objectives (from CEWO)	Dates
Action 1	Winter base flow	For native fish condition and movement, vegetation in-channel, longitudinal connectivity; refuge habitat during irrigation shut-down period	15/05/19 - 9/08/2019
Action 2	Winter to spring transition flow	At this time, there was no operational demand so CEW was used to prevent water levels reducing to low levels for a short period between action 1 and action 3.	10/08/19 - 27/08/19
Action 3a	Winter/spring early fresh	To provide early season rise in river level to contribute to connectivity, water quality, stimulating early growth of in-stream aquatic vegetation, pre-spawning condition of native fish and/or spawning in early spawning native fish.	28/08/19 - 4/09/19
Action 3b	Early spring elevated base flow	To maintain nesting habitat for Murray Cod, and inundation for aquatic vegetation growth.	5/09/19 - 22/09/19
Action 3c	Late spring fresh	To promote silver perch spawning, influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit. May also assist with dispersal of larvae and juveniles of a number of fish species.	23/09/19 - 11/10/19
Action 3d	Late spring elevated base flow	To influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit. May also assist with dispersal of larvae and juveniles of a number of fish species.	12/10/19 - 30/11/19
Action 3e	Recession	Slow recessions for instream water plants	1/12/19 - 22/12/19

3 Monitoring, evaluation and research

3.1 Approach to monitoring, evaluation and research

The overarching principle that underpins this monitoring, evaluation and research in the Edward/Kolety-Wakool Selected Area is that we are taking an ecosystem approach to evaluate the responses to Commonwealth environmental watering. Monitoring indicators have been selected that each have clear linkages to other components of the MER project (Figure 3.1). The monitoring and research has a strong focus on fish (including reproduction, recruitment and adult populations) and water quality. The Edward/Kolety-Wakool system is recognised as a priority area for fish diversity in the Murray-Darling Basin, and outcomes for fish and water quality have been the main focus of environmental watering actions in the Edward/Kolety-Wakool system since 2010. Some of the other indicators (e.g. stream metabolism and aquatic vegetation) strongly influence the health of the ecosystem, and thus a key goal of this MER Plan is to improve our understanding and interpretation of these interdependencies. Research projects will complement the monitoring and evaluation and where possible be undertaken collaboratively with the local community to address physical, ecological, and social questions that are key for supporting future environmental watering actions in the Edward/Kolety-Wakool system.

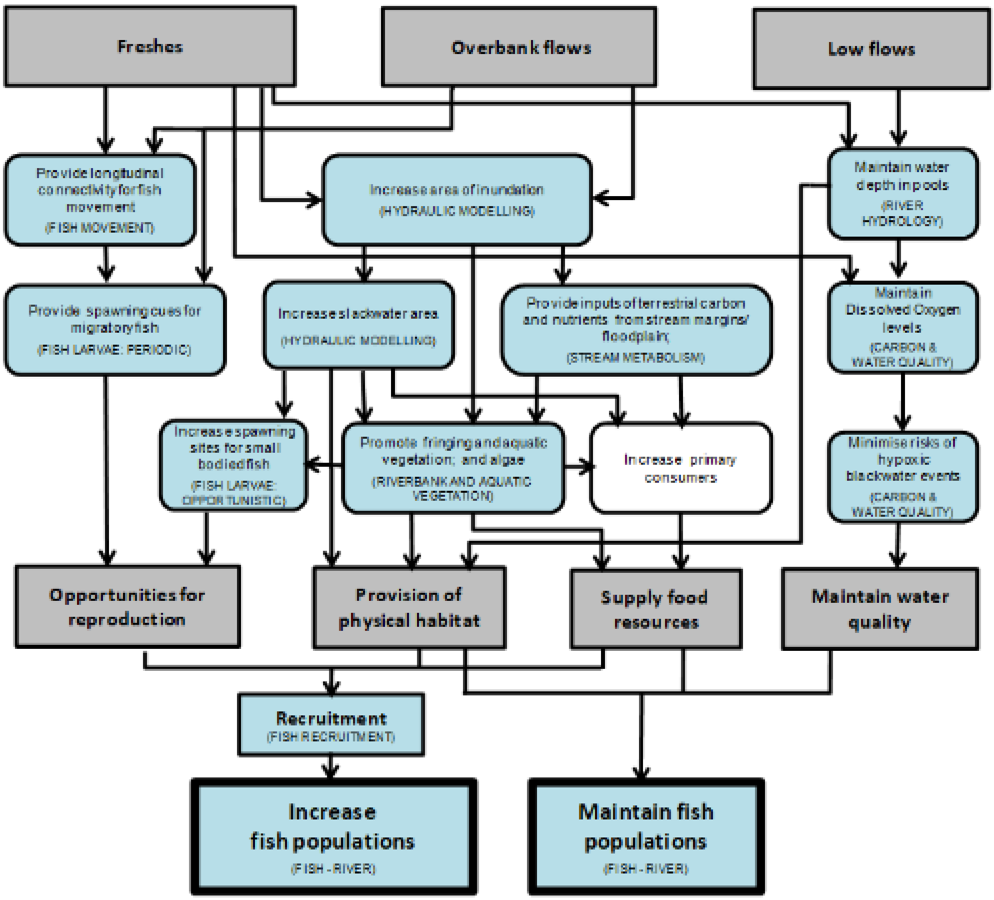
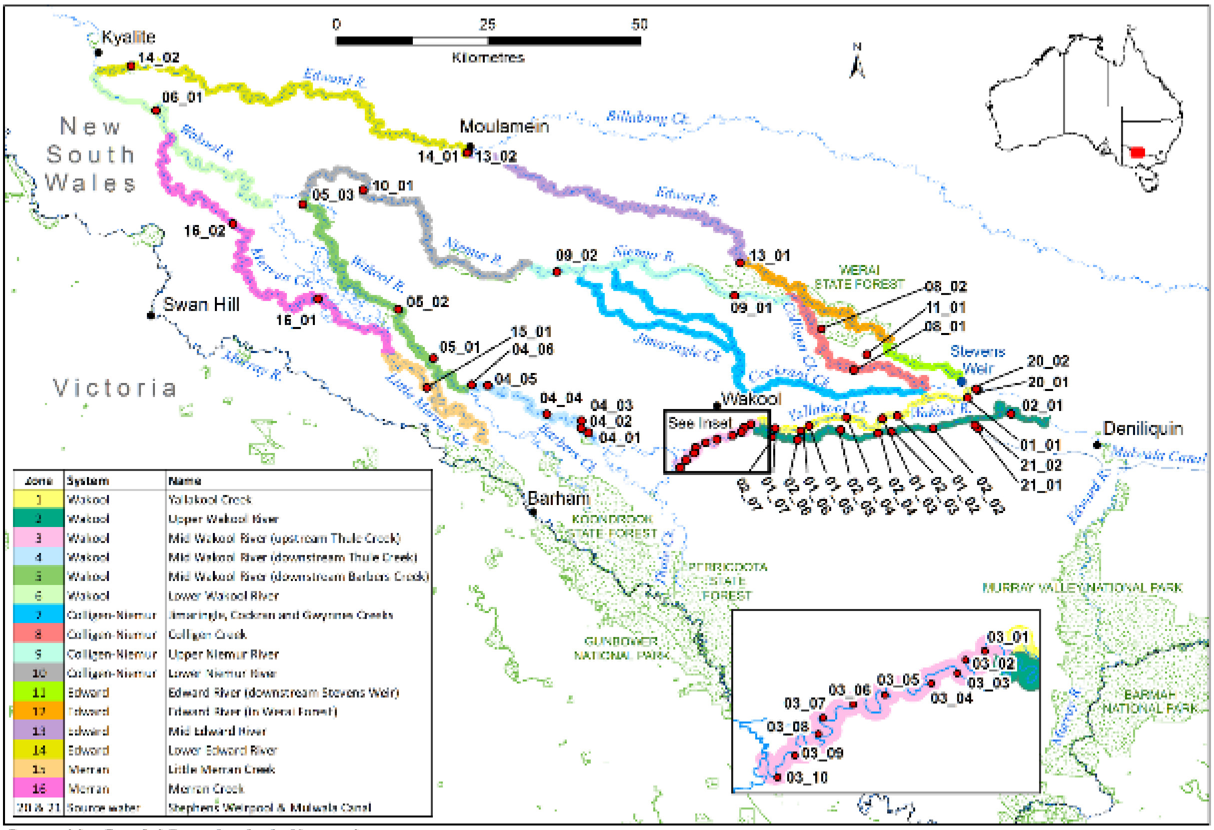


Figure 3.1 Conceptual diagram illustrating three main flow types (low flows, freshes, overbank flows) and their influence on ecosystem components and processes that, in turn, influence fish population dynamics. Indicators included in the Edward/Kolety-Wakool MER Plan are shown in brackets in boxes shaded blue.

3.2 Monitoring zones and sites

The monitoring of ecosystem responses to Commonwealth environmental watering in the Edward/Kolety-Wakool system in 2019-20 was undertaken following the methods outlined in the Edward/Kolety-Wakool MER Plan (Watts et al. 2019a).

At the commencement of the LTIM program daily discharge data from 14 hydrological stations in the Edward/Kolety-Wakool system were analysed along with information on geomorphology and location of major distributaries to classify the system into distinct hydrological zones (Watts et al. 2014). Fifteen distinct zones were identified (Figure 3.2, Table 3.1). Transitions between these zones occur where there are major inflows or outflows to a river or at locations where there are significant changes in geomorphology. The zones range from ephemeral watercourses (e.g. Jimaringle, Cockran and Gwynne's Creeks), to smaller creeks and rivers (Wakool River, Yallakool Creek, Colligen-Niemur system, and Merran Creek) to the larger Edward/Kolety River system.



Created by Spatial Data Analysis Network,
Charles Sturt University, May, 2015

Data Source: NSW 'Place Point' & 'Hydro line' spatial data: Digital Cadastral Database (CD-ROM), LPMA, 2008, New South Wales; Australian Reserves GEODATA TOPO 250K Series 3, 2006, OEH NSW National Parks 2012

Figure 3.2 Map showing 16 hydrological zones within the Edward/Kolety-Wakool system. Site names are listed in Table 3.1.

Table 3.1 List of site codes and site names for the CEWO MER Project in the Edward/Kolety-Wakool Selected Area.

Zone Name	Zone	Site Code	Site Name
Yallakool Creek	01	EDWK01_01	Yallakool/Back Creek Junction
Yallakool Creek	01	EDWK01_02	Hopwood
Yallakool Creek	01	EDWK01_03	Cumnock
Yallakool Creek	01	EDWK01_04	Cumnock Park
Yallakool Creek	01	EDWK01_05	Mascott
Yallakool Creek	01	EDWK01_06	Widgee, Yallakool Creek
Yallakool Creek	01	EDWK01_07	Windra Vale
Upper Wakool River	02	EDWK02_01	Fallonville
Upper Wakool River	02	EDWK02_02	Yaloke
Upper Wakool River	02	EDWK02_03	Carmathon Reserve
Upper Wakool River	02	EDWK02_04	Emu Park
Upper Wakool River	02	EDWK02_05	Homeleigh
Upper Wakool River	02	EDWK02_06	Widgee, Wakool River1
Upper Wakool River	02	EDWK02_07	Widgee, Wakool River2
Mid Wakool River (upstream Thule Creek)	03	EDWK03_01	Talkook
Mid Wakool River (upstream Thule Creek)	03	EDWK03_02	Tralee1
Mid Wakool River (upstream Thule Creek)	03	EDWK03_03	Tralee2
Mid Wakool River (upstream Thule Creek)	03	EDWK03_04	Rail Bridge DS
Mid Wakool River (upstream Thule Creek)	03	EDWK03_05	Cummins
Mid Wakool River (upstream Thule Creek)	03	EDWK03_06	Ramley1
Mid Wakool River (upstream Thule Creek)	03	EDWK03_07	Ramley2
Mid Wakool River (upstream Thule Creek)	03	EDWK03_08	Yancoola
Mid Wakool River (upstream Thule Creek)	03	EDWK03_09	Llanos Park1
Mid Wakool River (upstream Thule Creek)	03	EDWK03_10	Llanos Park2
Mid Wakool River (downstream Thule Creek)	04	EDWK04_01	Barham Bridge
Mid Wakool River (downstream Thule Creek)	04	EDWK04_02	Possum Reserve
Mid Wakool River (downstream Thule Creek)	04	EDWK04_03	Whymoul National Park
Mid Wakool River (downstream Thule Creek)	04	EDWK04_04	Yarranvale
Mid Wakool River (downstream Thule Creek)	04	EDWK04_05	Noorong1
Mid Wakool River (downstream Thule Creek)	04	EDWK04_06	Noorong2
Mid Wakool River (downstream Barbers Creek)	05	EDWK05_01	La Rosa
Mid Wakool River (downstream Barbers Creek)	05	EDWK05_02	Gee Gee Bridge
Mid Wakool River (downstream Barbers Creek)	05	EDWK05_03	Glenbar
Lower Wakool River	06	EDWK06_01	Stoney Creek Crossing
Colligen Creek	08	EDWK08_01	Calimo
Colligen Creek	08	EDWK08_02	Werrai Station
Upper Neimur River	09	EDWK09_01	Burswood Park
Upper Neimur River	09	EDWK09_02	Ventura
Lower Niemur River	10	EDWK10_01	Niemur Valley
Edward/Kolety River (downstream Stephens Weir)	11	EDWK11_01	Elimdale
Mid Edward/Kolety River	13	EDWK13_01	Balpool
Mid Edward/Kolety River	13	EDWK13_02	Moulamien US Billabong Creek
Lower Edward/Kolety River	14	EDWK14_01	Moulamien DS Billabong Creek
Lower Edward/Kolety River	14	EDWK14_02	Kyalite State Forest
Little Merran Creek	15	EDWK15_01	Merran Downs
Merran Creek	16	EDWK16_01	Erinundra
Merran Creek	16	EDWK16_02	Merran Creek Bridge
Edward/Kolety River, Stevens weir	20	EDWK20_01	Weir1
Edward/Kolety River, Stevens weir	20	EDWK20_02	Weir2
Mulwala canal	21	EDWK21_01	Canal1
Mulwala canal	21	EDWK21_02	Canal2

Due to funding constraints it is not possible to undertake monitoring and evaluation in all sixteen of the hydrological zones identified in the Edward/Kolety-Wakool system (Figure 3.2). The following factors were considered when prioritising the zones to include in the MER Plan:

- Likelihood of hydrological zones receiving Commonwealth environmental water or serving as a comparison zone (i.e. not receive Commonwealth environmental water)
- Location of hydrological gauging stations
- Availability of historical monitoring data in each zone and existing arrangements for access, including maintaining continuity of monitoring established during the LTIM project
- Ease of access for undertaking fieldwork under a range of weather conditions
- Need for a number of zones that experience a range of flows to facilitate predictive ecosystem response modelling and Selected Area gradient analysis
- Capacity to inform on specific objectives aligned with values and needs of local community, including Aboriginal people.

Taking all of these factors into account, the MER project includes monitoring and evaluation of ecosystem responses to Commonwealth environmental watering in the Edward/Kolety-Wakool system in the following hydrological zones:

- Monitoring sites established during the LTIM project that focus on the upper and mid reaches of the Wakool-Yallakool system (zones 1, 2, 3 and 4) were maintained for the MER project.
- Twenty sites that were established for fish community surveys in 2010 and were monitored in year one (2015) and year five (2019) of the LTIM project were maintained for the MER project and will be surveyed for fish community indices in year three of MER (2022).
- Additional sites were added to the existing network of water quality monitoring sites established during LTIM project. For the MER project there are 17 water quality monitoring sites throughout the whole system, including ongoing sites in Yallakool Creek, Wakool River zones 2 to 4, and source water sites in the Mulwala Canal and the Edward/Kolety River at Stephens Weir. New sites for MER expanded the water quality monitoring to further downstream in the Wakool River as well as in Tuppal Creek, the Edward/Kolety River and the Colligen-Niemur system to enable an evaluation of environmental water across the broader system.

The focus of the integrated research project is the Edward/Kolety River downstream of Stevens Weir to inform the adaptive management of environmental water in this River. The Edward/Kolety River was not monitored as part of LTIM program. The research questions that will be addressed will inform future monitoring and delivery of environmental water in the Edward/Kolety-Wakool system.

The Milewa Forest and Koondrook-Perricoota forest are not included in the MER project because they are currently monitored by other programs such as the MDBA Living Murray Program. The ephemeral creeks in zone 15, Jimaringle, Cockran and Gwynnes Creek, have not been included in the MER project to avoid duplication of monitoring, as environmental watering actions in these ephemeral creeks have previously been monitored by the NSW DPIE. We will seek to integrate outcomes of environmental watering in these systems in a qualitative evaluation of the outcomes of Commonwealth environmental water in the Edward/Kolety-Wakool system.


Upper Wakool R (zone 2)


Wakool River near Wakool Reserve (zone 3)


Wakool R near Moulamein Road bridge (zone 4)


Wakool River at Stoney Crossing (zone 6)


Colligen Creek, near Calimo (zone 8)


Edward/Kolety River (zone 13)


Mulwala Canal


Tuppal Creek

Figure 3.3 Photos of river sin the Edward/Kolety-Wakool system

3.2 Indicators for monitoring and evaluation

Table 3.2 provides a summary of the monitoring and evaluation activities for this MER Plan and provides a summary of the changes or additions relative to the Edward/Kolety-Wakool LTIM project (2014-2019). One of the main changes is that carbon and water quality monitoring has been extended so that evaluation can be undertaken across the entire Edward/Kolety-Wakool system (Table 3.2).

There are three categories of indicators for LTIM/MER monitoring:

- **Category I** –Mandatory indicators and standard operating protocols that are required to inform Basin-scale evaluation and may be used to answer Selected Area questions. Category 1 indicators monitored in the Edward/Kolety-Wakool system (Table 3.2) are: river hydrology, stream metabolism, nutrients and carbon, fish reproduction (larvae) and fish (river).
- **Category 2** –Optional indicators with mandatory standard protocols that may be used to inform Basin-scale evaluation and may be used to answer Selected Area questions. Fish movement is the only category 2 indicator monitored in the Edward/Kolety-Wakool system.
- **Category 3** – Selected Area specific monitoring protocols to answer Selected Area questions. Category 3 indicators monitored in the Edward/Kolety-Wakool system (Table 3.2) are: hydraulic modelling, additional water quality and carbon characterisation, riverbank and aquatic vegetation, fish reproduction (larvae), fish recruitment, and fish community survey (year 3 of MER).

The rationale regarding the selection of indicators is outlined in the Edward/Kolety-Wakool MER Plan (Watts et al. 2019a). Indicators are monitored to contribute to the Edward/Kolety-Wakool Selected Area Evaluation and/or the Whole of Basin-scale evaluation MER project that is led by CSIRO. Some indicators are expected to respond to environmental watering in short time frames (< 1 year), but others (e.g. fish community assemblage) are expected to respond over longer time frames (e.g. 2 to 5 years).

A summary of the long-term and short-term evaluation questions is provided in Table 3.3. Category 1 monitoring and evaluation questions follow those outlined in the CEWO LTIM Standard methods (Hale et al. 2014).

Table 3.2 Summary of monitoring and evaluation to be undertaken in the Edward/Kolety-Wakool system for the CEWO Monitoring, Evaluation and Research (MER) Project from 2019 to 2022. Changes and additions relative to the Edward/Kolety-Wakool LTIM project (2014-2019) are described. Zones and sites are described in Figure 3.2 and Table 3.1. Category 1 and 2 indicators are monitored using standard operating protocols to inform Basin-scale evaluation and may be used to answer Selected Area questions. Category 3 indicators are those monitored to answer Selected Area questions.

Theme	Cat	Zones	Changes or additions to the MER program compared to the LTIM project (2014-19)
Monitoring and Evaluation			
River hydrology	1	system	No changes to monitoring or evaluation from LTIM project. Discharge data will be obtained from WaterNSW website.
Hydraulic modelling			Hydraulic modelling was undertaken in zones 1,2,3,4 and 8 as part of the LTIM project. These models will continue to be used as part of MER evaluations but no new hydraulic modelling will be undertaken in these zones. Modelling of reaches in zones 11 and 12 will modelled as part of the integrated Edward/Kolety River research project.
Carbon and water quality	3	system	No changes in methods from LTIM. New sites have been added for the MER project so that the evaluation of this indicator will be undertaken across the whole Edward/Kolety-Wakool system.
Stream metabolism	1	1,2,3,4,8	For LTIM DO and light were logged continuously in 4 zones between August and April each year. MER logging will be continuous across the whole year and additional dissolved oxygen logger site was established in Colligen Creek.
Riverbank and aquatic vegetation	3	1,2,3,4,8	No changes in methods from LTIM. The composition and percent cover of riverbank and aquatic vegetation will be monitored monthly. Four reaches in Colligen Creek will be added to the MER project. These sites in Colligen Creek were previously monitored 2015-2019 through a project funded by Murray Local Land Services
Fish movement	2	system	Golden perch movement will be monitored from June-Sept 2019 to evaluate the 2019 winter environmental watering action. No fish movement will be monitored as part of the MER project after September 2019.
Fish reproduction	1	3	No changes to monitoring or evaluation from LTIM project. The abundance and diversity of larval fish will be monitored fortnightly between September and March using light traps and drift nets.
Fish reproduction	3	1,2,3,4,	No changes in methods from LTIM. Research on fish spawning will be undertaken in the Edward/Kolety River as part of the integrated research project
Fish recruitment	3	1,2,3,4	Minor changes to monitoring methods from LTIM project. No changes to monitoring sites.
Fish river (Cat 1)	1	3	No changes to monitoring or evaluation from LTIM project. Cat 1 fish community surveys will be undertaken once annually in zone 3 between March and May.
Fish community survey	3	system	No changes from LTIM project. Fifteen sites (in addition to the Cat1 fish sites) from throughout the system will be surveyed in 2022 only (year 3 of the MER project)

Table 3.3 Summary of the long-term and short-term evaluation questions for the Edward/Kolety-Wakool MER project.

Indicator	Evaluation questions
Hydrology	*Short and long-term questions* • What was the effect of CEW (Commonwealth environmental water) on the hydrology of the rivers in the Edward/Kolety-Wakool system? • What did CEW contribute to longitudinal connectivity?
Carbon and water quality	*Short and long-term questions* • What did CEW contribute to modification of the type and amount of dissolved organic matter through reconnection with previously dry or disconnected in-channel habitat? • What did CEW contribute to dissolved oxygen concentrations? • What did CEW contribute to nutrient concentrations? *Question for contingency monitoring* • What did CEW contribute to reducing the impact of hypoxic blackwater or other adverse water quality events in the system?
Stream metabolism (Cat 1)	*Short and long-term questions* • What was the effect of CEW on rates of GPP, ER and NPP • What did CEW contribute to total GPP, ER and NPP? • Which aspect of CEW delivery contributed most to productivity outcomes?
Riverbank and aquatic vegetation	*Long-term questions* • What has CEW contributed to the recovery (measured through species richness, plant cover and recruitment) of riverbank and aquatic vegetation that have been impacted by operational flows and drought and how do those responses vary over time? • How do vegetation responses to CEW delivery vary among hydrological zones? *Short-term questions* • What did CEW contribute to the percent cover of riverbank and aquatic vegetation? • What did CEW contribute to the diversity of riverbank and aquatic vegetation taxa?
Fish movement	*Short term questions* • Does CEW facilitate longitudinal connectivity for periodic species during winter?
Fish reproduction (Cat 1)	*Long term questions* • What did CEW contribute to native fish populations? • What did CEW contribute to native fish species diversity? *Short term questions* • What did CEW contribute to native fish reproduction? • What did CEW contribute to native fish survival
Fish reproduction	*Short and Long-term questions* • What did CEW contribute to the spawning of 'Opportunistic' (e.g. small bodied fish) species? • What did CEW contribute to spawning in 'flow-dependent' spawning species (e.g. golden and silver perch)?
Fish recruitment	*Short and Long-term questions* • What did CEW contribute to native fish recruitment to the first year of life? • What did CEW contribute to native fish growth rate during the first year of life?
Fish river (Cat 1)	*Long term questions* • What did CEW contribute to native fish populations? *Short term questions* • What did CEW contribute to native fish reproduction? • What did CEW contribute to native fish survival?
Fish community	*Long-term question* • How does the fish community in the Edward/Kolety-Wakool system vary over 3-5 years, and does this link with sequential flow characteristics?

3.3 Evaluation of monitoring outcomes

The outcome of Commonwealth environmental watering undertaken in 2019-20 was undertaken for the following indicators:

- Hydrology (Section 4)
- Water quality and carbon (Section 5)
- Stream metabolism (Section 6)
- Aquatic and riverbank vegetation (Section 7)
- Fish movement, reproduction, recruitment, and community (Section 8)

Responses to Commonwealth environmental water were evaluated in two ways:

iii) Indicators that respond quickly to flow (e.g. hydrology, water quality and carbon, stream metabolism, fish movement, fish spawning) were evaluated for their response to specific watering actions. Hydrological indicators were calculated on the discharge data with and without the environmental water.

iv) Indicators that respond over longer time frames (e.g. riverbank and aquatic vegetation, fish recruitment, fish community) were evaluated for their response to the longer-term environmental watering regimes. This was undertaken by comparing responses over multiple years, and/or comparing responses in reaches that have received environmental water to zones (e.g. upper Wakool River zone 2) that has received none or minimal environmental water.

3.4 Research

An integrated research project will address knowledge gaps that are necessary to improve the delivery, monitoring and evaluation of environmental water in the Edward/Kolety-Wakool system. The integrated research project focusses on the Edward/Kolety River; this part of the system is not monitored as part of LTIM/MER project, so there are considerable knowledge gaps that need to be addressed to inform the future delivery of environmental water to the Edward/Kolety River and the management of environmental water in relation to the Werai Forest, which is part of the NSW Central Murray Forests Ramsar site (NSW Office of Environment and Heritage 2018).

This integrated research project includes physical, ecological, and social research that will address questions (Table 3.4) relating to how managed flows in the Edward/Kolety River, and the operation of Stevens Weir, influence physical aspects (e.g. lateral connectivity and physical form) and ecological processes, such as river productivity, wetland plant emergence and survival, turtle movement and condition, and fish spawning. In addition, an e-DNA approach will be used to determine the presence and spatial distribution of threatened, uncommon and iconic or rare taxa that have not been the target of the LTIM/MER monitoring and evaluation. Integrated with these biophysical research themes, social research will be undertaken to examine stakeholder attitudes to, and acceptance of, the concept and use of Commonwealth environmental water. Some of the research components have different reporting timelines (Table 3.4). The research implementation will be undertaken throughout the MER project (2019-2022), and the integrated research outcomes will be in the final MER report in 2022.

An innovative aspect of this research project is that local stakeholder groups will participate in the integrated research. The Edward-Wakool Angler Association is a collaborative partner on the fish spawning research in the Edward/Kolety River. Yarkuwa Indigenous Knowledge Centre is a collaborative partner on the research on turtles and understory and groundcover vegetation in Werai Forest.

Table 3.4 Summary of research questions for the Edward/Kolety integrated research project, and timeline for reporting for each theme

Research Area	Research Question	Research timeframe	Final report
Hydraulic modelling	Inundation models will be developed to link with the research questions relating to the Edward/Kolety River and Werai Forest	2019-2022	2022
Physical condition of riverbanks	What are the features of the flow regime in the Edward/Kolety River that drive erosion and deposition?	2019-20	2020
Stream metabolism	Does connectivity of flows into Werai Forest contribute to primary productivity outcomes in the Edward/Kolety River?	2019-2022	2022
Understorey and groundcover vegetation in Werai Forest	How do understorey and groundcover vegetation species in low lying parts of Werai Forest respond to small inundation events via Tumudgery Creek?	2019-2022	2022
Turtles	How does connectivity of wetlands along the Edward/Kolety River affect turtle distribution, movement and body condition?	2019-2021	2021
Fish spawning	Do golden perch and silver perch spawn in the Edward/Kolety River?	2019-2022	2022
Biodiversity (e-DNA)	Can a targeted single-species e-DNA approach be used to identify the presence and spatial distribution of threatened, uncommon and iconic species of crustacean, turtles, fish and aquatic mamals in the Edward/Kolety system	2019-2020	2020
Social research	This will be a co-designed research project, with questions to be developed during the first phase in collaboration with community and managers. Focus may include: knowledge, information and learning; stakeholder attitudes to and acceptance of the concept and use of environmental water	2020-2021	2021

4 Hydrology

Author: Robyn Watts

Key findings	
Maximum and minimum discharge	The winter watering action (action 1) maintained base flows in Yallakool Creek, the mid and lower Wakool River, and the Colligen-Niemur system. In the absence of environmental water there would have been an extended period of cease to flow in these rivers. Watering action 3 increased the maximum discharge in all zones compared to operational flows.
Flow variability	Watering action 3 increased the coefficient of variation of discharge compared to operational flows. In the absence of this watering action there would have been extended period of low variability of flows.
Longitudinal connectivity	Watering action 1 (winter watering action) maintained longitudinal connectivity in Yallakool Creek, the mid and lower Wakool River, and the Colligen-Niemur system.
Lateral connectivity	Watering action 3 increased lateral connectivity compared to the modelled connectivity under operational flows.

4.1 Background

Like many rivers of the MDB, the flow regimes of rivers in the Edward/Kolety-Wakool system have been significantly altered by river regulation (Green 2001; Hale and SKM 2011). Natural flows in this system are strongly seasonal, with high flows typically occurring from July to November. Analysis of long-term modelled flow data show that flow regulation has resulted in a marked reduction in winter high flows, including extreme high flow events and average daily flows during the winter period (Watts et al. 2015). There is also an elevated frequency of low to median flows and reduced frequency of moderate high flows. These flow changes reflect the typical effects of flow-regime reversal observed in systems used to deliver dry-season irrigation flows (Maheshwari et al. 1995).

The Edward/Kolety-Wakool system has experienced a wide range of flow conditions over the past 15 years, and these antecedent conditions will influence the way in which the ecosystem responds to Commonwealth environmental watering. From 1998 to 2010 south-eastern Australia experienced a prolonged drought (referred to as the Millennium drought) and flows in the MDB were at record low levels (van Dijk 2013; Chiew et al. 2014). During this period the regulators controlling flows from the Edward/Kolety River into tributary rivers such as Yallakool Creek and the Wakool River were closed for periods of time. Consequently, between February 2006 and September 2010 there were periods of minimal or no flow in the Wakool River. During this period localised fish deaths were recorded on a number of occasions including in 2006 and 2009. At the break of the drought after many years without overbank flows, a sequence of unregulated flow events between September 2010 and April 2011 triggered a widespread hypoxic (low oxygen) blackwater event in the mid-Murray (MDBA 2011; Whitworth et al. 2012; Watts et al. 2017a).

In late 2016 there was a widespread flood in the southern-MDB associated with record-breaking rainfall in the catchment. Some areas of the floodplain were inundated that had not been flooded for more than 20 years. In the Murray catchment, Murray River flows at Yarrawonga in October were the highest since 1993 (MDBA River Murray Weekly Report, 7th Dec 2017). The unregulated flows from the Murray River inundated the floodplain including Barmah Forest and Koondrook–Perricoota Forests and agricultural land, and resulted in a very large flood event in the Edward/Kolety-Wakool system (BOM 2017). In association with the floods there was a hypoxic blackwater event that extended throughout the Murray River system, including the Edward/Kolety-Wakool system.

This chapter reports on the hydrology of the Edward/Kolety-Wakool system from 1 May 2019 to 30 June 2020. This reporting period commences in May 2019 to enable an evaluation of the winter watering action that commenced in May 2019.

4.2 Environmental watering actions targeting hydrology outcomes

Three watering actions were planned by the Commonwealth Environmental Water Office for the 2019-20 water year in the Wakool-Yallakool system and the Colligen-Niemur system (Table 4.1). Some of the water during these actions was sourced as return flows from the Southern Connected Flow in the Murray River. This influenced flows in the Edward/Kolety-Wakool system from 28 August to 9 September 2019, and 23 September to 1 October 2019. The return flows from Millewa Forest may have affected the water quality in the Edward/Kolety-Wakool system on these dates, and on later dates at sites further downstream. Actions 1 and 3a specifically targeted hydrology outcomes of connectivity.

Table 4.1 Planned Commonwealth environmental watering actions in 2019-20 in the Edward/Kolety-Wakool River system.

Watering Action No	Name	Objectives (from CEWO)	Dates
Action 1	Winter base flow	For native fish condition and movement, vegetation in-channel, **longitudinal connectivity;** refuge habitat during irrigation shut-down period	15/05/19 - 9/08/2019
Action 2	Winter to spring transition flow	At this time, there was no operational demand so CEW was used to prevent water levels reducing to low levels for a short period between action 1 and action 3.	10/08/19 - 27/08/19
Action 3a	Winter/spring early fresh	To provide early season **rise in river level to contribute to connectivity**, water quality, stimulating early growth of in-stream aquatic vegetation, pre-spawning condition of native fish and/or spawning in early spawning native fish.	28/08/19 - 4/09/19
Action 3b	Early spring elevated base flow	To maintain nesting habitat for Murray Cod, and inundation for aquatic vegetation growth.	5/09/19 - 22/09/19
Action 3c	Late spring fresh	To promote silver perch spawning, influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit. May also assist with dispersal of larvae and juveniles of a number of fish species.	23/09/19 - 11/10/19
Action 3d	Late spring elevated base flow	To influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit. May also assist with dispersal of larvae and juveniles of a number of fish species.	12/10/19 - 30/11/19
Action 3e	Recession	Slow recessions for instream water plants	1/12/19 - 22/12/19

4.3 Selected Area evaluation questions

- *What was the effect of Commonwealth environmental water on the hydrology of the Edward/Kolety-Wakool system?*
- *What did Commonwealth environmental water contribute to longitudinal hydrological connectivity?*
- *What did Commonwealth environmental water contribute to lateral connectivity?*

4.4 Methods

Daily discharge data for automated hydrometric gauges (Table 4.2) were obtained from the New South Wales Office of Water website (https://realtimedata.waternsw.com.au/water.stm). Daily discharge data for non-automated sites, such as the Wakool escape from Mulwala Canal, and daily usage of Commonwealth environmental water were obtained from WaterNSW.

Some of the study reaches do not have hydrometric gauging stations. The daily discharge data for sites in the Wakool River zone 2 was estimated by adding the discharge from gauge 409019 Wakool River offtake regulator to the discharge data from the Wakool escape from Mulwala canal. The daily discharge data for Wakool River zone 3 was estimated by adding daily discharge data from Yallakool Creek offtake (gauge 409020), the Wakool offtake regulator (gauge 409019) and the Wakool Escape from Mulwala Canal with an adjustment during regulated flows to account for travel time (4 days) and estimated 20% losses (V. Kelly, WaterNSW pers. comm.) between the offtakes and the confluence of Yallakool Creek and the Wakool River.

Table 4.2 Details of Water NSW hydrometric gauges used to obtain discharge data. Zone codes are as described in Figure 3.1 and Table 3.1.

River	LTIM zone	Gauge number	Name of gauge
Yallakool Creek	1	409020	Yallakool Creek @ Offtake
Wakool River	2	409019	Wakool River Offtake regulator
Wakool River	4	409045	Wakool @ Wakool-Barham Road
Wakool River	5	409062	Wakool River Gee Gee Bridge 2
Wakool River	6	409013	Wakool @ Stoney Crossing
Colligen Creek	8	409024	Colligen Creek B/L regulator
Niemur River	10	409086	Niemur at Mallan School
Edward/Kolety River		409008	Edward River Offtake
Edward/Kolety River	11	409023	Edward River DS Stevens weir
Edward/Kolety River	13	409104	Edward River at Moulamein
Edward/Kolety River	14	409035	Edward River at Liewah

Details of the daily volume of water (ML/d) accounted for as Commonwealth environmental water was provided by WaterNSW and the Commonwealth Environmental Water Office. These data were used to produce hydrographs showing the daily discharge and the proportion of that flow that is Commonwealth environmental water for the four hydrological zones.

To evaluate to what extent Commonwealth environmental water contributed to longitudinal hydrological connectivity, the hydrographs for the Wakool River at Gee Gee Bridge site 05_02 (gauge 409062) and Stoney Crossing, site 06_01 (gauge 409013) were plotted and visually compared to the shape of the hydrographs upstream that received Commonwealth environmental water.

4.5 Results

Hydrology in 2019-20

The hydrology of the rivers in the Edward/Kolety-Wakool system in 2019-20 was influenced by winter watering action, return flows during the southern connected flow, Commonwealth environmental watering actions from September through to December 2019, and an unregulated flow pulse in May 2020.

In the Edward/Kolety River system downstream of the Edward offtake the discharge was held steady at approximately 1550 ML/day for nine months between July 2019 and March/April 2020 (Figure 4.1). However, in the Edward/Kolety River downstream of Stevens Weir the discharge was considerably more variable and this variability in flows was evident right through the Edward/Kolety River to Liewah (Figure 4.3).

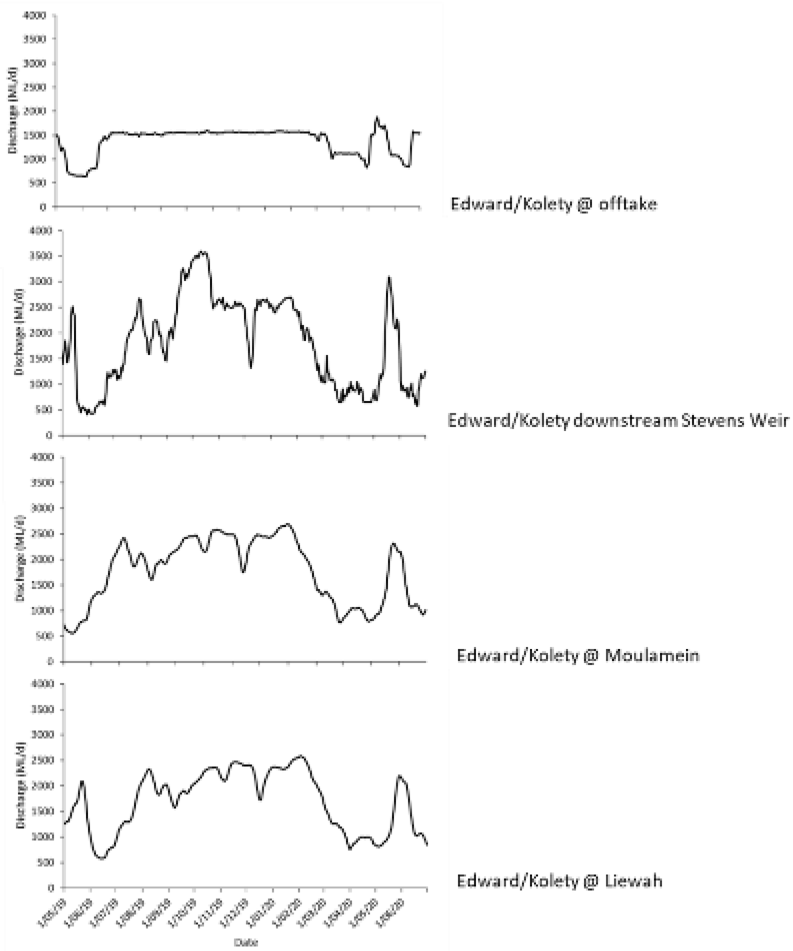


Figure 4.1 Hydrographs for the Edward/Kolety River at the Edward River offtake (gauge 409008), downstream of Stevens Weir (gauge 409023), Moulamein (gauge 409104) and at Liewah (gauge 409035) from 1 May 2019 to 30 June 2020.

A similar pattern was evident in the Wakool River. The discharge was considerably less variable at the offtake but more variable in the mid reaches and lower reaches of the Wakool at Stoney Crossing (Figure 4.2) after inputs of flows from the Murray system.

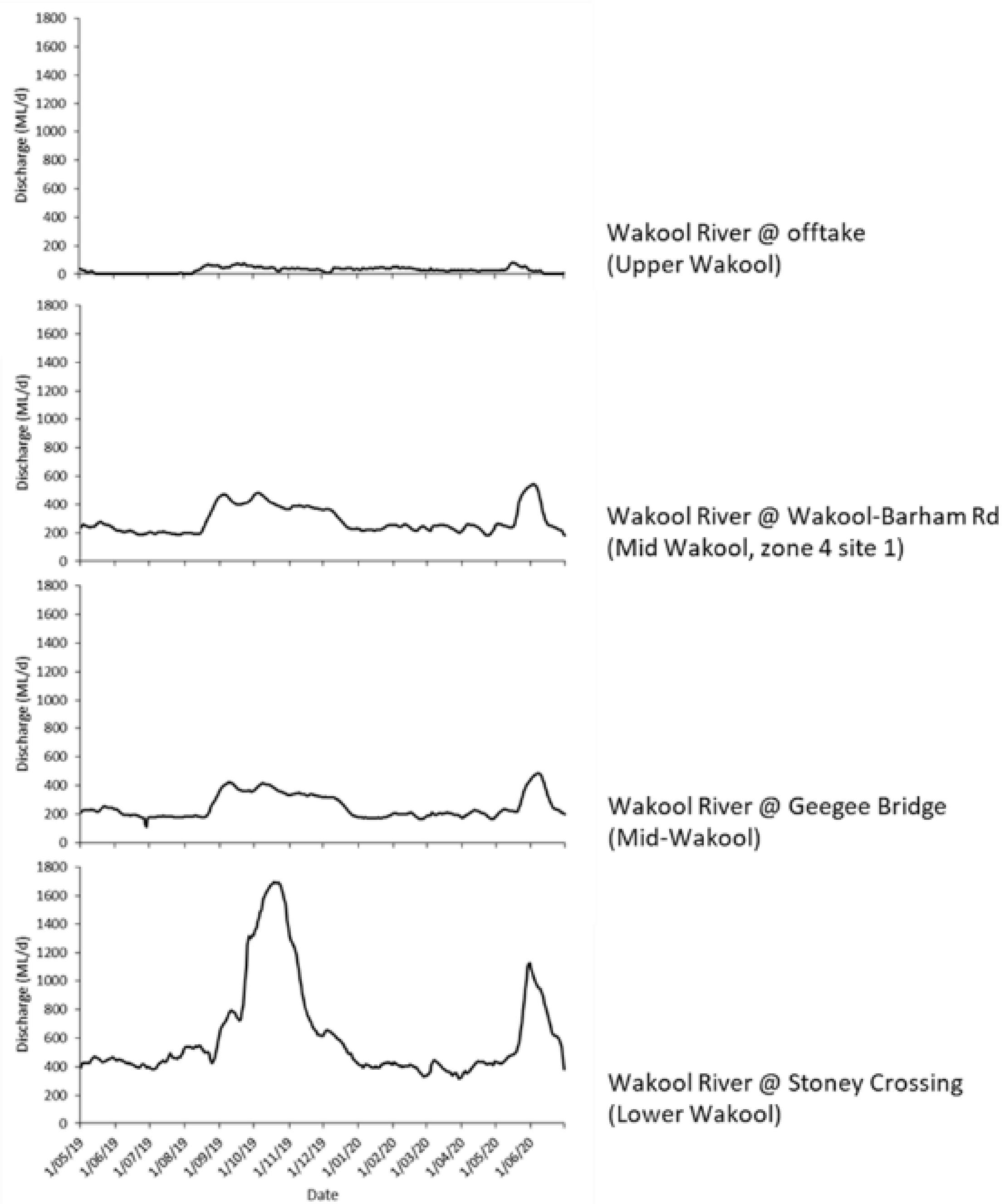


Figure 4.2 Hydrographs for the Wakool River at offtake (gauge 409019), Gee Gee Bridge (gauge 409062), and at Stoney Crossing (gauge 409013) from 1 July 2018 to 30 June 2019.

In the Colligen Niemur system there was a similar pattern where there was lower variability of flows at the upper reaches, with increased variability further downstream. The most upstream hydrological gauge below the Colligen regulator shows there was a long period of time when flows were held at approximately 200 ML/day (Figure 4.3). Whereas further downstream in the Niemur River at Barham-Moulamein Road and at the Mallan School gauges the influence of other inflows into the Niemur River from the Edward/Kolety River via Reed beds and Niemur regulators has resulted in a more variable hydrograph (Figure 4.5).

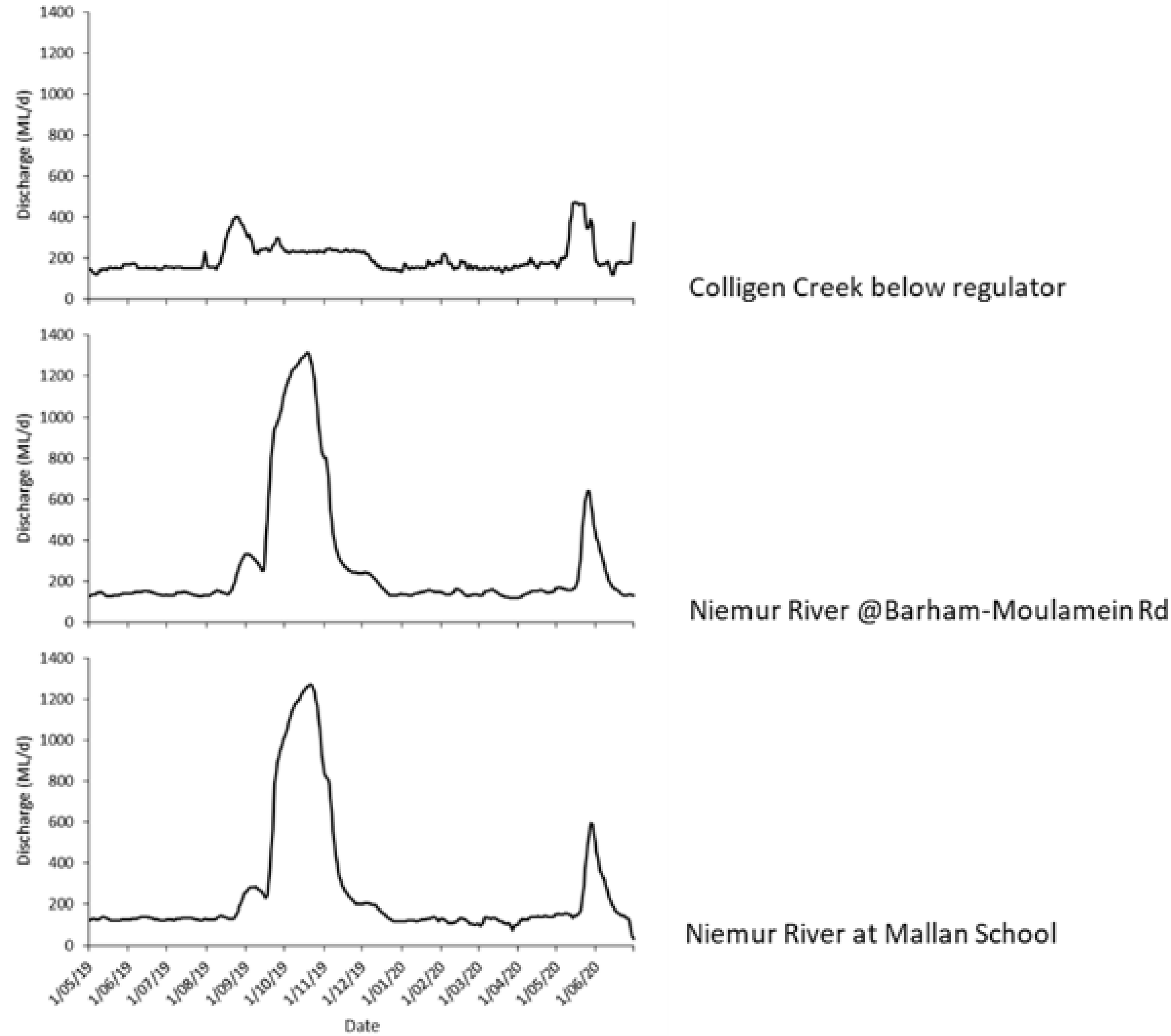


Figure 4.3 Hydrographs for the Colligen-Niemur system at Colligen Creek below regulator (gauge 409024), and in the Niemur River at Mallan School (gauge 409086) from 1 July 2018 to 30 June 2019.

Environmental Watering actions in 2019-20

The annual hydrographs (1 May 2019 to 30 June 2020) at gauges in the Yallakool-Wakool system shows the contribution of Commonwealth environmental water to the hydrograph (Figure 4.4).

The continuous winter flow (watering action 1) maintained longitudinal connectivity throughout winter 2019 in zones 1, 3 and 4 and the cease to flow in the upper Wakool River (zone 2) in 2019 is clear evident (Figure 4.4).

The Commonwealth environmental watering actions in the Wakool-Yallakool system from September through to December 2019 had a significant influence on the flows in zones 1, 3 and 4. This period of time includes actions 3a through to 3e (Table 4.1). There was minimal environmental water delivered to zone 2 (upper Wakool River)(Figure 4.4).

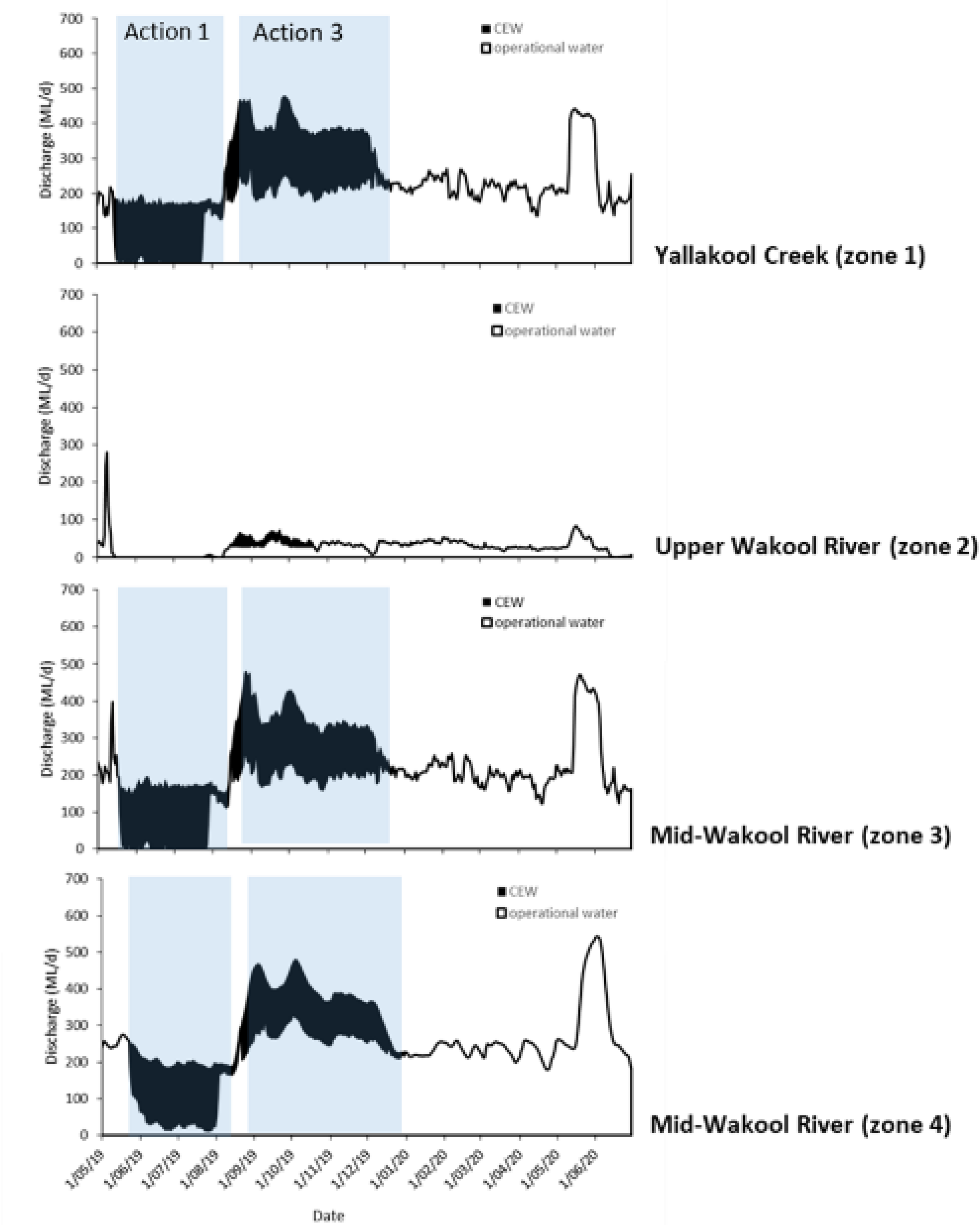


Figure 4.4 Hydrographs of zones 1 Yallakool Creek, and zones 2, 3 and 4 in the Wakool River from 1 May 2019 to 30 June 2020. The portion of the hydrographs coloured black is attributed to the delivery of Commonwealth Environmental Water. The blue shaded sections relate to the environmental watering actions 1 and 3.

The continuous winter flow (watering action 1) in zones 8 in Colligen Creek maintained longitudinal connectivity throughout winter 2019 (Figure 4.5).

Similarly, the Commonwealth environmental watering actions in Colligen Creek from September through to December 2019, had a significant influence on the hydrology of that zone, that would have otherwise have received minimal variation in discharge over almost 6 months of the year (Figure 4.5).

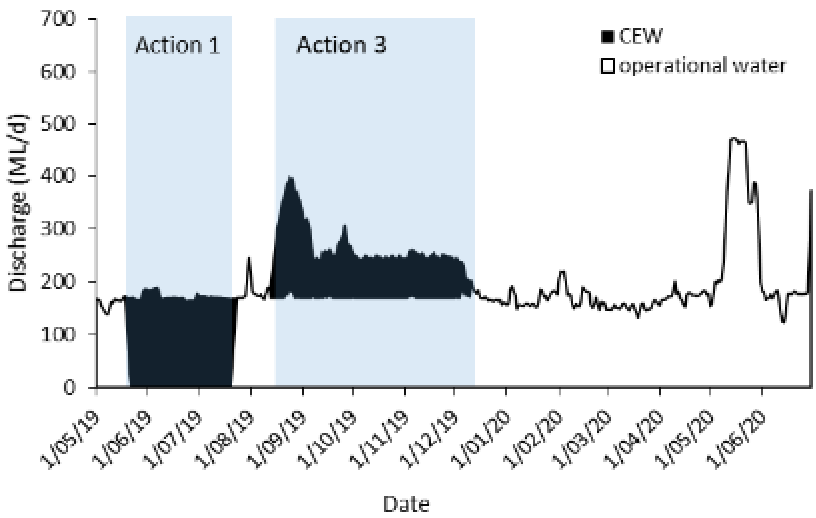


Figure 4.5 Hydrographs of zones 8 Colligen Creek from 1 May 2019 to 30 June 2020. The portion of the hydrographs coloured black is attributed to the delivery of Commonwealth Environmental Water. The blue shaded sections relate to the environmental watering actions 1 and 3.

Longitudinal connectivity

The benefits to longitudinal connectivity of the winter 2019 environmental watering action are evident when continuous river flows are mapped (Figure 4.6). In contrast, a map of refuge pools and dry sections of the Edward/Kolety-Wakool system during the millennium drought (Figure 4.7) shows that there are many section of the Wakool-Yallakool and Colligen-Niemur systems that do not continuously maintain water during cease to flow periods.

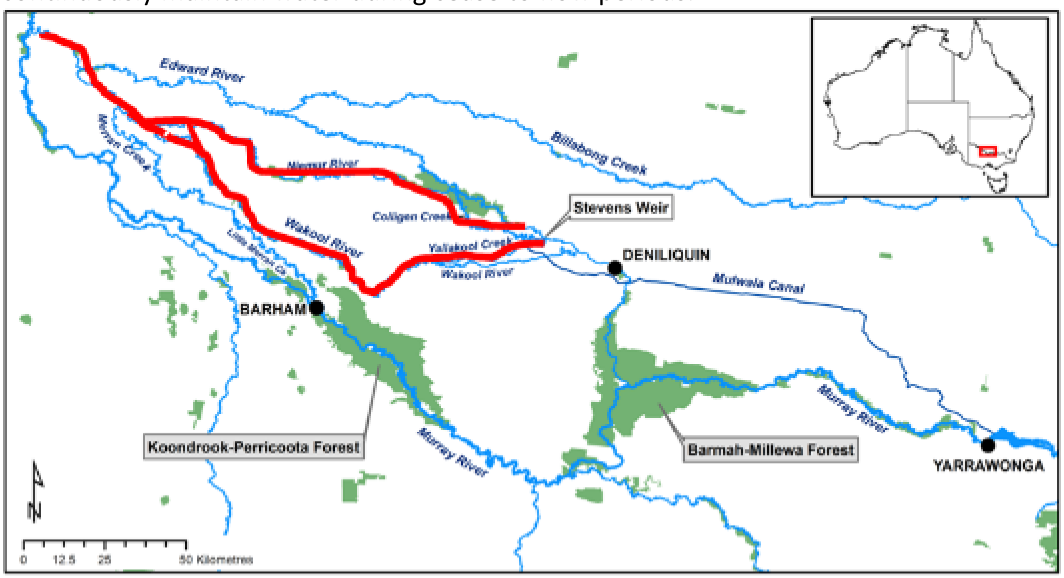


Figure 4.6 Map of the Edward/Kolety-Wakool system showing the length of river where connectivity was maintained (in red) due to winter watering action 1 in Wakool-Yallakool system and Colligen Creek.

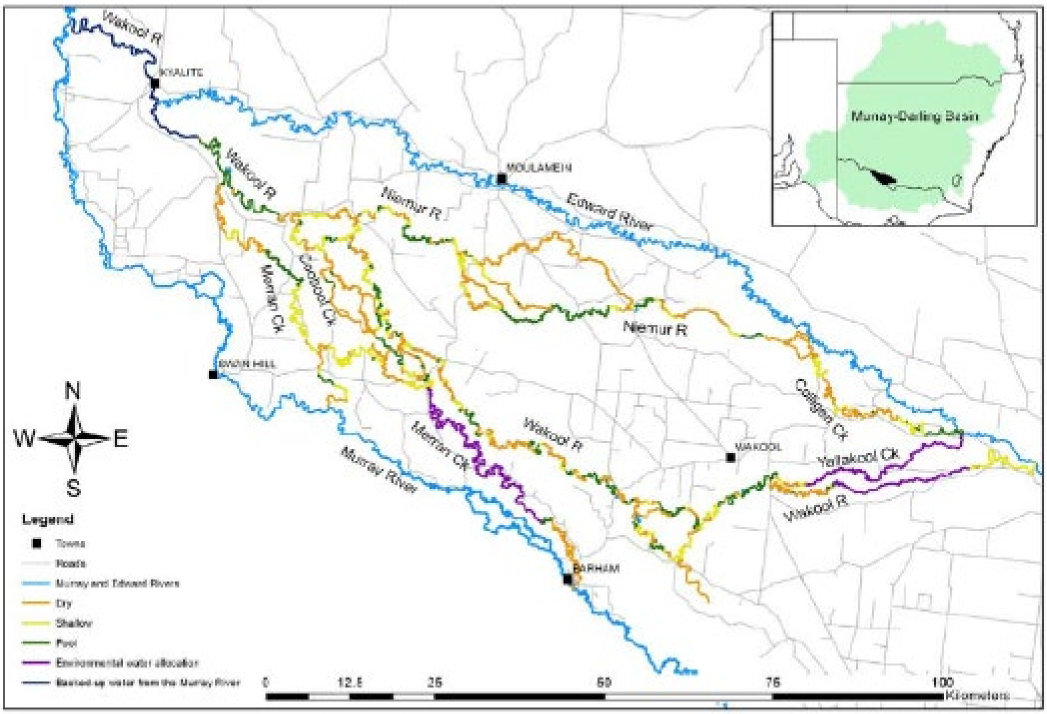


Figure 4.7 Map of the Edward/Kolety-Wakool system showing the distribution of aquatic refugia, and areas that were dry on 27th November 2007 during the Millenium drought (Source Gilligan, Vey and Asmus 2009).

Lateral connectivity

An example of lateral connectivity during watering action 3 is shown in Figure 4.8 using results of hydraulic models. This demonstrates the increased lateral connectivity between the river channel and low lying wetlands that would have occurred during watering action 3, compared to what would have occurred under operational flows. Results of hydraulic modelling at other reaches in the Edward/Kolety-Wakool system are presented in Watts et al. (2015).

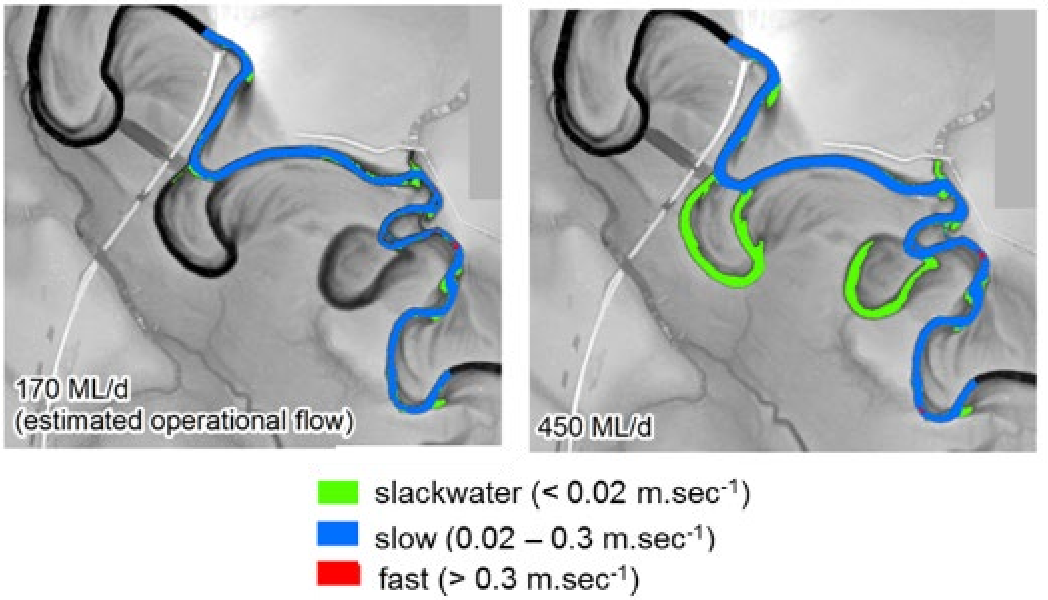


Figure 4.8 Results of hydralic modelling for the Wakool River at the Wakool-Barham Road showing difference in inundated area under 170 ML/day operational flow (left) and 450 ML/day environmental flow (right) that was similar discharge to the peak of the watering action in September 2019.

4.6 Discussion

What was the effect of Commonwealth environmental water of the Edward/Kolety-Wakool system?

Watering actions in the Edward/Kolety system in 2019-20 increased the maximum discharge in all zones compared to operational flows. The maximum discharge was considerably more than would have been experienced under operational flows.

What did Commonwealth environmental water contribute to longitudinal hydrological connectivity?

Watering action 1 (winter flow) maintained longitudinal connectivity in Yallakool Creek, the mid and lower Wakool River, and the Colligen-Niemur system. Maintaining longitudinal connectivity and preventing cease to flow in winter has many ecosystem benefits including preventing exposure of acid sulphate soils in the lower part of the system, enabling movement of fish into and out of the river system, enabling sedentary fish and other organisms to maintain their local habitat over winter, and preventing frost damage of aquatic vegetation and exposure of rhizomes to damage by pigs.

What did Commonwealth environmental water contribute to lateral connectivity?

Watering action 3 (spring watering action) increased lateral connectivity within the river system, and would have resulted in a considerable increase in wetted area. Increasing the extent and duration of lateral connectivity can play an important role in river productivity, increasing the opportunity for dissolved carbon inputs to the stream from the sediment or organic materials, such as leaves, biofilms, grasses and other inundated vegetation. The inundation also provides opportunities for growth and increased cover of submerged and amphibious macrophytes which can increase habitat for invertebrates, frogs and fish.

5 WATER QUALITY AND CARBON

Authors: Xiaoying Liu and Nicole McCasker

Key findings	
Dissolved oxygen concentrations	In 2019-20 water year, dissolved oxygen concentration was consistently higher during late summer and early autumn in zones 1, 3 and 4 than zone 2. Zones 1, 3 and 4 received more Commonwealth environmental water than zone 2. Concentrations of dissolved oxygen in the Edward/Kolety River, Wakool River and the Colligen-Niemur River were above the range of concern to fish populations (4 mg/L). The expected seasonal variations were observed, with higher concentrations in winter and lower concentrations correlating to the periods of higher water temperature.
Nutrient concentrations	Total Phosphorus and Total Nitrogen were slightly elevated, likely due to greater turbidity (particles suspended in the water column) but bioavailable nutrient remained low. The absence of overbank flows meant that substantial nutrient inputs were not expected in the system, although a general downstream increase in TN and TP were observed in the zones which received the majority of flow (zones 1, 3 and 4). Nutrients in the Edward/Kolety River, Wakool River and Colligen-Niemur River were similar, remaining in the acceptable range.
Temperature regimes	None of the watering actions targeted temperature. Water temperatures in the system were primarily controlled by the prevailing weather conditions.
Type and amount of dissolved organic matter	There was no detectable effect of environmental watering actions on this indicator in 2019-20 and there were no adverse water quality outcomes. The watering actions in 2019-20 did not specifically target the transport of dissolved organic matter. Dissolved organic carbon was not elevated outside the normal range and remained well below concentrations associated with blackwater events. Dissolved organic carbon in the Edward/Kolety River, Wakool River and Colligen-Niemur River were similar, remaining in the acceptable range.

5.1 Background

Water quality is a key indicator of aquatic ecosystem health, and flow plays an important role in the maintenance of water quality in lowland rivers. Water quality parameters will often respond to changes in flow regimes very quickly. Changes in flow in a river system can influence water quality both positively and negatively with the outcome dependent on the source of the water, magnitude and duration of the flow, time of the year and other catchment conditions. High flow events caused increases in wetted benthic areas can result in exchange of nutrients and carbon between the river and the adjacent floodplain, and/or previously disconnected in-channel areas (Baldwin 1999; Baldwin and Mitchell 2000; Robertson et al. 2016) and environmental flows play a key role in restoring carbon exchange that has been lost due to extensive river regulation and modification of channel and bank features (Baldwin et al. 2016).

A range of parameters can be measured as indicators of water quality in river systems and many of these parameters as water quality targets in the Murray-Darling Basin Plan 2012 are directly or indirectly influenced by alterations in flow. For example, DO can be influenced by flow through changes in water volume and turbulence, and through indirect processes such as alterations in rates of bacterial metabolism and photosynthesis. This, in turn, will directly influence the suitability of the water quality for aquatic organisms, such as fish. Nutrients and organic matter concentrations may be influenced by flow, either by dilution or through inputs associated with water contacting parts of the channel or floodplain which were previously dry and which have stores of nutrients and carbon in both plant materials and the soil (Baldwin 1999; Baldwin and Mitchell 2000).

Australian riverine ecosystems can be heavily reliant on both algal and terrestrial dissolved organic matter for microbial productivity and can be limited by dissolved organic carbon concentrations (Hadwen et al. 2010). Aquatic environments naturally have quite variable dissolved organic matter concentrations and there are no optimal concentrations or trigger values provided for organic matter (ANZECC 2000).

Organic matter is made up of a complex mixture of compounds with different properties and variable availability to the microbial population. This mixture contains many different types of compounds with a diverse range of sources and the most fundamental use of broad categories of organic matter in natural waters are non-humic substances and humic substances (Choudhry 1984). Non-humic substances include relatively simple compounds belonging to recognised groups such as carbohydrates, proteins, peptides, fats and other low molecular weight organic compounds. However, the much larger molecules that make up of humic substances can dominate in many waters and in contrast are poorly characterised (Choudhry 1984). Humic substances can be further classified into two groups (including humic and fulvic acids) based on their properties rather than chemical structure. Humic substances are predominantly derived from the processing of plant residues and can involve complex chains and aromatic rings which contribute to their strong yellow-brown colour.

Microbial communities do not respond to all types of organic matter in the same way (Baldwin 1999; O'Connell et al. 2000; Howitt et al. 2008) although it has been shown that bacterial communities can respond to changes in organic carbon source quite rapidly (Wehr et al. 1999). The very large, complex type of organic matter referred to as humic substances has been shown to be less available to bacterial communities than simpler non-humic carbon (Moran and Hodson 1990) although this can be altered over time with exposure to ultraviolet light (Moran and Zepp 1997; Howitt et al. 2008). These differences in microbial response to different types of organic matter mean that it is important to consider not just the total amount of dissolved organic matter in the rivers but to monitor changes in the type of organic matter present. Both absorbance and fluorescence spectra are used to examine the organic matter in this study. As a general guide, absorbance at longer wavelengths indicates larger, more complex organic matter (Bertilsson and Bergh 1999). Absorbance at a particular wavelength may be increased by increasing concentration of organic matter or a change in the type of organic matter.

Reconnection of the stream channel with backwater areas and dry sections of the floodplain and channel may result in additional nutrients and organic carbon. Inputs of these substances may have a positive influence on the river community through the stimulation of productivity and increased food availability for downstream communities (Robertson et al. 1999) and the connection between the river and floodplain has been shown to generate essential carbon stores to sustain the system through drier periods (Baldwin et al. 2013). However, excessive nutrient and organic carbon inputs can result in poor water quality through the development of algal blooms or blackwater events resulting in very low dissolved oxygen concentrations (Howitt et al. 2007; Hladyz et al. 2011). Inputs of large amounts of organic matter and nutrients during hot weather are particularly problematic due to the influence of temperature on the rates of microbial processes and organic matter leaching (Howitt et al. 2007; Whitworth et al. 2014). This chapter reports on changes in water quality in response to flows from July 2019 to 30 June 2020 and will consider changes in both the quantity and type of organic matter present in the system. Specifically, this work will address the evaluation questions in section 5.3.

5.2 Environmental watering actions targeting water quality outcomes

Three Commonwealth environmental watering actions were delivered in the Edward/Kolety-Wakool system in 2019-2020 (Table 5.1). This report will cover water quality data from July 2019 to June 2020.

Table 5.1 Commonwealth environmental watering actions in 2019-20 in the Edward/Kolety-Wakool system.

Watering Action	Name	Objectives (from CEWO)	Dates
Action 1	Winter base flow	For native fish condition and movement, vegetation in-channel, longitudinal connectivity; refuge habitat during irrigation shut-down period	15/05/19 - 9/08/2019
Action 2	Winter to spring transition flow	At this time, there was no operational demand so CEW was used to prevent water levels reducing to low levels for a short period between action 1 and action 3.	10/08/19 - 27/08/19
Action 3a	Winter/spring early fresh	To provide early season rise in river level to contribute to connectivity, water quality, stimulating early growth of in-stream aquatic vegetation, pre-spawning condition of native fish and/or spawning in early spawning native fish.	28/08/19 - 4/09/19
Action 3b	Early spring elevated base flow	To maintain nesting habitat for Murray Cod, and inundation for aquatic vegetation growth.	5/09/19 - 22/09/19
Action 3c	Late spring fresh	To promote silver perch spawning, influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit. May also assist with dispersal of larvae and juveniles of a number of fish species.	23/09/19 - 11/10/19
Action 3d	Late spring elevated base flow	To influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit. May also assist with dispersal of larvae and juveniles of a number of fish species.	12/10/19 - 30/11/19
Action 3e	Recession	Slow recessions for instream water plants	1/12/19 - 22/12/19

5.3 Selected Area evaluation questions

As described above, the relationship between flow and water quality is complex and can be influenced by how changes in flow influence wetted benthic area, water depth, rate of flow and connectivity to the floodplain. Water quality parameters may be affected in different ways due to the direct effects of changes in flow, or due to interactions between the parameters. In order to obtain an understanding of the impact of environmental water deliveries to the Edward/Kolety-Wakool system on the water quality in a broader range of sites (the Wakool-Yallakool system, Edward/Kolety River, the Colligen-Niemur River and Tuppal Creek), we monitor a number of parameters at each site through a combination of continuous logging, spot readings on site and sample collection for laboratory analysis. Water quality will generally respond very rapidly to changes in flow, but trends may also develop over a longer period, so the questions below are considered on a 1-3 year basis.

In 2019-20 the key questions relating to the CEW actions were:

- What did Commonwealth environmental water contribute to DO concentrations?
- What did Commonwealth environmental water contribute to nutrient concentrations?
- What did Commonwealth environmental water contribute to modification of the type and amount of dissolved organic matter through reconnection with previously dry or disconnected in-channel habitat?

The remaining question was not addressed as these conditions were not present in the system:

- What did Commonwealth environmental water contribute to reducing the impact of blackwater or other adverse water quality events in the system?

5.4 Monitoring sites

The core carbon fluorescence and water quality data have been collected at sites shown in Table 5.2 and Figure 5.1 and includes ongoing monitoring at established sites in Yallakool Creek (Zone 1), Wakool River (Zones 2 to 4), and source water for these sites from the Mulwala Canal and the Edward/Kolety River at Stevens Weir. New sites for the Flow-MER project expanded the water quality monitoring further downstream in the Wakool River and to Tuppal Creek, the Edward/Kolety River, and the Colligen-Niemur River to better capture the impact of environmental water in the broader system. Sites 5 and 6 (Edward/Kolety River) together with 9, 10 and 11 (the Colligen-Niemur River system) may be used in combination to assess carbon and nutrient exchange between the river systems and the Werai Forest should an appropriate overbank flow occur.

The focus of the annual monitoring is the assessment of organic matter inputs and water quality changes during in-stream flows. Sampling consists of water samples collected from each site on a monthly basis throughout the year.

Table 5.2 Sites for water quality and carbon routine monitoring. Colours in this table relate to the colours on the map in Figure 5.1.

No.	Site name	River system	Gauge number	LTIM DO logger	WaterNSW DO logge	New Flow-MER DO logger	Sites labels on figures
1	Tuppal Creek	Tuppal Creek	409056			ˇ (yellow)	Aratula_Rd
2	Mulwala Canal						Canal
3	Four Post	Edward River	409047		ˇ (green)		Four_post
4	Stevens Weirpool	Edward River	409101				Weir
5	Downstream Stevens Weir	Edward River	409023			ˇ (yellow)	Eastman_bridge
6	Downstream Werai Forest	Edward River				ˇ (yellow)	Balpool_rd_bridge
7	Moulamein	Edward River	409014		ˇ (green)		Moulamein
8	Liewah	Edward River	409035			ˇ (yellow)	Liewah
9	Colligen	Colligen-Niemur River	409024			ˇ (yellow)	Colligen-Old Morago Rd
10	Niemur Barham Road	Colligen-Niemur River	409048		ˇ (green)		Niemur-Moulamein Rd
11	Niemur Mallan School	Colligen-Niemur River	409086		ˇ (green)		Niemur-Mallan School
12	Zone 1 site 5	Yallakool Creek		ˇ (red)			Zone 1
13	Zone 2 site 4	Wakool River		ˇ (red)			Zone 2
14	Zone 3 site 5	Wakool River		ˇ (red)			Zone 3
15	Wakool Barham Road	Wakool River	409045	ˇ (red)			Zone 4
16	Zone 4 site 5	Wakool River		ˇ (red)			Zone 4
17	Gee Gee Bridge	Wakool River	409062		ˇ (green)		Zone 5
18	Stony Crossing	Wakool River	409013		ˇ (green)		Zone 6

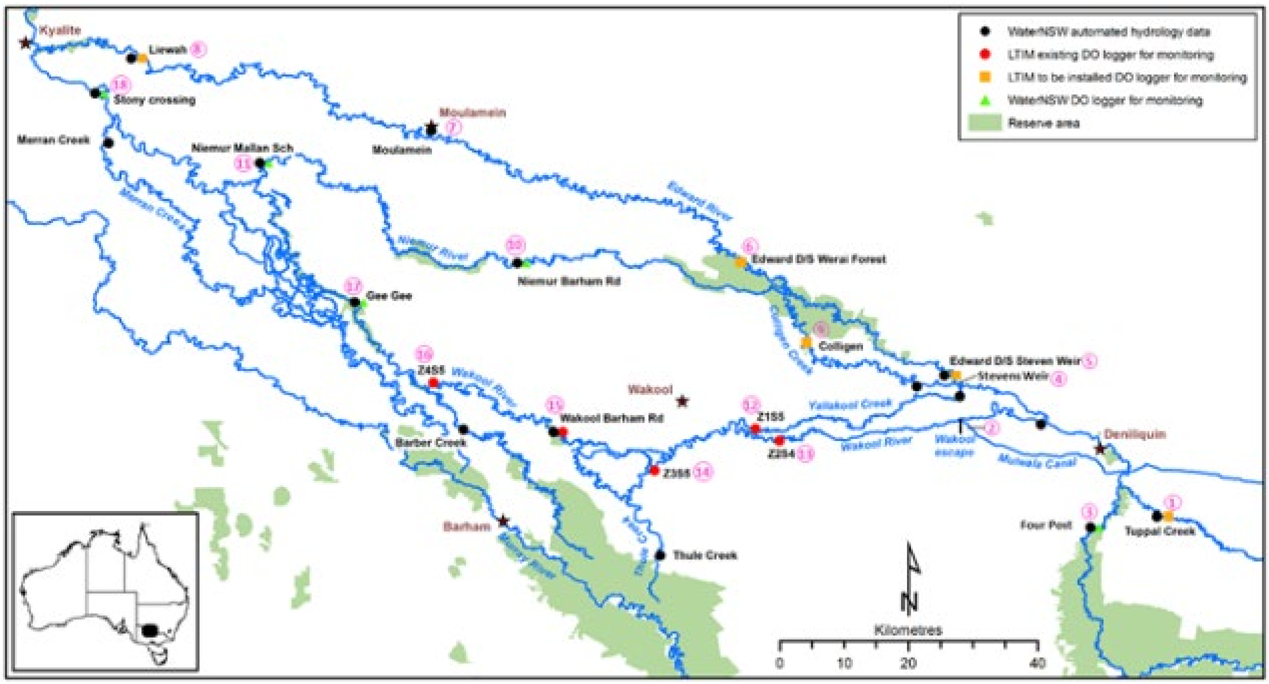


Figure 5.1 Map of the Edward/Kolety-Wakool Selected Area showing existing LTIM sites that are continued (red), sites where water quality sampling are supplemented with data from WaterNSW loggers (green), sites where new loggers are installed (yellow).

5.5 Methods

Water temperature and DO were logged every ten minutes at ten monitoring sites including Tuppal Creek, downstream Stevens Weir, downstream Werai Forest, Colligen, Zone 1 Site 5, Zone 2 Site 4, Zone 3 Site 5, Zone 4 Site 1, Zone 4 Site 5 and Liewah (Figure 5.1). Data were downloaded and loggers calibrated approximately once per month depending on access to survey sites. Light and depth loggers were also deployed, and data were downloaded on a monthly basis. The data collected by the loggers was used to calculate daily average temperature and DO concentrations for each of the river/creek system from 1 May 2019 to 30 June 2020. This period was used so the evaluation of the winter 2019 watering action could be included in this report.

From July 2019 to June 2020 water quality parameters (temperature (°C), electrical conductivity (mS/cm), DO (%), pH, and turbidity (NTU)) were measured as spot recordings monthly at monitoring sites within each river/creek system, and from Stevens Weir on the Edward/Kolety River and the Mulwala Canal. Water samples were collected once per month from monitoring sites within each river/creek system, and from Stevens Weir on the Edward/Kolety River, and the Mulwala Canal.

Water samples were processed according to the methods detailed in Watts et al. (2014a) to measure:

- Dissolved Organic Carbon (DOC)
- Nutrients (Ammonium (NH_4^+), filtered reactive phosphorus (FRP), dissolved nitrate + nitrite (NOx), Total Nitrogen (TN) and Total Phosphorus (TP))
- Chlorophyll-*a* (Chl *a*)
- Absorbance and fluorescence spectroscopy for organic matter characterisation

Water samples for organic matter characterisation, DOC and bioavailable nutrients (FRP, NOx, NH_3) were filtered through a 0.2 µm pore-sized membrane at the time of sampling and then stored on ice until returned to the laboratory. DOC and nutrient samples were frozen and sent to CSIRO NATA certified lab in CSU Albury campus for analysis. Carbon characterisation samples were sent to NaLSH, CSU Wagga Wagga campus and analysed within a day of returning from the field.

Absorbance scans were collected using a Varian Cary 4000 instrument across a wavelength range of 550 nm to 200 nm (green through to ultraviolet) with a 1 nm step size. Absorbance is a measure of light absorbed by the sample and is a logarithmic scale. An absorbance of 1 indicates that only 10% of the light of that wavelength is transmitted through the sample. Fluorescence scans were collected using a Varian Eclipse spectrofluorometer scanning both emission and excitation wavelengths to give an excitation-emission matrix. Excitation wavelengths were scanned from 200 to 400 nm with a 10 nm step size and for each excitation wavelength, emission of light at 90° to the source was recorded from 200 nm to 550 nm with a 1 nm step size. Fluorescence results were corrected for sample absorption and plotted as contour plots (Howitt et al. 2008). To correct for drift in the instrument zero position, each contour plot was scaled by subtracting the average emission intensity across the range 200-210 nm for an excitation of 250 nm from all fluorescence intensities effectively setting this region of the contour plot to zero on all plots.

An example of a fluorescence contour plot is shown in Figure 5.2. The contour plots have the excitation wavelength (light shone into the sample) on the y-axis. On the x-axis is the emission wavelength (light given off by the sample). The intensity of the fluorescence (how much light is given off, corrected for absorbance by the sample) is represented by the colours of the contour plot, with more intense fluorescence represented by the blue end of the scale. The two blue diagonal lines are artefacts of the technique and will be present in all samples- key data is found between these two lines.

The monitoring results were assessed against the lowland river trigger levels for aquatic ecosystems in south-east Australia from the ANZECC (2000) water quality guidelines. If the concentration of a particular water quality parameter exceeds the trigger level or falls outside of the acceptable range, the guidelines are written with the intention that further investigation of the ecosystem is 'triggered' to establish whether the concentrations are causing ecological harm. Systems may vary in their sensitivity to various parameters and therefore exceeding a trigger level is not an absolute indicator of ecological harm. It is quite common for water quality parameters to briefly fall outside of guideline values during large overbank flows. The ANZECC water quality guidelines do not provide trigger levels for total organic carbon and dissolved organic carbon, and this reflects the expectation that there will be large variation in the 'normal' concentrations of organic carbon between ecosystems and also in the chemical and biological reactivity of the mixture of organic compounds making up the DOC and TOC at a particular site. Given the variable make-up of organic carbon, and the possible range of ecological responses to this mixture, a trigger level for this parameter would not be appropriate. However, trigger levels are provided for a number of nutrients and these are discussed below.

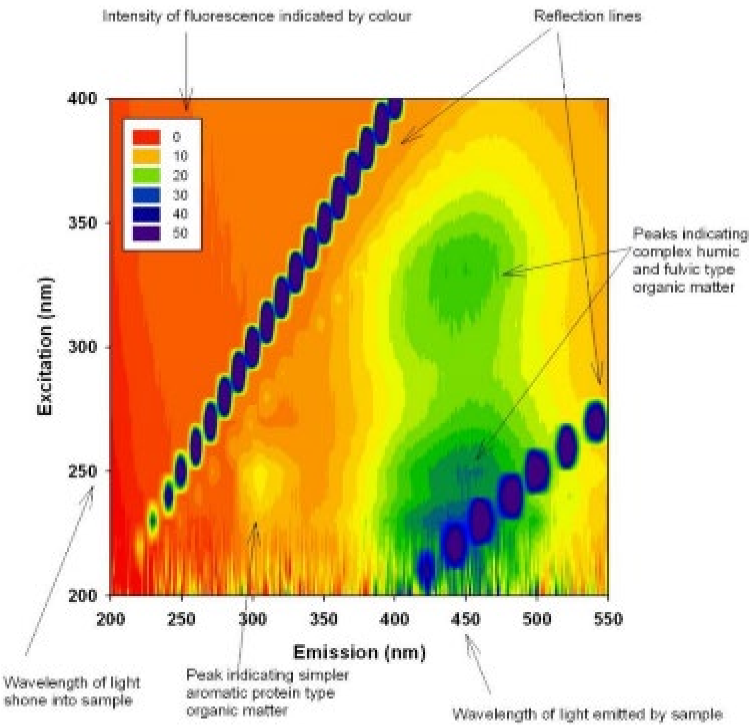


Figure 5.2 Sample excitation emission contour plot indicating key features of the data (Watts et al. 2013).

5.6 Results

The collected water quality and carbon data have been grouped based on the major rivers; the Yallakool-Wakool system, the Edward/Kolety River, the Colligen-Niemur River system and Tuppal Creek. The data collected by the loggers was used to calculate daily average temperature results and DO concentrations for selected sites of each river system between July 2019 and 30 June 2020. The water samples for the assessment of water quality changes during in-stream flows were collected from each site on a monthly basis throughout the year from July 2019 to June 2020. The water samples for the assessment of organic matter inputs were collected from each site on a monthly basis from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions. In general, downstream sites affected by water actions were later and experienced longer periods than upstream sites.

The Wakool-Yallakool system

Temperature and dissolved oxygen and spot water quality parameters

Water temperature was consistent across study sites (zones 2, 3 and 4) in the Wakool-Yallakool system with water temperature exceeding 25 °C briefly during summer and staying below 10 °C for several weeks during winter. The results indicate that water temperature is influenced predominantly by seasonal rather than site-specific factors. There was no discernible effect of Commonwealth environmental watering action on water temperature, with all sites displaying the same seasonal variation and influence of weather patterns. This was consistent with the trend observed in previous years.

The plot of average daily DO concentrations in zones 1 to 4 in the Wakool-Yallakool system (Figure 5.3) shows the expected seasonal variations with higher concentrations in the winter and lower concentrations correlating to the periods of higher water temperature. Yallakool Creek (zone 1) and the Wakool River in zones 3 and 4 (all receiving base flows and small freshes of Commonwealth environmental water) were similar to each other throughout most of the study period. In all cases a decline in DO was observed during the hotter months, as expected with the increased water temperature (which decreases oxygen solubility and increases the rate of many microbial processes).

The difference in DO concentration between zones does not reflect water temperature differences and likely reflects differences in input of oxygenated water from upstream and different rates of re-aeration and oxygen consumption associated with flow. Concentrations of DO in the Wakool River zone 2 briefly dropped into the range of concern to fish populations (below 4 mg/L) in late December 2019 and in late January/early February 2020, no values below 2 mg/L were recorded. It is common the Wakool River in zone 2 (shown in orange) had slightly lower DO than the other sites throughout the study period especially in summer when discharge is much lower at this reach. The difference in DO between zone 2 and the other zones was more in 2019-20 than was observed in 2018-19 and the period where DO was close to 4 mg/L was a bit longer, likely due to the lower discharge in this zone than in the water year 2018-19. Typically flow is extremely low in zone 2 over the summer, the higher than usual flow conditions in 2018-19 demonstrate that there is potential to use Commonwealth environmental water to improve water quality in this part of the system in the future.

Spot water quality parameters (electrical conductivity (EC), turbidity and pH) remained stable and within the normal range for this system throughout the study period and were very similar to results from the 2014-15, 2017-18 and 2018-19 sampling years in the absence of extensive overbank flows or excessive algae bloom.

The EC values at all sites were well below the ANZECC (2000) trigger levels on all sampling dates. The increase in EC values sometimes observed in the upper Wakool River zone 2 during autumn was not observed in the 2019-20 water year and the relatively stable water levels during this period may have reduced the impact or amount of groundwater seeping into the system which was hypothesised to be the source of this increase in some years.

Turbidity measurements were generally above the ANZECC (2000) trigger level but within the range commonly observed in the 2014-15, 2017-2018 and 2018-19 sampling years.

Most pH values were within the acceptable range throughout the year and values were very similar between sites. The high pH from December 2019 through to February 2020 may indicate increased algal activity at that time. The greater range of pH results observed towards the end of the water year may reflect declining instrument performance and are not of concern.

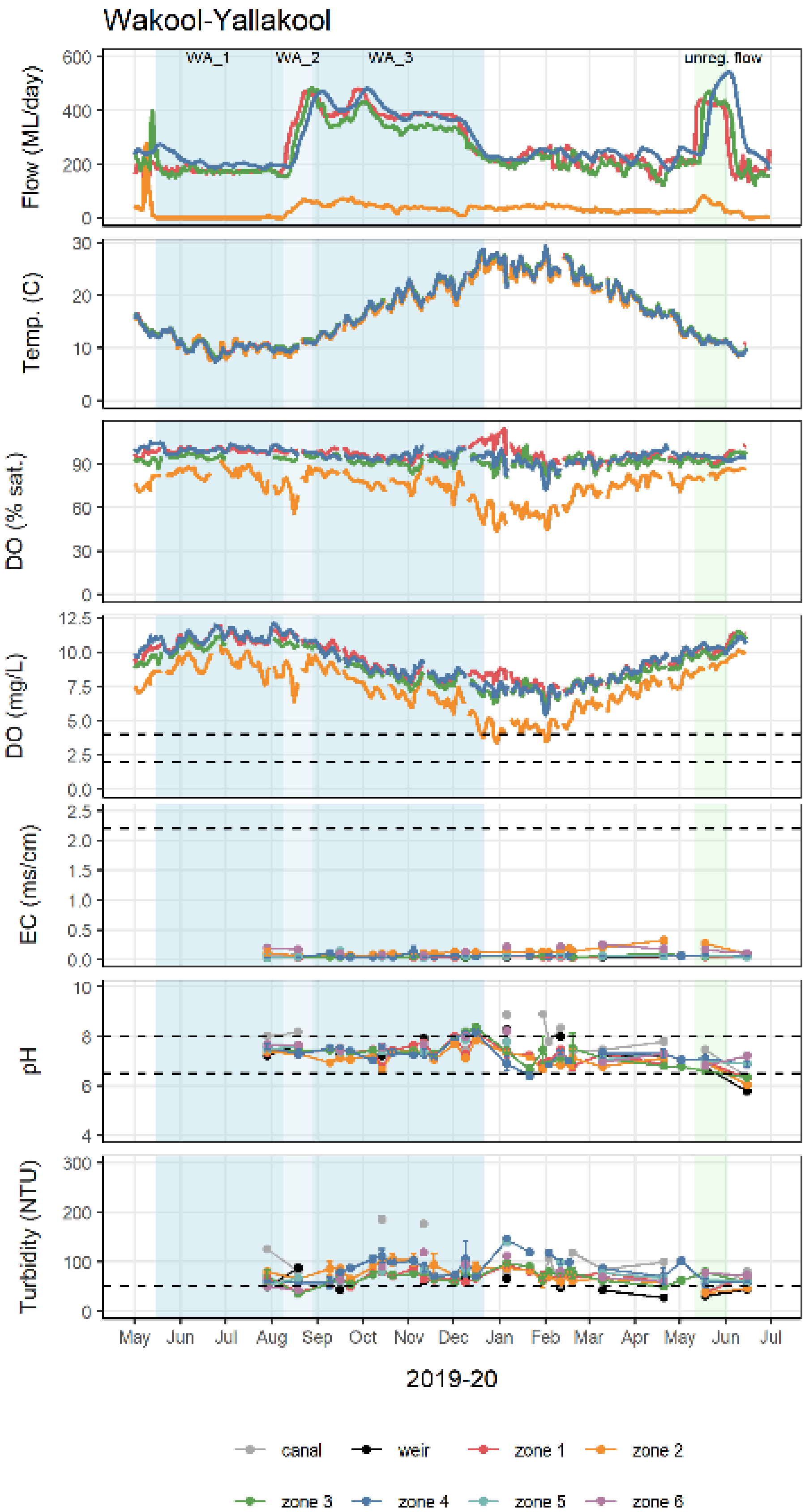


Figure 5.3 Daily average temperature results, daily dissolved oxygen (DO) concentrations, electrical conductivity (EC), turbidity and pH for the study sites and source water over the 2019-20 watering year in the Wakool-Yallakool system.

Nutrients and dissolved organic carbon

In general, nutrients and DOC were not elevated outside the normal range (Figure 5.4) in the Wakool-Yallakool system and were very similar to the concentrations recorded in previous years, with the exception of the periods of the bloom of cyanobacteria (*Chrysosporum ovalisporum*) in 2015-16 and the extensive unregulated overbank flooding in 2016-17. TP generally increased downstream zones and Zone 2 frequently has higher concentrations. This is consistent with the pattern in TN and trends in Chl *a*.

Excessive algae growth was observed in the Wakool-Yallakool system in early January 2020 (left image of Figure 5.5) corresponding with an increase in Chl *a* concentration at all sites. And a red alert for the Stony Crossing (Zone 6) on the Wakool River was issued in January 2020 but there was no clear influence on other water quality parameters. An outlier of Chl *a* measured in mid-Wakool River Zone 3 in March 2020 was higher than other zones, possibly due to floating algae that was present and may have contaminated the water sample. Although a red alert was issued for the Edward/Kolety River at Deniliquin at the beginning of April 2020 there was no clear increases in Chl *a* at monitoring sites, possibly because of water temperature had reduced.

Both TP and TN were increased during 2019-20 water actions which might have been associated with higher turbidity (suspended particles keeping adsorbed nutrients in the water column). TP and TN routinely exceeded the ANZECC (2000) trigger values of 0.05 mg/L and 0.5 mg/L respectively but remained within the normal range observed in this system. There were generally lower concentrations in Yallakool Creek zone 1 than in Wakool River (zones 2 to 6) suggesting slight increases in TP and TN as the water progresses through the system.

The NOx remained below the trigger levels and was similar to previous observations under normal conditions. The high NOx values were only in source water of Mulwala Canal on two occasions in August and November 2019. The high ammonia values occurred in several sampling sites could possibly be due to ammonia introduced from the source water or from a disturbance upstream or disturbance of the sediments while sampling. The FRP form of bioavailable phosphorus remained at the very low concentrations normally seen in this system in the absence of overbank flooding.

During 2019-20 DOC remained in the range of concentrations normally observed in this system in the absence of overbank flows or excessive algal growth. Although a pulse of dark coloured water was observed in the system in January 2020 (right image of Figure 5.5), this corresponds with a slight increase in DOC concentrations, but these remained within the normal range. The timing of this pulse corresponds with the lowest DO concentrations observed over the water year, although these were within the range normally measured at that time of year. Small inputs of DOC to the river can increase microbial productivity which are a source of food for aquatic organisms such as fish. Increased algal growth over the summer was insufficient to produce an increase in the dissolved fraction of the organic matter in these river systems. It is noted that DOC increases do not all occur at the same time in all zones. This suggests there may be local sources of DOC at times during this study period, possibly due to water that was in backwaters or on low lying benches during the higher summer flows draining back into the river system.

A slight increase in DOC was recorded during the unregulated flow event in 2020. It is noted that the upstream site in the Wakool River (Zone 2) has lower DOC than the downstream sites in this reach, however the results remain within the scatter of concentrations observed for the other sampling sites. This likely indicates that the pulse was of very brief duration and had already begun to clear from the top of the system at the time of sampling.

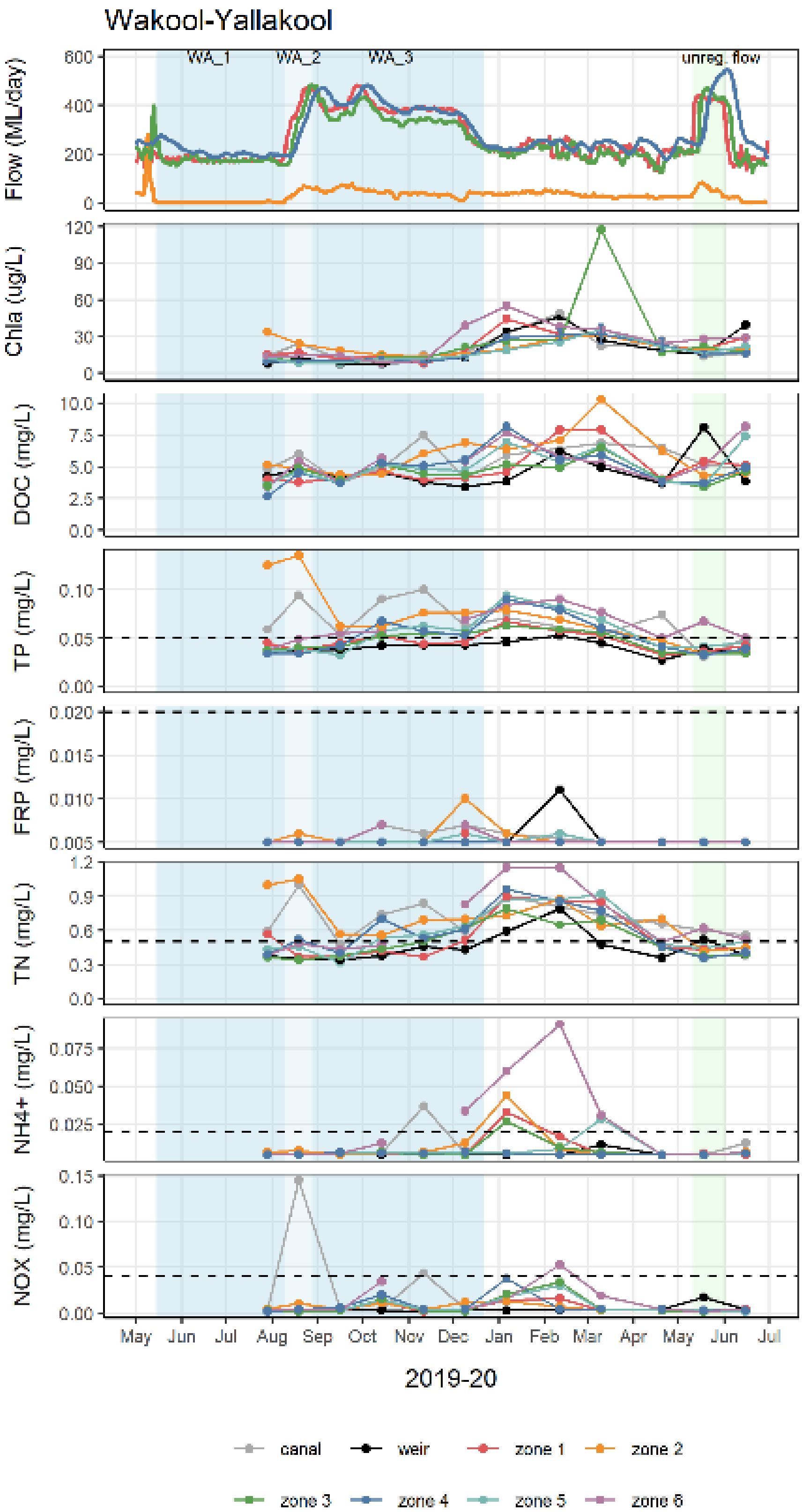


Figure 5.4 Chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), phosphorus (total phosphorus (TP) and filtered reactive phosphorus (FRP)), nitrogen (total nitrogen (TN), ammonium (NH_4^+) and dissolved nitrate + nitrite (NOx)) concentrations for the study sites and source water over the 2019-20 watering year in the Wakool-Yallakool system.

Figure 5.5 Poor water quality was observed at the upper-Wakool River system (Bolton Rd Bridge) (left) and Niemur-Moulamein Rd Bridge (right) during the contingency water quality monitoring between the end of January and late February 2020. (Photo: Xiaoying Liu)

Monitoring sites in the Edward/Kolety River, the Colligen-Niemur River and Tuppal Creek were additional sites for Flow-MER project 2019-22 and the interpretation of the results of these sites in 2019-20 is the first year of data.

The Edward/Kolety River

Temperature and dissolved oxygen and spot water quality parameters

Water temperature was consistent across study sites in the Edward/Kolety River with water temperature exceeding 25 °C briefly during summer and staying below 10 °C for a week during winter (Figure 5.6). The results indicate that water temperature is influenced predominantly by seasonal rather than site-specific factors with all sites displaying the same seasonal variation and influence of weather patterns.

The plot of average daily DO concentrations at Balpool Road Bridge and Eastman Bridge were similar to each other throughout most of the study period. Concentrations of DO in the Edward/Kolety River were above the range of concern to fish populations (below 4 mg/L) over the study season. It shows the expected seasonal variations with higher concentrations in the winter and lower concentrations correlating to the periods of higher water temperature. However, there was a discernible effect of the Southern Connected Flow in the Edward/Kolety River system from mid-October through to early November 2019 at the study site of Eastman Bridge with fluctuated water temperature results and DO concentrations. The difference in DO concentration between zones does not reflect water temperature differences and likely reflects differences in input of oxygenated water from upstream and different rates of re-aeration and oxygen consumption associated with flow. Unregulated flow occurred between 11 May and 2 June 2020 did not influence DO levels, possibly due to cool water temperatures in winter.

Spot water quality parameters (electrical conductivity (EC), turbidity and pH) remained stable and within the acceptable range for this system throughout the study period. EC remained stable within the lower end of the range expected for lowland rivers indicating in ANZECC (2000). Turbidity measurements generally fluctuated above and below the ANZECC (2000) trigger level and values were very similar between sites, with the exception on just one occasion at Moulamein and Liewah in December 2019. This possibly was caused by the input from Billabong Creek with high turbidity.

Most pH values were within the acceptable range throughout the year and values were very similar between sites. The high pH values from January to February 2020 at Four Post may indicate increased algal activity at that time. The greater range of pH results observed towards the end of the water year may reflect declining instrument performance and are not of concern.

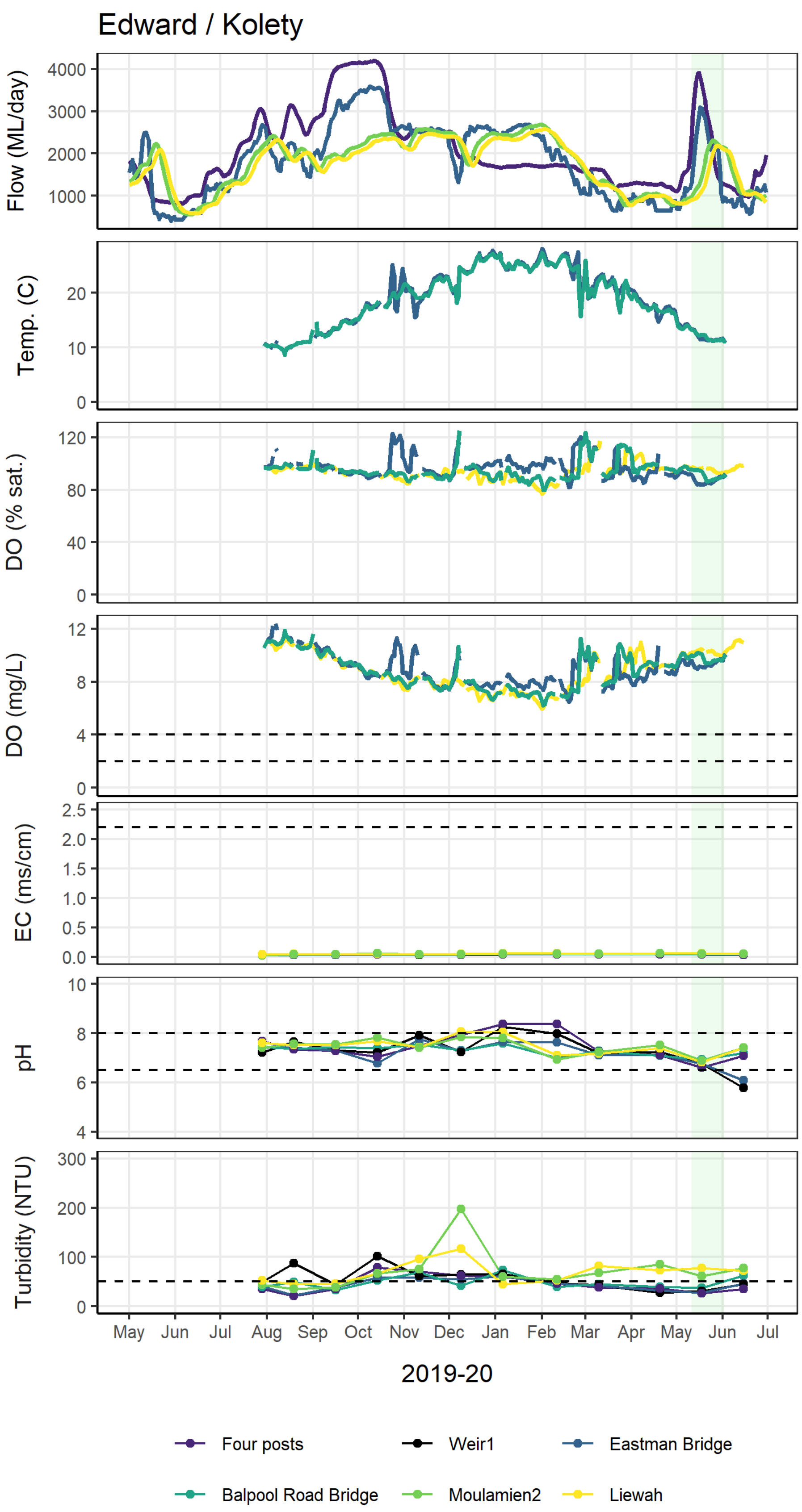


Figure 5.6 Daily average temperature results, daily dissolved oxygen (DO) concentrations, electrical conductivity (EC), turbidity and pH for the study sites and source water over the 2019-20 watering year in the Edward/Kolety River system.

Nutrients and dissolved organic carbon

In general, the range of DOC concentrations in the Edward/Kolety River was similar to the concentrations measured in the Wakool-Yallakool system in 2019-20 water year, with slightly lower nutrients levels. TP generally increased at downstream sites which is consistent with the pattern in TN and trends in Chl *a* (Figure 5.7).

Chl *a* concentrations remained stable and values were very similar between sites below 20 µg/L from July to December 2019. Increases in Chl *a* concentrations along the Edward/Kolety River between January and February corresponded with observed excessive algae growth, suggesting increases in photosynthesis which is quite common during the summer months with high water temperatures and light levels. Chl *a* concentrations declined between March and May 2020.

During 2019-20 the range of DOC concentrations in the Edward/Kolety River were similar to the Wakool-Yallakool system, remaining in the acceptable range. A slight increase in DOC concentrations occurred in February and March 2020 but these remained within the acceptable range, the timing of this pulse corresponds with the lowest DO concentrations observed over the water year (Figure 5.7). Elevated DOC in May 2020 indicates greater carbon inputs associated with the unregulated flows during this time where larger areas of Werai Forest were wetted and downstream sites had a later pulse. Large parts of the Edward/Kolety River in particular were quite dark and clearer (lower turbidity) than normal, but the oxygen in these sections was quite high and were not of concern. It is noted that the upstream site in the Edward/Kolety River had lower DOC than the downstream sites in this reach, however the results remain within the range of concentrations observed for the other sampling sites. This likely indicates that the pulse was of very brief duration and had already begun to clear from the top of the system at the time of sampling.

Small inputs of DOC to the river can help with supporting microbial productivity which become available food for aquatic organisms such as fish. Increased algal growth over the summer was insufficient to produce a substantial increase in the dissolved fraction of the organic matter in these river systems. It is noted that DOC increases did not all occur at the same time at all zones, suggesting there might be a separate source of DOC for the part of the system during this period.

Both TN and TP were increased corresponding with higher discharges in 2019-20 water year which was possibly caused by in-stream processes during the flow discharge keeping particles suspended (suspended particles keeping adsorbed nutrients in the water column). TP and TN concentrations fluctuated above and below the ANZECC (2000) trigger values of 0.05 mg/L and 0.5 mg/L respectively. There were generally lower concentrations in upstream sites than downstream sites suggesting slight increases in TP and TN as the water progresses through the system.

The bioavailable nutrient (FRP, NH4+, NOx) concentrations did not exceed ANZECC (2000) trigger values, with the exception of NOx on just one occasion during the unregulated flow in at Four Post in May 2020.

A pulse of DOC and nutrients from Millewa Forest was introduced to the Edward/Kolety-Wakool system in October 2019 through the Southern Connected Flow watering action in Murray River. The impact of the Southern Connected Flow watering action on the Edward/Kolety River system was slight and it is unlikely to be ecologically significant due to the change in DOC and nutrients concentrations was very small.

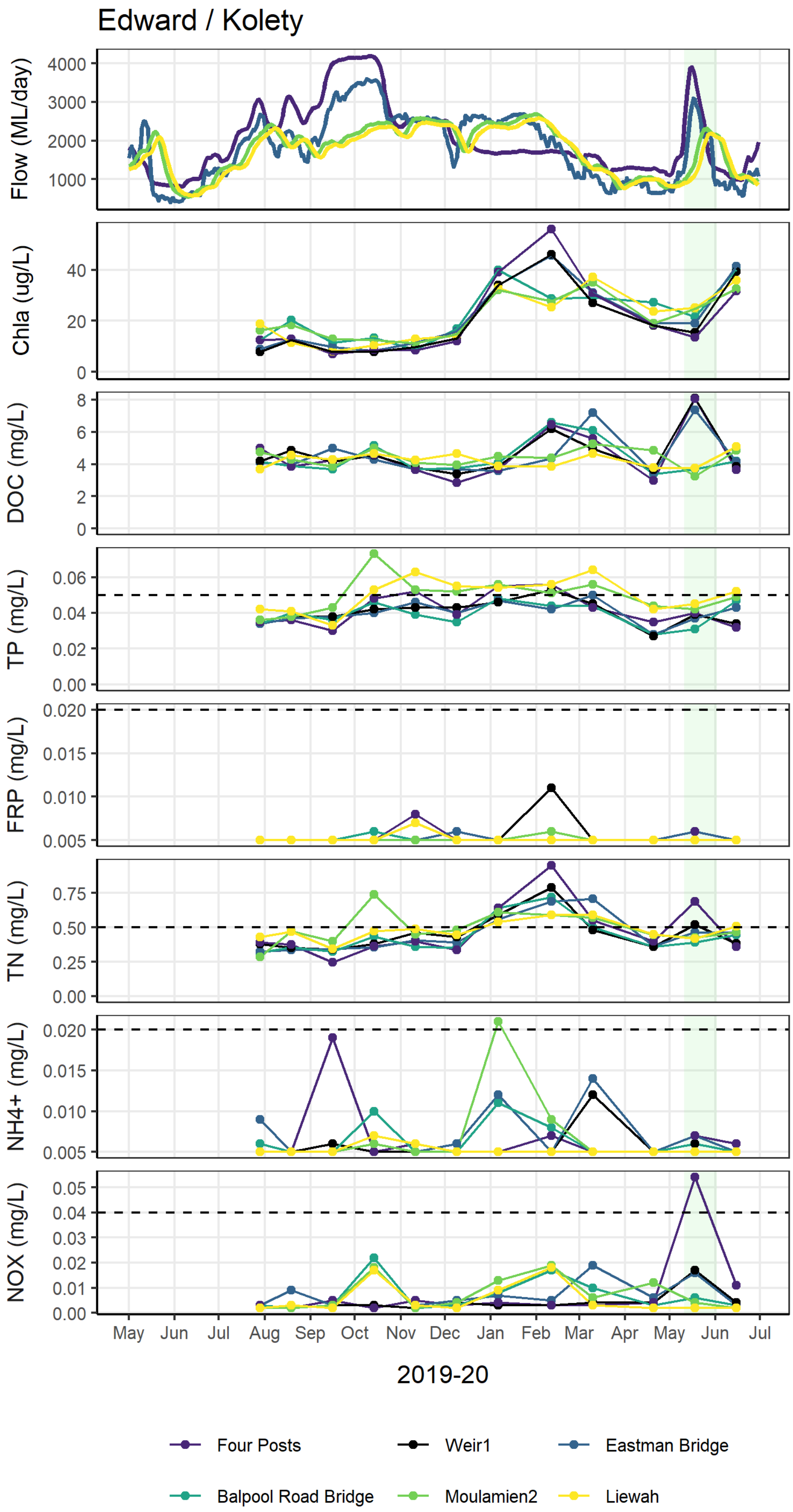


Figure 5.7 Chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), phosphorus (total phosphorus (TP) and filtered reactive phosphorus (FRP)), nitrogen (total nitrogen (TN), ammonium (NH_4^+) and dissolved nitrate + nitrite (NOx)) concentrations for the study sites and source water over the 2019-20 watering year in the Edward/Kolety River system.

The Colligen-Niemur system

Temperature and dissolved oxygen and spot water quality parameters

Water temperature in the Colligen-Niemur system at Old Morago Rd exceeded 25 °C briefly during summer and staying below 10 °C for a couple of weeks during winter (Figure 5.8). The results indicate that water temperature was influenced predominantly by seasonal rather than site-specific factors with all sites displaying the same seasonal variation and influence of weather patterns.

The plot of average daily DO concentrations in the study site at Old Morago Rd shows the expected seasonal variations with higher concentrations in the winter and lower concentrations correlating to the periods of higher water temperature. However, the water temperature in the study site at Old Morago Rd moderately increased between mid-October and early November 2019 with lowered DO concentrations, this probably resulted from receiving minor to no amount discharge during that period.

Concentrations of DO in the Colligen-Niemur system were above the range of concern to fish populations (below 4 mg/L) but generally were lower than the DO levels in the Edward/Kolety River. DO declined to below 5 mg/L during a period of very high temperature in late January, and this trend reversed as water temperatures cooled with a change in the weather conditions. Unregulated flow did not cause discernible influence on DO levels might due to cool water temperatures in winter.

Spot reading of samples sites within the Colligen-Niemur system were similar to the sites along the Edward/Kolety River remaining within the acceptable range throughout the study period of 2019-20. EC remained stable within the lower end of the range expected for lowland rivers indicating in ANZECC (2000). Turbidity measurements were slightly fluctuated above and below the ANZECC (2000) trigger level and values were very similar between sites with increasing turbidity in downstream sites.

Most pH values were within the acceptable range throughout the year and values were very similar between sites. The high pH value only occurred once in Niemur-Moulamein Rd Bridge in August 2019 and it was buffered immediately after receiving flow discharge. The greater range of pH results observed towards the end of the water year may reflect declining instrument performance and are not of concern.

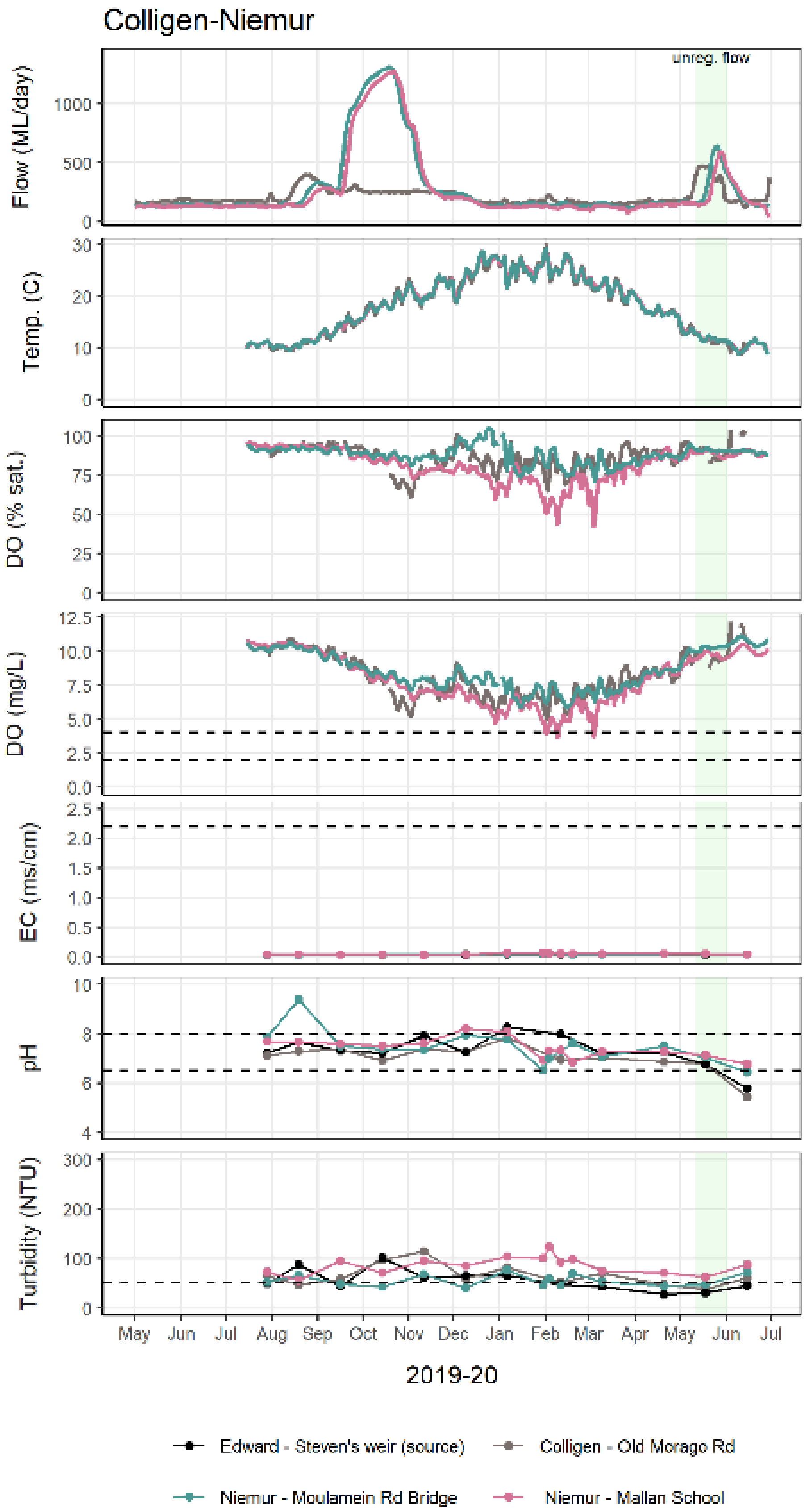


Figure 5.8 Daily average temperature results, daily dissolved oxygen (DO) concentrations, electrical conductivity (EC), turbidity and pH for the study sites and source water over the 2019-20 watering year in the Colligen-Niemur system.

Nutrients and dissolved organic carbon

In general, the range of nutrients and DOC concentrations in the Colligen-Niemur system was slightly higher than the concentrations recorded in the Edward/Kolety River in 2019-20 water year. TP was higher at downstream sites which is consistent with the pattern in TN and trends in Chl *a* (Figure 5.9). Concentrations of Chl *a*, DOC and TP and TN increased from December 2019 through to February 2020, probably due to low discharge during hot months.

Increased Chl *a* levels in the Colligen-Niemur system between January and February 2020 corresponded with observed poor water quality, suggesting increases in photosynthesis which is quite common during the summer months when water temperatures are high. A sharp rise in Chl *a* at the downstream site (Niemur-Mallan School) may have been associated with higher nutrient levels and low discharge during hot months.

Both TN and TP were increased corresponding with flow discharges in October and November 2019 which might have been associated with higher turbidity (suspended particles keeping adsorbed nutrients in the water column). TP and TN concentrations routinely exceeded the ANZECC (2000) trigger values of 0.05 mg/L and 0.5 mg/L respectively. There were generally lower concentrations in upstream sites than downstream sites suggesting slight increases in TP and TN as the water progresses through the system.

The bioavailable nutrient concentrations did not exceed ANZECC (2000) trigger values, with the exception of ammonia on one occasion during the unregulated flow at Mallan School in May 2020, possibly due to ammonia introduced from a disturbance upstream or disturbance of the sediments while sampling.

During 2019-20 the range of DOC concentrations in the Colligen-Niemur system was similar to the Edward/Kolety River remaining in the acceptable range. A slight increase in DOC concentrations occurred in February and March 2020 and the timing of this pulse corresponds with the lowest DO concentrations observed over the water year (Figure 5.9). Elevated DOC in May and June 2020 corresponding with observed dark coloured water indicates greater carbon inputs associated with the unregulated flows during this time where larger low-lying areas were wetted, but the oxygen in these sections was quite high and are not of concern.

A pulse of DOC and nutrients from Millewa Forest was introduced to the Edward/Kolety-Wakool system in October 2019 through the Southern Connected Flow watering action in the Murray River. The impact of Southern Connected Flow watering action on the Colligen-Niemur system was slight and it is unlikely to be ecologically significant as the change in DOC and nutrients concentrations was too small.

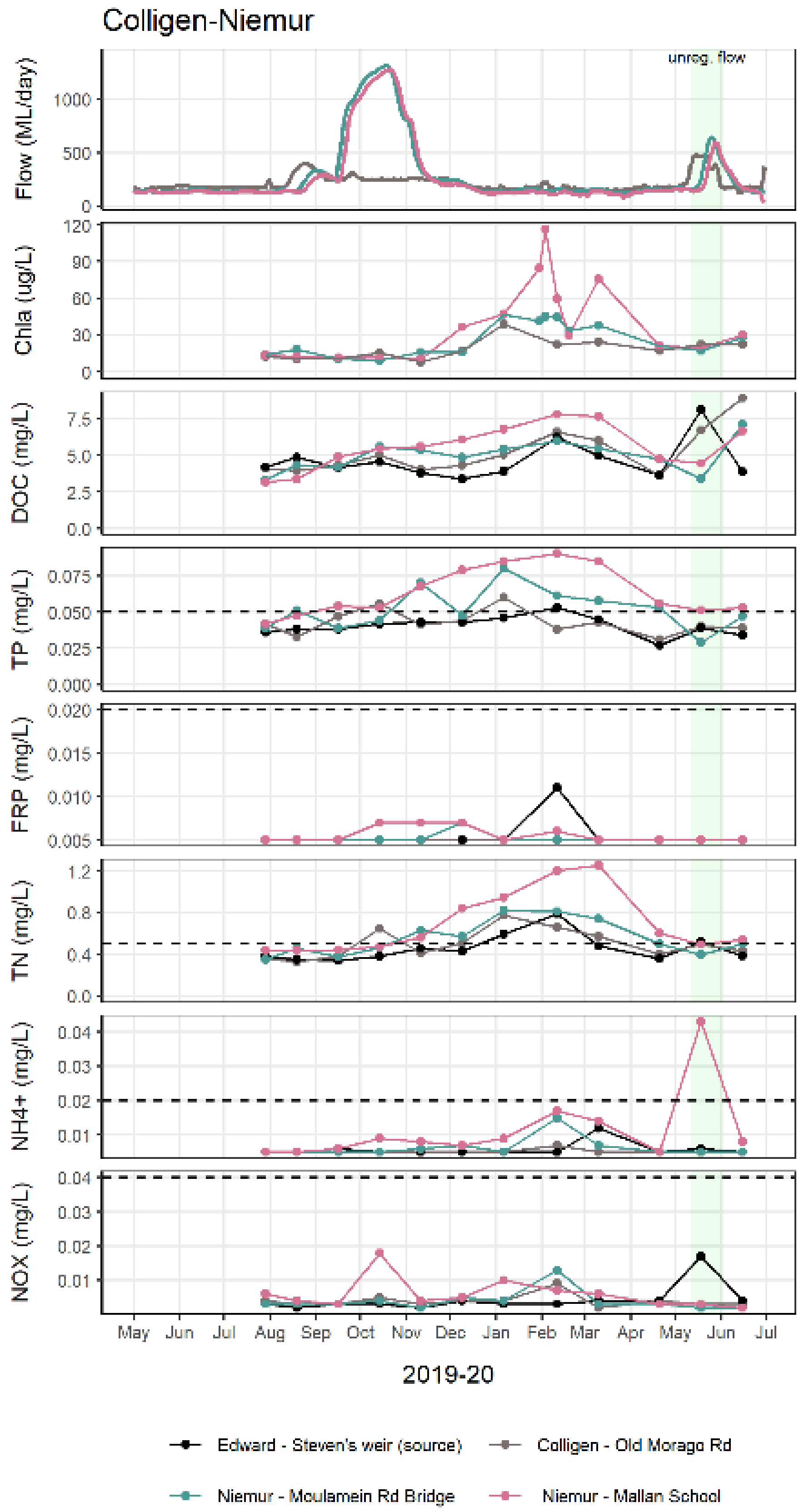


Figure 5.9 Chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), phosphorus (total phosphorus (TP) and filtered reactive phosphorus (FRP)), nitrogen (total nitrogen (TN), ammonium (NH_4^+) and dissolved nitrate + nitrite (NOx)) concentrations for the study sites and source water over the 2019-20 watering year in the Colligen-Niemur system.

Tuppal Creek

Temperature and dissolved oxygen and spot water quality parameters

Most of the spot readings at Aratula Road in Tuppal Creek remained within the acceptable range throughout the study period of 2019-20 (Figure 5.10). EC remained stable within the lower end of the range expected for lowland rivers indicating in ANZECC (2000). Turbidity measurements fluctuated above and below the ANZECC (2000) trigger level. Most pH values were within the acceptable range throughout the year. The greater range of pH results observed towards the end of the water year may reflect declining instrument performance and was not of concern. Concentrations of DO in the Tuppal Creek dropped into the range of concern to fish populations (below 4 mg/L) between October 2019 and mid-March 2020, values below 2 mg/L were briefly recorded in mid-October 2019, in early January 2020 and in early February 2020. Tuppal Creek is an ephemeral creek and received low base flows and DO remained quite low when there was no larger pulsed flows.

Nutrients and dissolved organic carbon

The nutrients and DOC concentrations in Tuppal Creek was generally higher than the concentrations recorded in the Edward/Kolety-Wakool system in 2019-20 water year. Tuppal Creek is an ephemeral creek and received low base flows interspersed by a few larger pulsed flows that can be the source of carbon and nutrients to the Edward/Kolety-Wakool River system if it’s connected to the main river channel.

The pattern of TP fluctuation is consistent with the pattern in TN and trends in Chl *a* in 2019-20 water year (Figure 5.11). Elevated nutrients, DOC and Chl *a* in Tuppal Creek in September 2019 suggests there were local sources of nutrients and DOC at times during this study period, possibly due to commence to flow conditions where water that was in backwaters or on low lying benches started to flow down the system. Concentrations of Chl *a*, DOC and nutrients did not keep increasing from December 2019 through to February 2020 probably due to higher discharge during these months. The increase in Chl a in Tuppal Creek in April 2020 may have been associated with higher concentrations of nutrients in the water.

There were some water pulses throughout the study year, and both TN and TP increased in Tuppal Creek during that period which were associated with higher turbidity (suspended particles keeping adsorbed nutrients in the water column). TP and TN concentrations consistently exceeded the ANZECC (2000) trigger values of 0.05 mg/L and 0.5 mg/L respectively.

The FRP and NOx remained below the ANZECC (2000) trigger values. The high ammonia values measured in Tuppal Creek on two occasions in February 2020 and May 2020 could possibly be due to ammonia introduced during flow peaks.

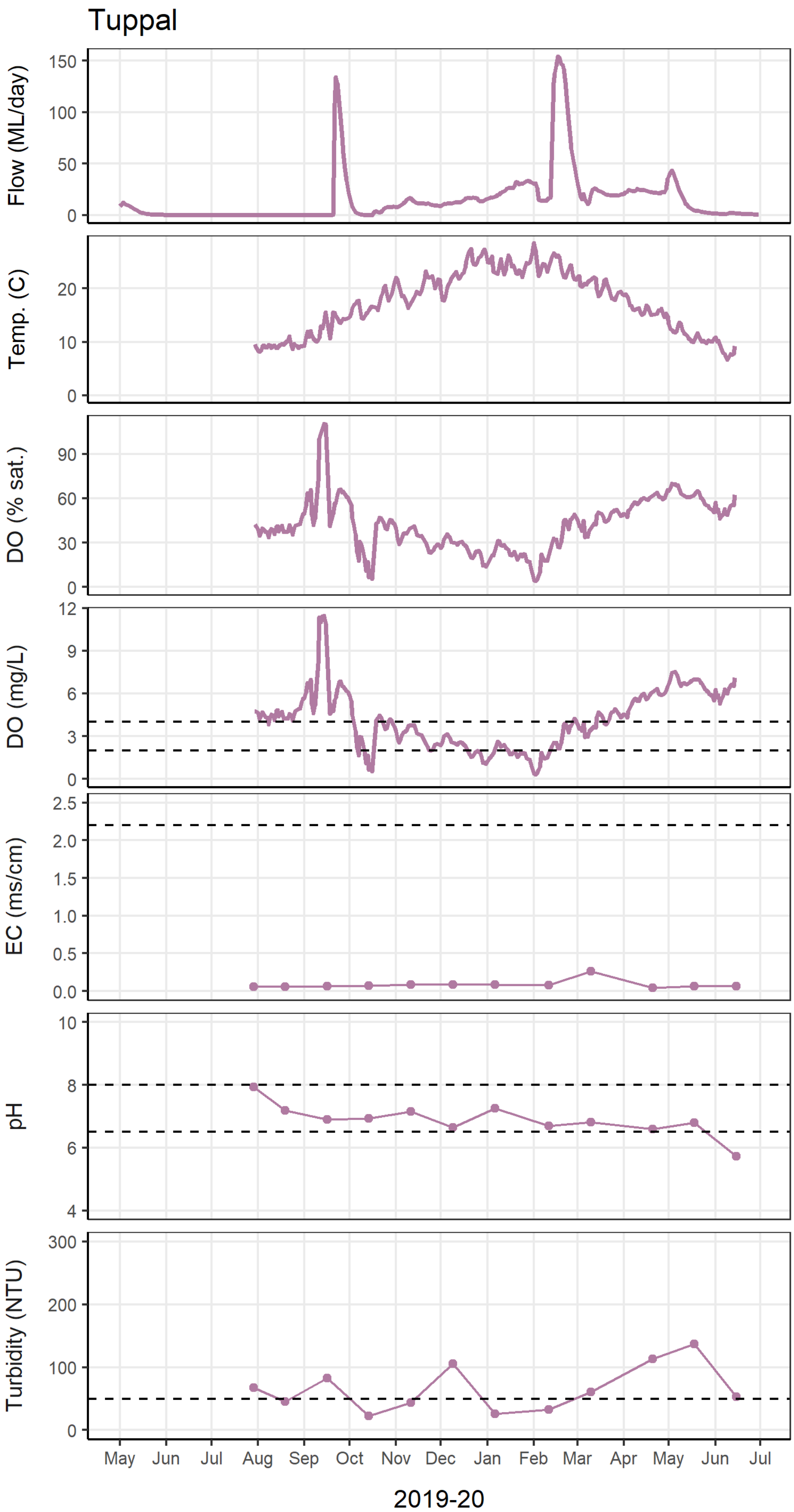


Figure 5.10 Daily average temperature results, daily dissolved oxygen (DO) concentrations, electrical conductivity (EC), turbidity and pH for the study sites and source water over the 2019-20 watering year in Tuppal Creek.

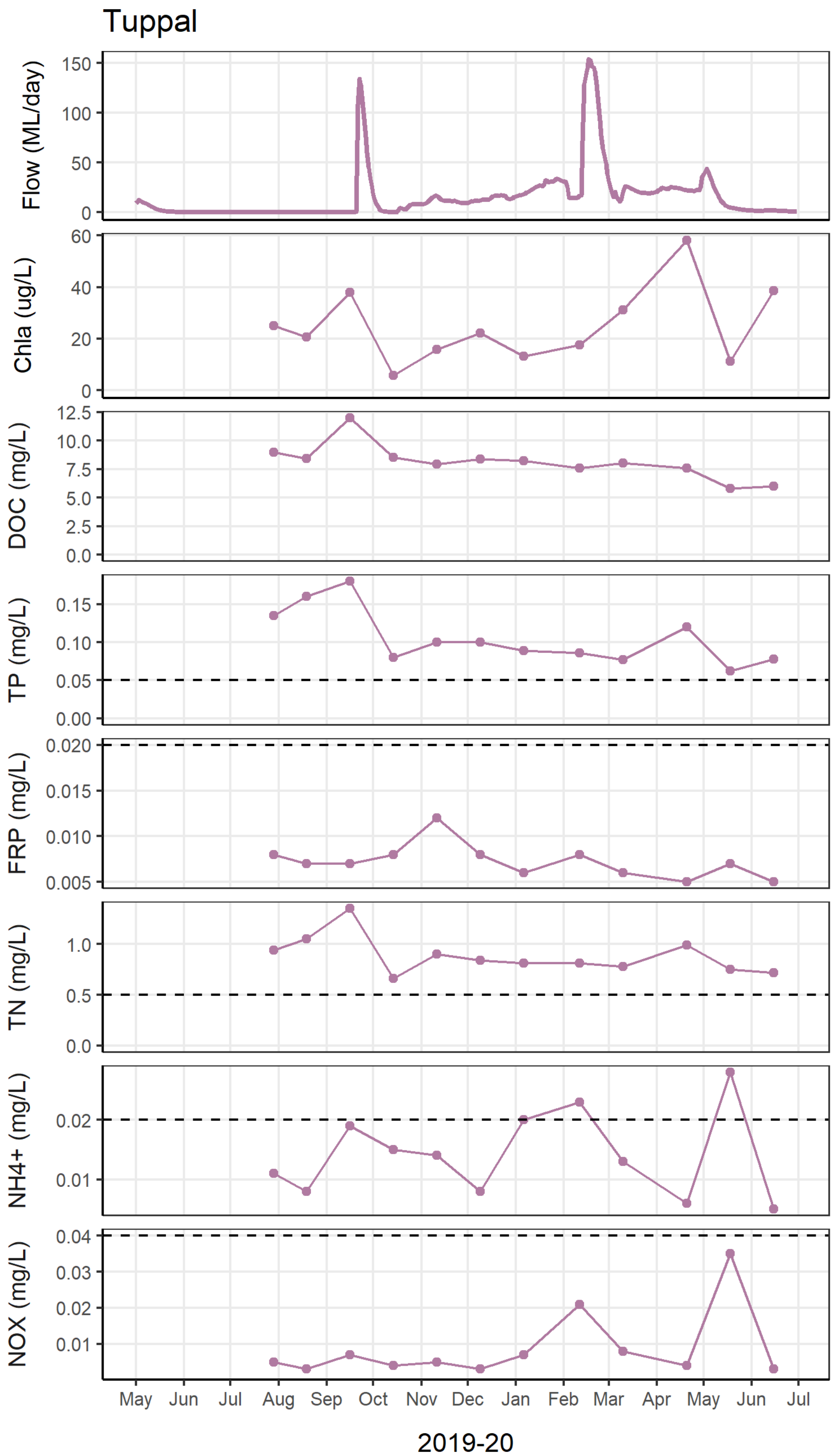


Figure 5.11 Chlorophyll *a* (Chl *a*), dissolved organic carbon (DOC), phosphorus (total phosphorus (TP) and filtered reactive phosphorus (FRP)), nitrogen (total nitrogen (TN), ammonium (NH_4^+) and dissolved nitrate + nitrite (NOx)) concentrations for the study sites and source water over the 2019-20 watering year in Tuppal Creek.

Organic matter characterisation

The Wakool-Yallakool system

Organic matter characterisation - Absorbance

The absorbance spectra for water samples collected from the Wakool-Yallakool system are shown in Figure 5.12. It is noted that during July 2019 the spectrum for the sample at Weir has a different shape to the other water samples. This may indicate either a localised water quality difference (algae, run off from the bank etc.) or sample contamination. The absorbance in zone 2 was slightly steeper (more small organic molecules) than at other sites, otherwise study zones are similar in organic matter composition. In October 2019 there is a trend towards increasing organic matter absorbance at downstream sites while in November 2019 this trend is reversed. This covers the period during the Southern Connected Flow in the Murray River pulse and may indicate a transfer of different organic carbon quality downstream. By January 2020 the absorbance spectra for water samples were very similar and through the summer the sites remain similar, with slightly higher absorbance at the most downstream sampling site. In February 2020 there is some increase in absorbance but the pattern between sites is maintained.

Organic matter characterisation - Fluorescence

Fluorescence excitation-emission matrices for water samples at the Wakool-Yallakool system through the sampling period are shown in Figure 5.13. As observed in the absorbance scans, the water sample collected from the Weir in July 2019 is different from all other sites and the region showing strong fluorescence indicates either organic material of different origin to the rest of the sites is present in the water (run-off or algal material) or sample contamination may be an issue. There was a discharge action in the Edward/Kolety River system between June and August 2019 and it peaked upstream of Edward/Kolety-Wakool system in July 2019. Organic material of different origin from runoff present in the water is the most likely explanation for this result. In October 2019 slightly higher fluorescence was observed at all sites with a very gradual increase downstream, consistent with the absorbance results. The downstream sites in zones 4, 5 and 6 were different to the other sites, also consistent with the absorbance results and the decreasing trend downstream in November 2019 is also evident here. The floating algae was observed in zone 2 in December 2019 and in January 2020, broadly similar fluorescence is present as a number of broad peaks distributed across the region between the two blue scatter lines. This is suggestive of a mixture of humic and fulvic substances and smaller fluorescent molecules, possibly a combination of aged organic matter and very fresh leachates or algal organic matter. Zone 3 has a similar distribution of peaks, zones 4, 5 and 6 have fluorescence more heavily dominated by aged organic matter, possibly suggesting floodplain organic matter inputs (e.g. reconnection of a billabong or low-lying floodplain). This is consistent with slight increases in DOC concentrations over that period. Fluorescence is generally low through summer and autumn although in February 2020 zone 2 clearly has a stronger humic and fulvic signature. Low water levels at this site may concentrate localised leaching of organic matter at this site.

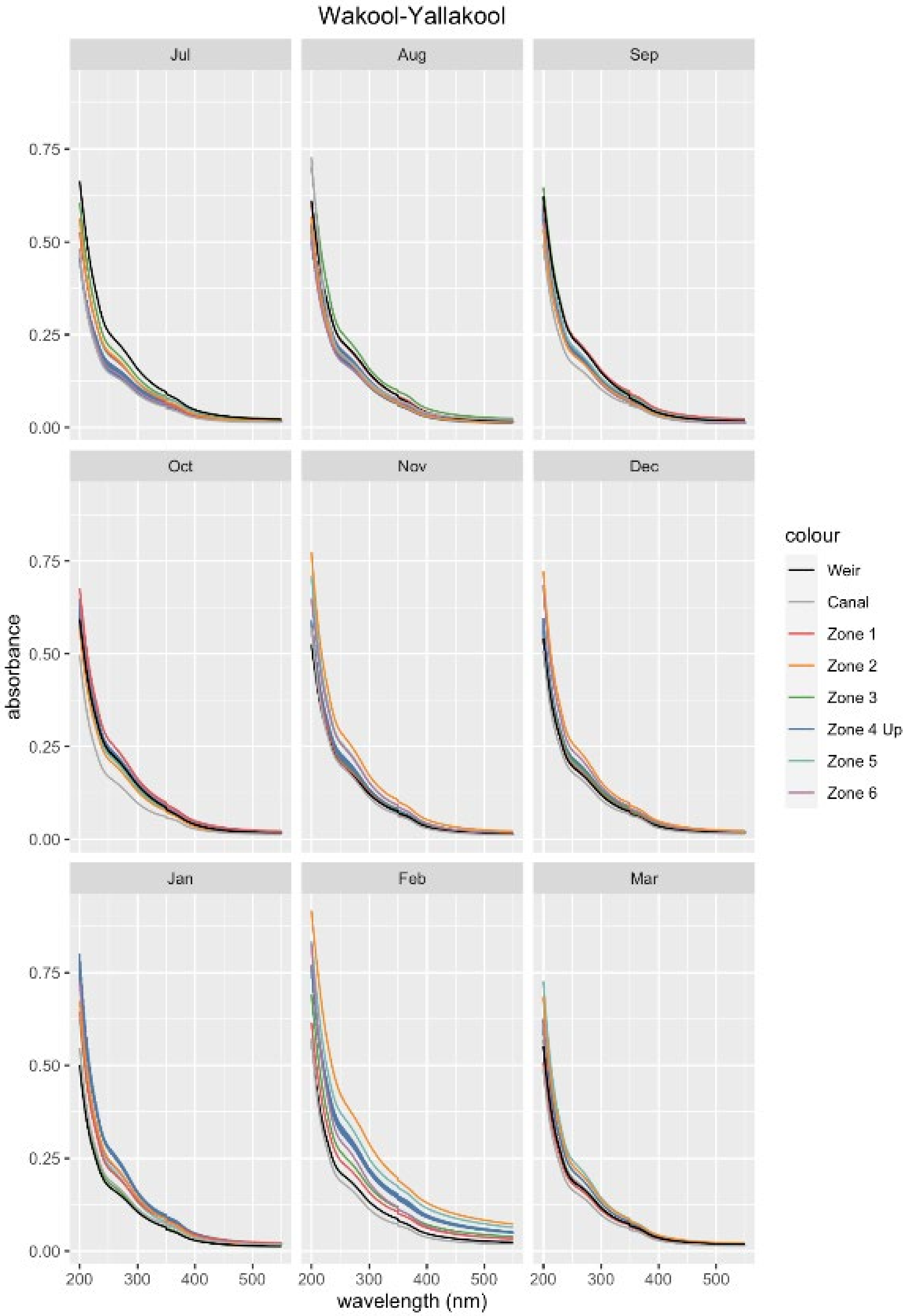


Figure 5.12 Absorbance of water samples at the Wakool-Yallakool system in 2019-20. The water samples for the assessment of organic matter inputs were collected from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions.

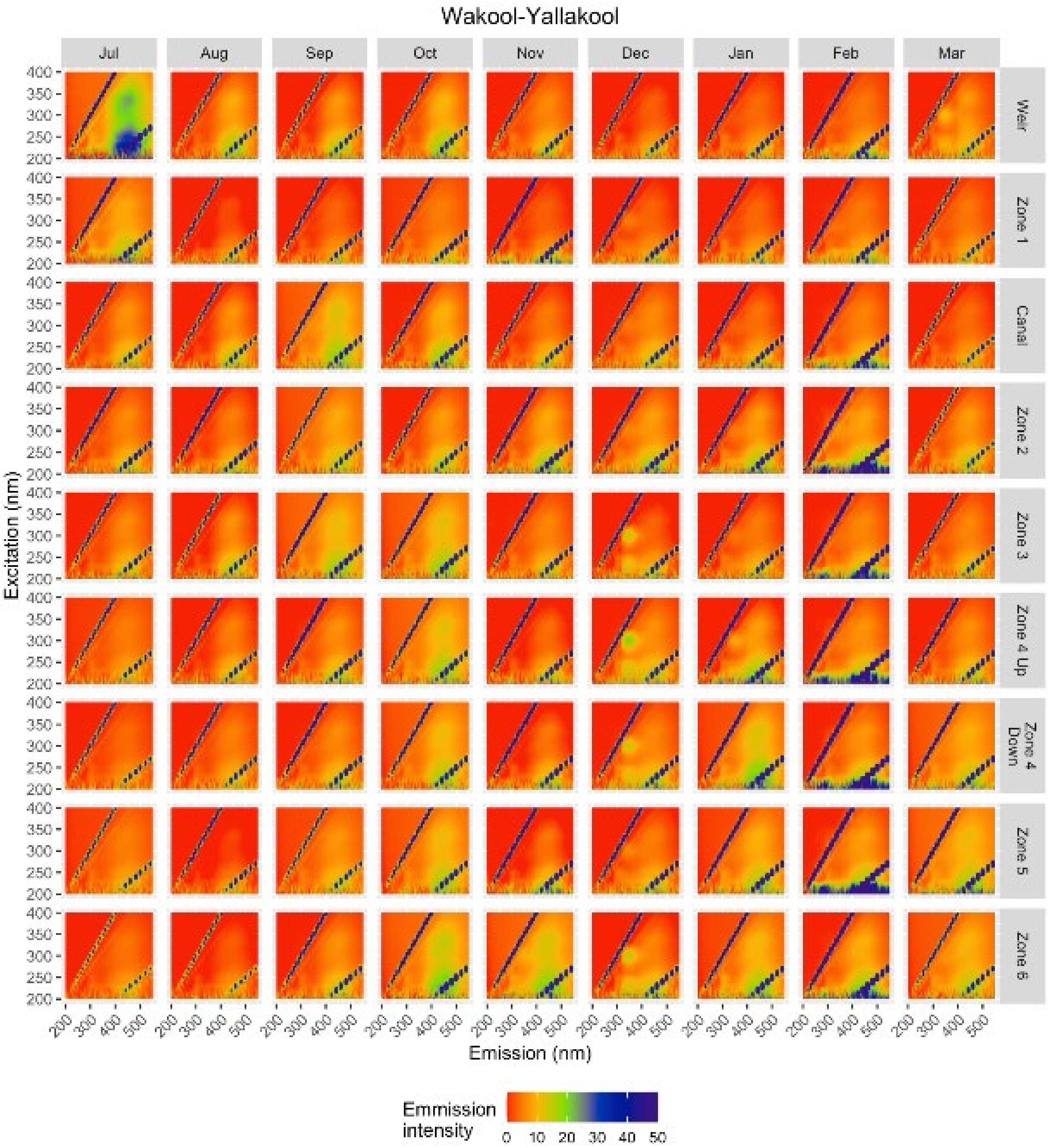


Figure 5.13 Fluorescence scans of water samples from the Wakool-Yallakool system in 2019-20. The water samples for the assessment of organic matter inputs were collected from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions.

The Edward/Kolety River

Organic matter characterisation - Absorbance

Absorbance scans (Figure 5.14) indicate that throughout most of the 2019-20 water year the mixture of organic compounds making up the DOC was fairly consistent across sites with no clear upstream/downstream trends in variation between the scans in the Edward/Kolety River. It is noted that during July 2019 the spectra for the samples at Weir and Four Post have different shapes to the other water samples. This may indicate either a localised water quality difference (algae, run off from the bank etc.) or sample contamination. In October 2019 there is a slight trend towards increasing organic matter absorbance at all sites. This covers the period during the Southern Connected Flow in the Murray River pulse and may indicate a transfer of different organic carbon quality downstream. By December 2019 the absorbance spectra for water samples were very similar and through the summer the sites remain similar, with slightly higher absorbance at the most downstream sampling site. In February 2020 there is some increase in absorbance but the pattern between sites is maintained.

Organic matter characterisation - Fluorescence

Fluorescence excitation-emission matrices for water samples at the Edward/Kolety River through the sampling period are shown in Figure 5.15. As observed in the absorbance scans, the water samples collected from the Weir, Four Post and Eastman Bridge in July 2019 are different from all other sites and the region showing strong fluorescence indicates either organic material of different origin to the rest of the sites is present in the water (run-off or algal material) or sample contamination may be an issue. There was a discharge action in the Edward/Kolety River between June and August 2019 and it peaked at upstream of the Edward/Kolety River around the sampling time in July 2019. Organic material of different origin from runoff present in the water is the most likely explanation for this result. In October 2019 slightly higher fluorescence was observed at all sites with a very gradual increase downstream, consistent with the absorbance results. Broadly similar fluorescence is present in downstream sites of Edward/Kolety River (Moulamein and Liewah) in December 2019, as a number of broad peaks distributed across the region between the two blue scatter lines. This is suggestive of a mixture of humic and fulvic substances and smaller fluorescent molecules, possibly a combination of aged organic matter and very fresh leachates or algal organic matter. Fluorescence is generally low through summer and autumn although in February 2020 the downstream Werai Forest (Balpool Bridge) has different fluorescence signature which may reflect algal carbon present at this sampling site. This is consistent with a slight increase in DOC and Chl *a* concentration.

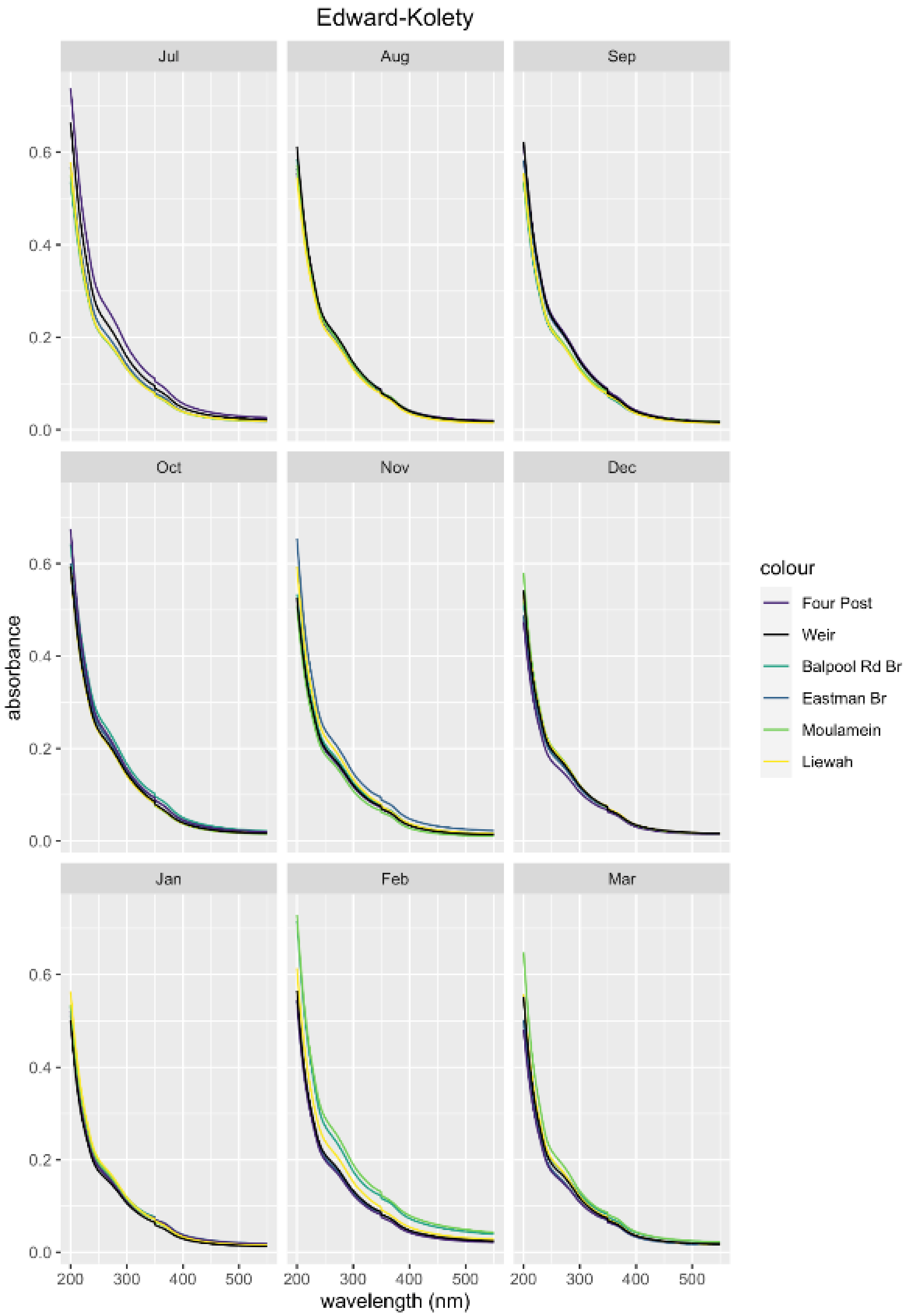


Figure 5.14 Absorbance of water samples at the Edward/Kolety River system in 2019-20. The water samples for the assessment of organic matter inputs were collected from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions

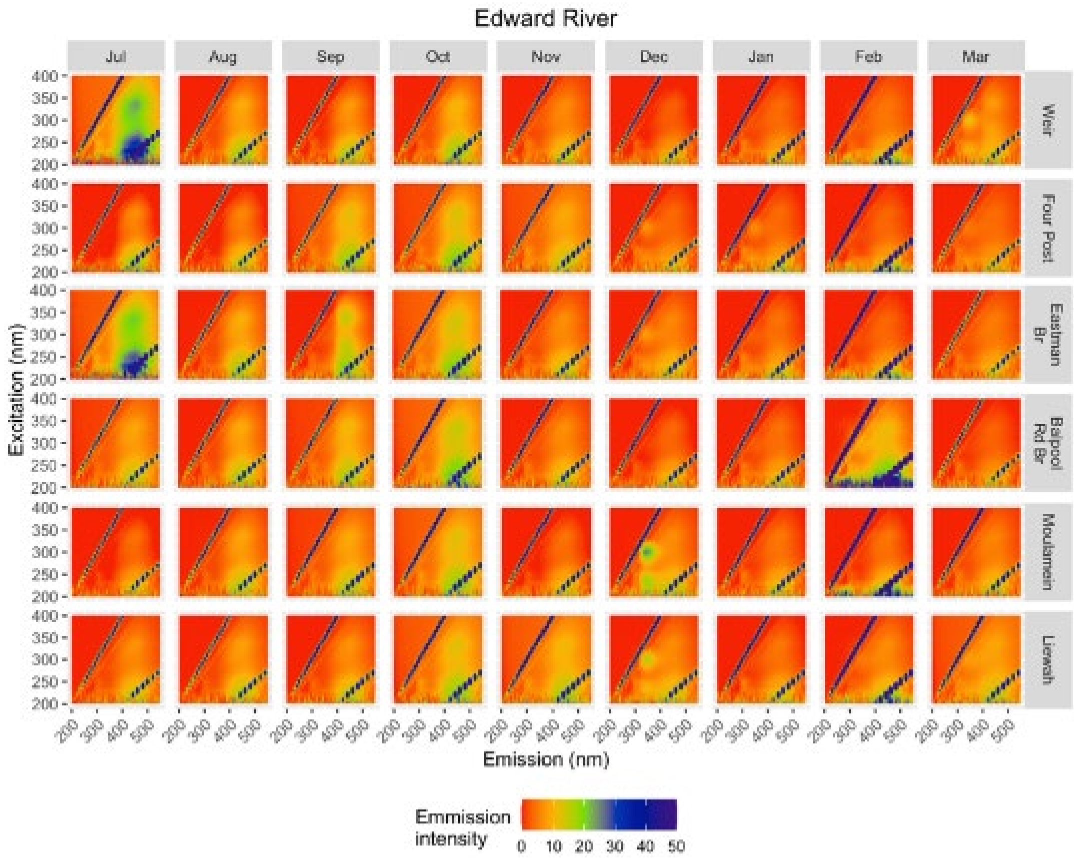


Figure 5.15 Fluorescence scans of water samples from the Edward/Kolety River in 2019-20. The water samples for the assessment of organic matter inputs were collected from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions.

The Colligen-Niemur River system

Organic matter characterisation - Absorbance

Absorbance scans (Figure 5.16) indicate that throughout most of the 2019-20 water year the mixture of organic compounds making up the DOC was fairly consistent across sites with no clear upstream/downstream trends in variation between the scans in the Colligen-Niemur River system. It is noted that during July 2019 the spectrum for the sample at Weir has different shape to the other water samples. This may indicate either a localised water quality difference (algae, run off from the bank etc.) or sample contamination. In October 2019 there is a trend towards increasing organic matter absorbance at all sites and downstream sites experienced longer periods than upstream sites. This covers the period during the Southern Connected Flow in the Murray River pulse and may indicate a transfer of different organic carbon quality from Edward/Kolety River (source water) to the Colligen-Niemur River system. By December 2019 the absorbance spectra for water samples were very similar and through the summer the sites remain similar, with slightly higher absorbance at the most downstream sampling site. In February 2020 there is some increase in absorbance but the pattern between sites is maintained.

Organic matter characterisation - Fluorescence

Fluorescence excitation-emission matrices for water samples at all sites through the sampling period (Figure 5.17) indicate that the organic matter mix was similar across sites at the Colligen-Niemur

River system in the 2019-20 water year. As observed in the absorbance scans, the water sample collected from the Weir at Edward/Kolety River (source water) in July 2019 is different from all other sites and the region showing strong fluorescence indicates either organic material of different origin to the rest of the sites is present in the water (run-off or algal material) or sample contamination may be an issue. As mentioned earlier, organic material of different origin from runoff present in the water is the most likely explanation for this result. In October 2019 clearly higher fluorescence was observed at all sites with a very gradual increase downstream, consistent with the absorbance results. Fluorescence is generally low through summer and autumn although monitoring sites at Niemur River (Niemur Moulamein Rd Bridge and Niemur Mallan School) in February 2020 clearly have stronger humic and fulvic signature which may reflect algal carbon present at these sampling sites. Floating algae was observed at these two sites and this is consistent with a slight increase in DOC and Chl *a* concentration in these sites.

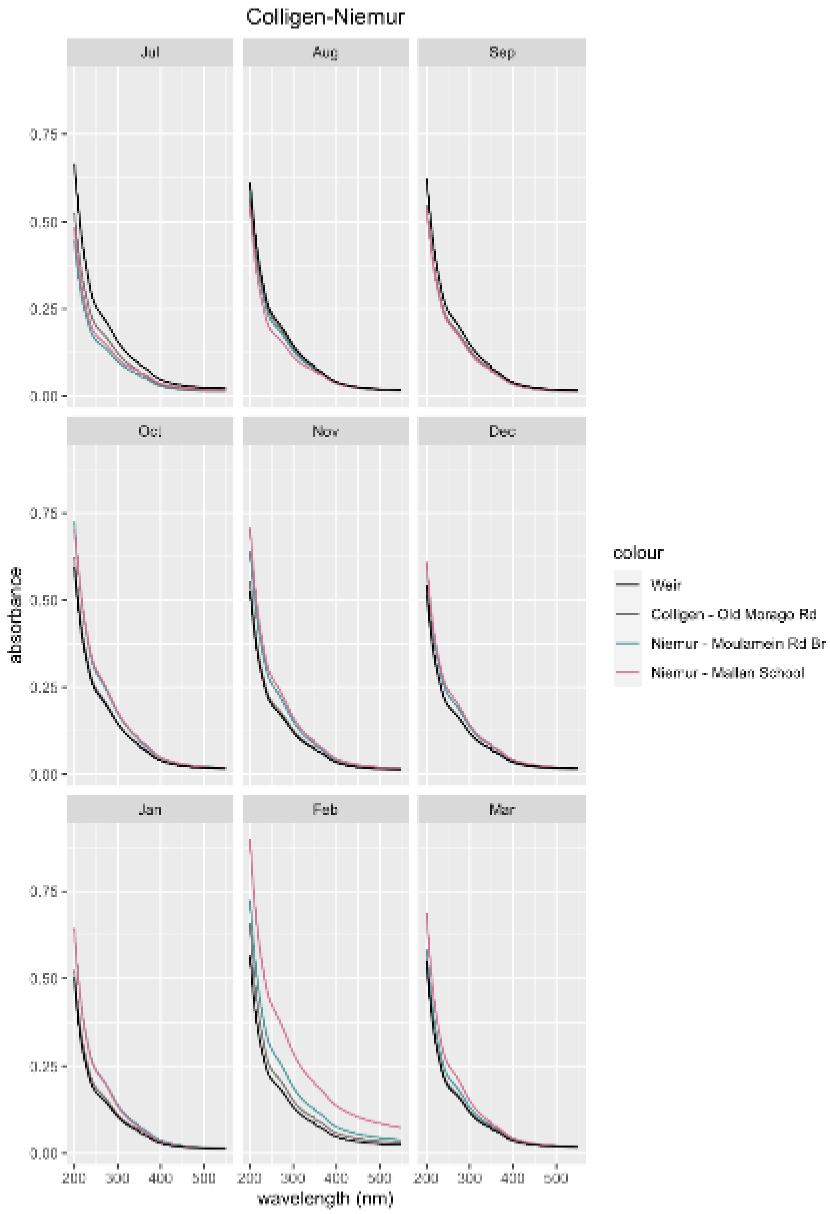


Figure 5.16 Absorbance of water samples at the Colligen-Niemur River system in 2019-20. The water samples for the assessment of organic matter inputs were collected from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions.

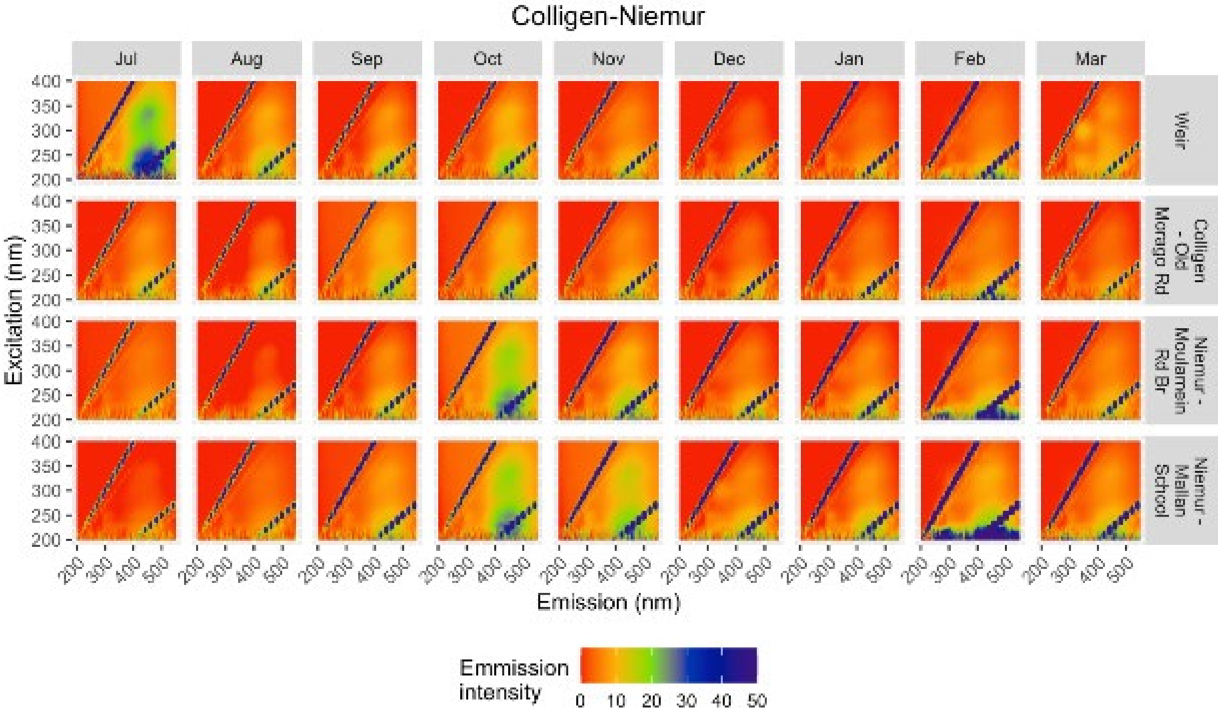


Figure 5.17 Fluorescence scans of water samples from the Colligen-Niemur River system in 2019-20 study season. The water samples for the assessment of organic matter inputs were collected from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions.

Tuppal Creek

Organic matter characterisation - Absorbance

Absorbance scans (Figure 5.18) indicate that the mixture of organic compounds making up the DOC was consistent in Tuppal Creek throughout most of the 2019-20 water year. In February 2020 there is a slight increase in absorbance, but the pattern is maintained.

Organic matter characterisation - Fluorescence

Fluorescence excitation-emission matrices for water samples at Tuppal Creek through the sampling period (Figure 5.19) indicate that the organic matter mix was similar at Tuppal Creek system with stronger humic and fulvic signatures. September 2019 has the strongest humic and fulvic signature which is consistent with an increase in DOC concentration in Tuppal Creek.

In February 2020, a number of broad peaks distributed across the region between the two blue scatter lines. This is suggestive of a mixture of humic and fulvic substances and smaller fluorescent molecules. It is possibly a combination of aged organic matter and very fresh leachates or algal organic matter which is consistent with receiving a discharge during period.

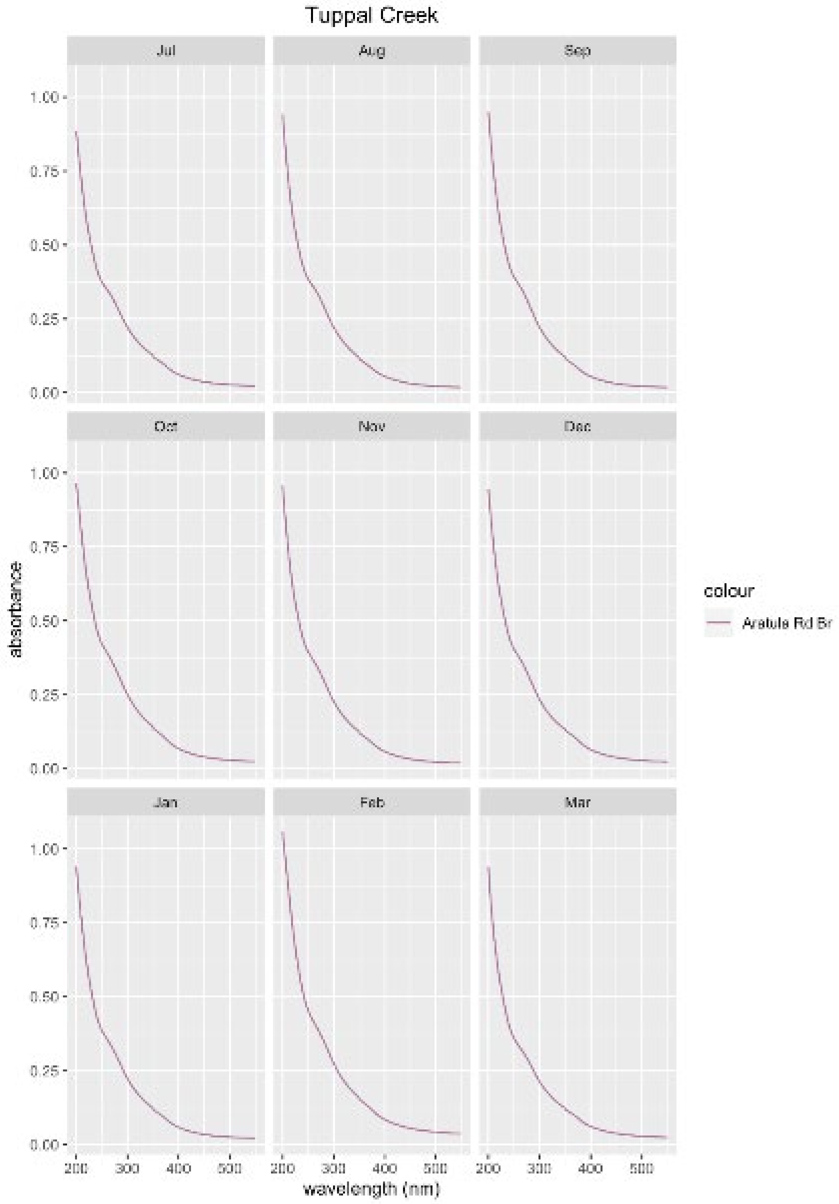


Figure 5.18 Absorbance of water samples at Tuppal Creek in 2019-20. The water samples for the assessment of organic matter inputs were collected from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions.

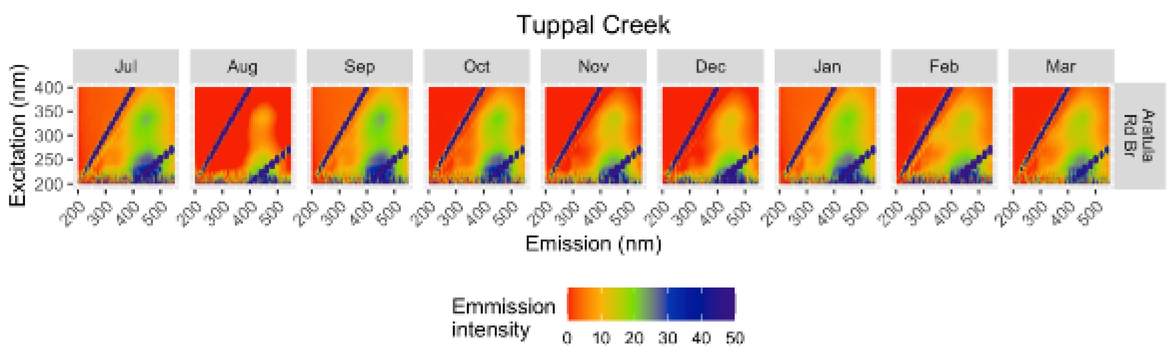


Figure 5.19 Fluorescence scans of water samples from Tuppal Creek in 2019-20 study season. The water samples for the assessment of organic matter inputs were collected from July 2019 to March 2020 and the data are not available for April, May and June 2020 due to COVID-19 travel restrictions.

5.7 Discussion

Short and long-term evaluation questions for core monitoring

Overall the water quality in the Edward/Kolety-Wakool Selected Area during the 2019-20 water year was characterised by normal conditions (similar to 2014-15, 2017-18 and 2018-19) following two extreme events (the 2015-16 cyanobacteria bloom and the 2016-17 hypoxic blackwater event).

In 2019-20 the key questions relating to the CEW actions were:

- What did Commonwealth environmental water contribute to dissolved oxygen concentrations?

For Wakool-Yallakool system, zones 1, 3 and 4 received more environmental water than Wakool River zone 2. Commonwealth environmental water maintained DO concentrations in zones 1, 3 and 4 during winter 2019 to spring and early summer. Zones receiving environmental water had higher dissolved oxygen concentrations than that in zone 2. DO concentration was consistently higher during late summer and autumn to winter 2020 in zones 1, 3 and 4. This difference among zones with and without environmental water persisted beyond the end of the watering action, thus Commonwealth environmental water assisted in the maintenance of dissolved oxygen concentrations over the summer period in the zones receiving the additional flow.

It needs to be noted that it is common for DO to be lower in zone 2 than the other study sites during summer when discharge is much lower in zone 2 and the risk of temperature induced hypoxia during heatwaves is greater in this part of the system. However the difference between zone 2 and the other study zones was less in 2018-19 than was commonly observed in other years (Watts et al. 2019) and the period where DO was close to 4 mg/L was shorter, likely due to the higher discharge in this zone than in other years. The higher than usual flows in zone 2 in 2019-20 demonstrate that there is potential to use Commonwealth environmental water to improve water quality in this part of the system in the future.

- What did Commonwealth environmental water contribute to nutrient concentrations?

Nutrient concentrations remained within the expected range throughout the Edward/Kolety-Wakool River system during the 2019-20 water year. The absence of overbank flows meant that substantial nutrient inputs were not expected in the system, although a general downstream increase in TN and TP were observed in the zones which received the majority of flow. TP and TN were slightly elevated over watering actions, possibly caused by higher turbidity and in-stream processes during the watering action. TN and TP were generally higher in the Wakool River zone 2 (receiving minor to no amount environmental water) during the environmental watering action, suggesting either dilution of these nutrients by Commonwealth environmental water at the other study sites, or that conditions in Zone 2 favoured the retention of nutrients associated with organic matter or particulates (e.g. algal cells) within the water column. Bioavailable nutrient remained low and were similar across study sites and do not appear to have been influenced by Commonwealth environmental water.

Chl *a* content is closely associated with nutrient concentrations and acts as an indicator of trophic status in freshwater systems. Chl *a* levels fluctuate naturally over time and higher concentrations are common during the summer months when water temperature and light level are higher. The increase in the concentrations of TP and TN in the summer months with no water actions is accompanied by the consistently higher Chl *a* concentrations observed throughout the system.

The MER project 2019-20 study season was the first time that monitoring of organic matter inputs and water quality changes were monitored across the whole year, including winter. Water samples were collected on a monthly basis between July 2019 and June 2020. Thus we don't have water quality data (nutrients, DOC, Chl a) during the unregulated flow event in May 2019 and are unable to discuss the water quality during that action.

- What did Commonwealth environmental water contribute to modification of the type and amount of dissolved organic matter through reconnection with previously dry or disconnect in-channel habitat?

There was no detectable effect of Commonwealth environmental watering actions on this indicator in 2019-20. The type and amount of DOC in the system was similar to previous years where blackwater and major algal blooms were not present. Poor water quality was observed in the Wakool-Yallakool system in summer during heatwaves. This corresponds with a slight increase in DOC concentration but these remained within the normal range.

It is common for DOC concentration to be higher during summer and early autumn in zone 2 (receiving minor to no amount of environmental water). The generally lower flow in Zone 2 means the risk of temperature induced hypoxia during heatwaves is greater in this part of the system. As shown in Watts et al. (2019), the DOC concentration in zone 2 was lower than the other study zones in February 2019, likely due to the higher discharge in this zone than in other years. Typically, flow is extremely low in zone 2 over the summer, the higher than usual flow conditions demonstrate that there is potential to use Commonwealth environmental water to improve water quality in this part of the system in the future.

Broader system monitoring

Water quality monitoring across the broader Edward/Kolety-Wakool system in 2019-20 water year expanded the monitoring further downstream in the Wakool River and to Tuppal Creek, the Edward/Kolety River, and the Colligen-Niemur River system to better capture the impact of environmental water in the broader system.

Concentrations of DO in the Edward/Kolety River system and the Colligen-Niemur River system were above the range of concern to fish populations (below 4 mg/L) over the study season. It shows the expected seasonal variations with higher concentrations in the winter and lower concentrations correlating to the periods of higher water temperature. During 2019-20 the range of DOC concentrations in the Edward/Kolety River system and Colligen-Niemur system was quite similar remaining in the acceptable range. A slight increase in DOC concentrations occurred in February and March 2020 but these remained within the acceptable range, the timing of this pulse corresponds with the lowest DO concentrations observed over the 2019-20 water year. Elevated DOC in May 2020 corresponding with observed dark coloured water indicates greater carbon inputs associated with the unregulated flows but the oxygen in these sections was quite high and are not of concern. A pulse of DOC and nutrients from the Barmah-Millewa Forest was introduced to the Edward/Kolety-Wakool River system in October 2019 through the Southern Connected Flow watering action in Murray River. The impact of this watering action on the Edward/Kolety-Wakool River system was slight and the change in DOC and nutrients concentrations was small.

Evaluation questions for targeted contingency monitoring

The remaining question was not addressed as these conditions required to generate blackwater were not present in the Edward/Kolety-Wakool River system during this 2019-20 water year.

- What did Commonwealth environmental water contribute to reducing the impact of hypoxic blackwater or other adverse water quality events in the system?

Links to other indicators

Water quality and river flows are fundamentally linked. Water quality can be positively and negatively influenced by river flows and this can directly or indirectly influence productivity, aquatic vegetation and aquatic organisms including fish.

Small inputs of DOC to the river can support microbial productivity which become available as food for aquatic organisms such as fish. As mentioned in Section 6, watering actions in 2019-20 water year had a beneficial effect on stream metabolism. Small pulses of DOC were introduced to the Edward/Kolety-Wakool River system over watering action events in 2019-2020. Input of DOC to the river system during watering action 3 was more than in watering action 1, because higher flow events can result in exchange of large amounts of nutrients and carbon between the river and the in-channel geomorphic features. This corresponds with that carbon production and consumption during watering action 3 was higher than in watering action 1. Under certain temperature and flow conditions the input of DOC from large scale events can have the positive outcome of increasing productivity in the river ecosystem. However, large scale events also have the potential to result in negative outcomes. For example, an extensive unregulated overbank flooding event in 2016 inundated the Edward/Kolety-Wakool floodplain (including forested areas, cropping and grazing land and urban areas) and introduced considerable quantities of DOC into the river system, causing a widespread hypoxic blackwater event that resulted in the death of native fish.

6 STREAM METABOLISM

Authors: Nick Bond and Andre Siebers

Key findings	
Gross Primary Production (GPP)	Watering actions did not substantially affect areal rates of gross primary productivity (GPP)(mg $O_2/m^2/day$), which largely followed seasonal trends. However, when GPP was calculated as the amount of organic carbon ('fish food') produced per day (kg C/day) then watering actions were shown to have a beneficial effect (more 'food' is better). The size of the beneficial impact was largely related to the proportion of total flow that came from the watering action, with greater proportional effects of environmental water in winter low-flow periods. Carbon production was enhanced by between 15% and 278% during the watering actions, with a median across all sites and watering actions of 50% more carbon produced during Commonwealth environmental watering actions compared to no Commonwealth environmental water.
Ecosystem Respiration (ER)	As with GPP, areal rates of ecosystem respiration (ER)(mg $O_2/m^2/day$) were largely driven by seasonal trends. However, when ER was calculated as the amount of organic carbon consumed per day (kg C/day), then watering actions had a beneficial effect. A higher amount of organic carbon consumed means more nutrient recycling and hence greater nutrient supply to fuel GPP. Carbon consumption was enhanced by between 18% and 263% during the watering actions, with a median across all sites and watering actions of 51% more carbon consumed during Commonwealth environmental watering actions compared to no Commonwealth environmental water.

6.1 Background

Whole stream metabolism measures the production and consumption of dissolved oxygen (DO) gas, which occur as a result of the key ecological processes of photosynthesis and respiration (Odum 1956). Healthy aquatic ecosystems need both processes to generate new biomass, which becomes food for organisms higher up the food chain, and to break down plant and animal detritus and to recycle nutrients to enable growth to occur. Hence metabolism assesses the energy base underpinning aquatic food webs. The relationships between these processes are shown in Figure 6.1.

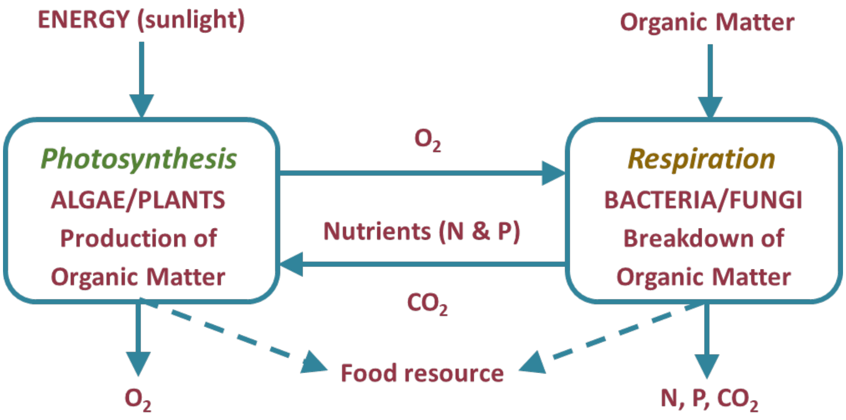


Figure 6.1 Relationships between photosynthesis, respiration, organic matter, dissolved gases and nutrients

Metabolism is expressed as the increase through photosynthesis or decrease through respiration of DO concentration over a given time frame; most commonly expressed as the change in milligrams of DO per litre per day (mg O_2/L/day). Typical rates of primary production and ER range over two orders of magnitude, from around 0.2 to 20 mg O_2/L/day, with most measurements falling between 2–20 mg O_2/L/day (Bernot *et al.*, 2010; Marcarelli *et al.*, 2011).

If process rates are too low, this will limit the amount of food resources (bacteria, algae and water plants) for consumers. This limitation will then constrain populations of larger organisms including fish and amphibians. Rates are expected to vary on a seasonal basis as warmer temperatures and more direct, and longer hours of, sunlight contribute to enhancing primary production during summer and into early autumn. Warmer temperatures and a supply of organic carbon usually result in higher rates of ER (Roberts, Mulholland & Hill, 2007).

In general, there is concern when process rates are too high. Greatly elevated primary production rates usually equate to algal bloom conditions or excessive growth of plants, which may block sunlight penetration, killing other submerged plants, produce algal toxins and large diel DO swings - overnight elevated respiration rates can decrease DO to the point of anoxia (no DO in the water). When an algal bloom collapses, the large biomass of labile organic material is respired, often resulting in extended anoxia. Very low or no DO in the water can result in fish kills and unpleasant odours.

Sustainable rates of primary production will primarily depend on the characteristics of the aquatic ecosystem. Streams with higher concentrations of nutrients especially those with very open canopies, and hence a lot of sunlight reaching the water, will have much higher natural rates of primary production than forested streams, where rates might be extremely low due to heavy shading and low concentrations of nutrients. Habitat availability, climate and many other factors also influence food web structure and function. Uehlinger (2000) demonstrated that freshes with sufficient stream power to cause scouring can ‘reset’ primary production to very low rates which are then maintained until biomass of primary producers is re-established. These scouring freshes are normally found in high gradient streams and are considered unlikely to occur in lowland streams such as those in the Edward/Kolety-Wakool system.

This chapter reports on stream metabolism in response to flows in the 2019-20 water year and will consider changes in GPP and ER in the system in response to watering actions.

6.2 Environmental watering actions in 2019-20

Three Commonwealth environmental watering actions were delivered in the Edward/Kolety-Wakool system in 2019-20 (Section 4). The response of stream metabolism to two of these watering actions (Table 6.1) was evaluated. Watering action 2 was not evaluated as it was a short (18 day) transition period between watering actions 1 and 3.

Table 6.1 Environmental watering actions assessed for ecosystem metabolism in the Edward/Kolety-Wakool system in 2019-20

	Watering action	Type of action	Dates	Rivers
1	Winter base flow	Minimum base flow (200 ML/day)	15/05/19 - 9/08/2019	Yallakool Creek, mid- and lower Wakool River, Colligen Creek-Niemur River
3	Spring-summer freshes and elevated base flow	a) Winter / spring early fresh b) Early spring elevated base flow c) Late spring fresh d) Late spring elevated base flow e) Recession	28/08/19 - 22/12/19	Yallakool Creek, mid- and lower Wakool River, Colligen Creek-Niemur River

6.3 Selected area questions

The Edward/Kolety-Wakool MER reports follow the previous Long-term Intervention Monitoring (LTIM) Selected Area evaluation of stream metabolism responses to Commonwealth environmental water delivery (Watts et al. 2019). The questions addressed addresses the importance of new organic (plant) matter, created through photosynthesis, supplying essential energy to the food web and the critical role of respiration in breaking down organic detritus and therefore resupplying nutrients to enable such growth to occur (Table 6.2).

Table 6.2 Selected Area evaluation questions relating to the effect of Commonwealth environmental water on stream metabolism

Key components	Selected Area questions
GPP, ER, NPP	• What was the effect of Commonwealth environmental watering actions on rates of GPP, ER, and NPP? • What did Commonwealth environmental water contribute to total GPP, ER, and NPP? • Which aspect of Commonwealth environmental water delivery contributed most to productivity outcomes?

6.4 Methods

Data collection

Stream metabolism measurements were performed in accordance with the LTIM Standard Operating Procedure (Hale *et al.*, 2014). Water temperature and dissolved oxygen were logged every ten minutes with at least one logger placed in each of the four study zones; in zones 1, 3 and 4, loggers were placed at the upstream and downstream end of these zones. For 2019-20, water temperature and dissolved oxygen were also logged at the Old Morago Road site on Colligen Creek. Data were downloaded and loggers calibrated approximately once per month if sites were accessible, and more frequently (often fortnightly) during summer to avoid problems with probe biofouling. Light and depth loggers were deployed alongside oxygen loggers and data were downloaded on an approximately monthly basis. The data collected by the loggers was also used to calculate daily average temperature and dissolved oxygen concentrations (see Section 5) for each of the zones.

In accord with the LTIM Standard Protocol, temperature (°C), electrical conductivity (µS/cm), dissolved oxygen (%), pH and turbidity (NTU) were also measured as spot recordings fortnightly within each zone. For 2019-20, average water depth was also estimated from hydraulic flow modelling (undertaken by Marine Solutions on behalf of CSU) which derived 5 cross-sectional wetted areas of each zone at varying discharge (approximately 25 to 1900 ML/day). A 2nd-order polynomial trendline was derived from the 5 discharge-depth relationships and used to predict average depth from daily discharge data.

Data analysis

Acceptance criteria for inclusion of daily results from the BASEv2 model (updated from Grace *et al.*, 2015; according to Song *et al.*, 2016) followed Watts et al. (2019) as established at the July 2015 LTIM Workshop and then refined at the equivalent meeting in July 2016. These criteria were that the fitted model for a day must have (i) an R^2 value of at least 0.90 *and* a coefficient of variation for the GPP, ER, and K parameters of < 50%, (ii) a reaeration coefficient (K) within the range 0.1 to 15, and (iii) model fit parameter PPfit within the range 0.1 to 0.9. Values outside these parameters indicate that the 'best fit' to the data was still an implausible model.

The original units of GPP and ER estimation from BASE are volumetric (mg O_2/L/day) and can be affected by concentration and dilution effects from varying discharge (Watts *et al.*, 2019). We therefore converted all GPP and ER estimates to areal rates (g O_2/m^2/day) by multiplication with estimated average depth. This approach addresses issues associated with the fact that higher flows are often associated with lower rates of production per litre.

For the environmental watering action periods 1 and 3, the estimation of the additional daily carbon production (kg) attributable to Commonwealth environmental watering actions entailed the following steps.

1. Rates of carbon produced and consumed each day were calculated by multiplying GPP or ER in mg O_2/L/Day by the number of litres discharged that day. Conversion to organic carbon involves a factor of 12/32 (ratio of atomic mass of C to molecular mass of O_2). This factor does not include any physiological efficiency factor for converting oxygen to organic carbon which typically is in the range 0.8 to 1. Given the exploratory use of this metric, concern over conversion efficiency at this stage is unwarranted.
2. Total production for each day was estimated by multiplying the rate of production derived for that day (in kg C/L/day) by the observed discharge on that day (L).

3. To calculate the discharge predicted to have occurred in the absence of Commonwealth Environmental Water (CEW), we subtracted the volume of CEW from the total discharge (observed discharge – CEW).
4. To calculate the average depth of the water column in the absence of CEW, we applied the estimation equations for average depth to the discharge predicted to have occurred in the absence of CEW.
5. To estimate volumetric rates of GPP and ER in the absence of CEW, we divided areal rates of production and consumption (in g O_2/m^2/day) for each day by the estimated average depth of the water column in the absence of CEW. Rates were then converted to units of organic carbon as above.
6. These alternative rates of production and consumption were then multiplied by the non-CEW discharge volume to determine the total production predicted to have occurred on that day in the absence of CEW.
7. The above steps produced two time-series of estimated total daily production with and without CEW.
8. The daily estimates of CEW/non-CEW derived production were averaged to determine the mean daily additional production during watering actions and total additional production over the entire watering action.

6.5 Results

Rates of stream metabolism

Using the acceptance criteria for each day's diel DO curve, the acceptance rate ranged from a low of 3% of all days with data available for zone 3 Upstream to a high of 63% at zone 1 Downstream (Table 6.3). These values are low compared to 2018-19 (29-65%), 2017-18 (58-79%), 2016-17 (17-48%) and 2015-16 (14-67%) (Watts *et al.*, 2019). Examination of the underlying oxygen logger data suggests that many of the unusable data days in 2019-20 are due to measurement errors (e.g., temporary logger malfunctions) rather than variation in environmental conditions (e.g., flow events).

Table 6.3 Summary of data availability for the eight data logger sites, May 2019 – June 2020.

Hydrological zone	Site	Total days	Days with acceptable data	% Acceptable data days
Zone 1: Yallakool Creek	Upstream	100	14	14
	Downstream	397	251	63
Zone 2: Wakool River	Downstream	397	229	58
Zone 3: Wakool River upstream Thule Creek	Upstream	100	3	3
	Downstream	373	44	12
Zone 4: Wakool River downstream Thule Creek	Upstream	98	13	13
	Downstream	403	194	48
Old Morago: Colligen Creek		300	70	23

Median GPP values for all eight sites fell within a narrow range of 0.9 to 4.2 mg O_2/L/day, similar to the range in 2018-19 (1.2 to 2.0 mg O_2/L/day), 2017-18 (1.1 to 2.6 mg O_2/L/day), 2015-16 (1.4 to 4.1 mg O_2/L/Day) and 2016-17 (1.6 to 3.9 mg O_2/L/Day). When converted to areal rates, the median GPP values had a similarly narrow range (from 0.9 to 2.9 g O_2/m^2/day) (Table 6.4). Major events such as large flows and anoxia can often preclude data meeting acceptance criteria. These comparisons are therefore made using metabolic rates obtained primarily during in-channel flow conditions.

Table 6.4 Summary of gross primary production (GPP) and ecosystem respiration (ER) rates and GPP/ER ratios for the eight sites in five hydrological zones, May 2019 – June 2020. Each metabolic parameter is expressed as a median and mean with minimum and maximum values also included. 'n' is the number of days for which successful estimates of metabolic parameters were obtained.

	Zone 1 upstream (n = 14)				Zone 1 downstream (n = 251)			
	Median	Mean	Min	Max	Median	Mean	Min	Max
GPP (g O_2/m^2/day)	1.53	0.94	0.40	6.28	2.85	1.53	0.33	30.47
ER (g O_2/m^2/day)	2.41	1.53	0.68	9.67	3.44	2.82	.017	19.88
GPP / ER	0.67	0.63	0.54	1.11	0.75	0.68	0.11	5.93

	Zone 2 downstream (n = 229)			
	Median	Mean	Min	Max
GPP (g O_2/m^2/day)	2.19	1.96	0.15	9.84
ER (g O_2/m^2/day)	5.08	4.32	0.77	22.17
GPP / ER	0.48	0.44	0.07	1.13

	Zone 3 upstream (n = 3)				Zone 3 downstream (n = 44)			
	Median	Mean	Min	Max	Median	Mean	Min	Max
GPP (g O_2/m^2/day)	0.92	0.98	0.80	0.99	1.18	0.94	0.50	3.32
ER (g O_2/m^2/day)	4.72	2.77	1.70	9.69	2.04	1.90	0.79	5.06
GPP / ER	0.31	0.35	0.10	0.47	0.63	0.58	0.19	1.16

	Zone 4 upstream (n = 13)				Zone 4 downstream (n = 194)			
	Median	Mean	Min	Max	Median	Mean	Min	Max
GPP (g O_2/m^2/day)	1.31	1.45	0.64	2.12	2.45	1.65	0.34	18.38
ER (g O_2/m^2/day)	2.62	2.31	0.98	5.82	3.56	2.86	0.23	21.48
GPP / ER	0.59	0.59	0.26	1.09	0.67	0.68	0.13	3.50

	Old Morago (n = 70)			
	Median	Mean	Min	Max
GPP (g O_2/m^2/day)	2.43	2.47	7.46	4.76
ER (g O_2/m^2/day)	4.76	4.04	1.43	15.28
GPP / ER	0.55	0.59	0.06	1.10

There was a seasonal increase in GPP from spring into summer in zones 1, 2, 3 and 4. The Colligen Creek (Old Morago Road) site had too little useable data during this period to quantify a trend. At all sites, GPP decreased from the end of summer into autumn. Warmer days, and more hours and higher intensity of sunshine during summer, likely drive this trend. GPP rates can also increase during summer after the solstice due to increasing plant biomass. Despite the constrained range of median values, there were many days at each site with higher rates of GPP and ER (from 10 to 30 g O_2/m^2/day), indicating that elevated rates were possible when conditions were conducive. Peaks were particularly noticeable in early summer in zone 1, 2 and 4 for both GPP and ER, coinciding with the drawdown period at the end of watering action 3. Zones 1 and 2, and Colligen Creek, showed additional pulses in ER in autumn coinciding with the unregulated flow pulse (Figure 6.2).

For most of the time each system was strongly heterotrophic (GPP < ER), even during early-summer GPP peaks (Figure 6.2). Zones 1, 2 and 4 also showed seasonal trends with GPP/ER increasing from winter into summer and then decreasing in autumn. The notable exception is the early summer peak in GPP at zone 1. This indicates that at most times, much more carbon is being consumed by respiration within the river than is being produced by photosynthesis, and that respiration rates do not decrease to the same extent as GPP during colder seasons. Much of the organic carbon being respired must therefore have been transported into the systems from upstream or from the surrounding catchment. Flows were also likely too low during the 2019-20 period to connect anabranches or low-lying floodplains, i.e., shallow wetted habitat where primary productivity can often reach very high areal rates.

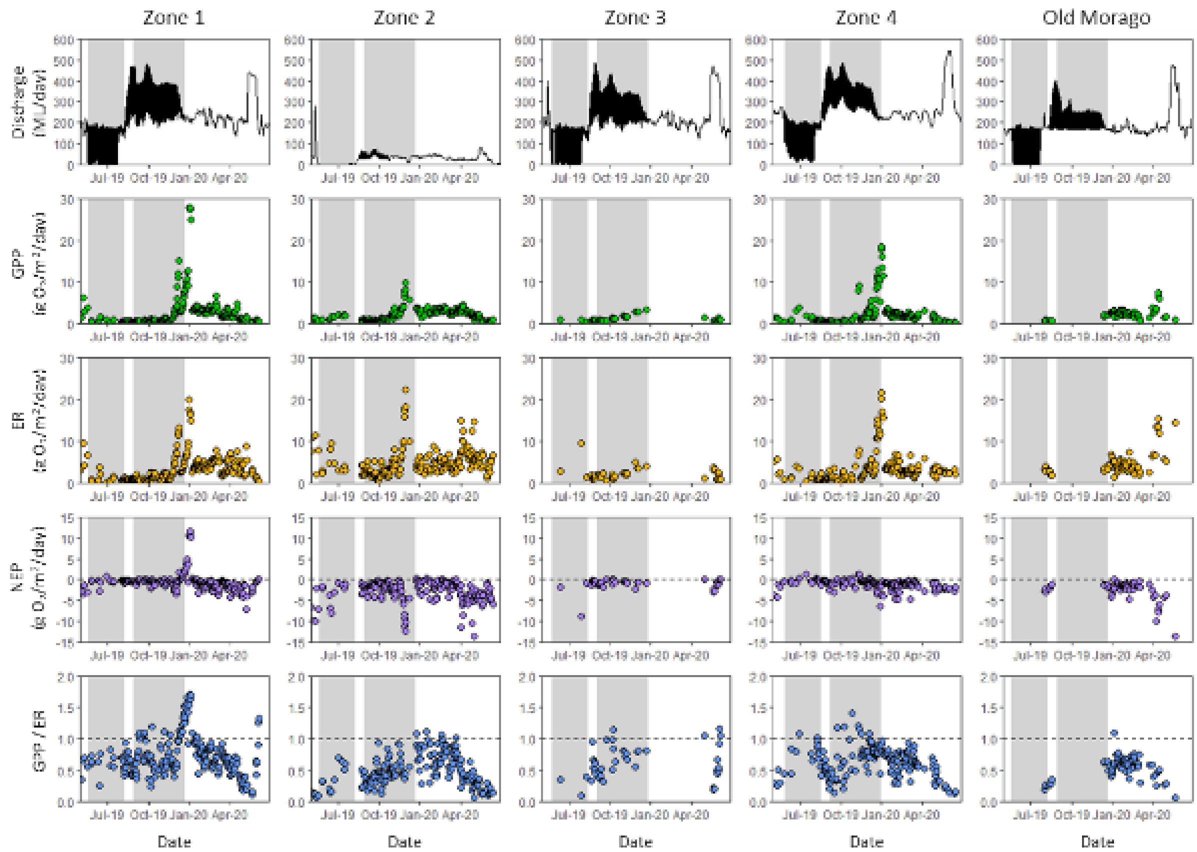


Figure 6.2 Plots of discharge, oxygen production (GPP), consumption (ER), net production (NPP) and production: consumption ratio (GPP / ER) over all sites in five hydrological zones in 2019-20. Watering action 1 (15/5/19 - 9/8/19) and 3 (28/8/19 - 22/12/19) are indicated by shaded bars. Shaded bars are adjusted for travel time for zones 3 (4 days) and 4 (9 days).

Response of stream metabolism to Commonwealth environmental watering actions

The Colligen Creek, Old Morago Road site had too little useable data available for watering action 1 and 3 to provide meaningful analyses (Fig. 6.2). Consequently, it is not shown in the following section.

Environmental watering action 1 in winter 2019: GPP rates mostly fell within a narrow range (0 to 3 g O_2/m^2/day) across all zones during watering action 1. ER rates were more variable, with notably higher rates at zones 2 and 3 than those at zones 1 and 4. This contributed to zones 2 and 3 being more strongly heterotrophic during the watering action (Figure 6.3). Delivery of environmental water resulted in noticeably increased production and consumption of C at zones 1 and 4 (Figure 6.4). The effect of CEW was more difficult to predict at zone 3 due to deficient data, and at zone 2 due to a large number of zero-discharge days.

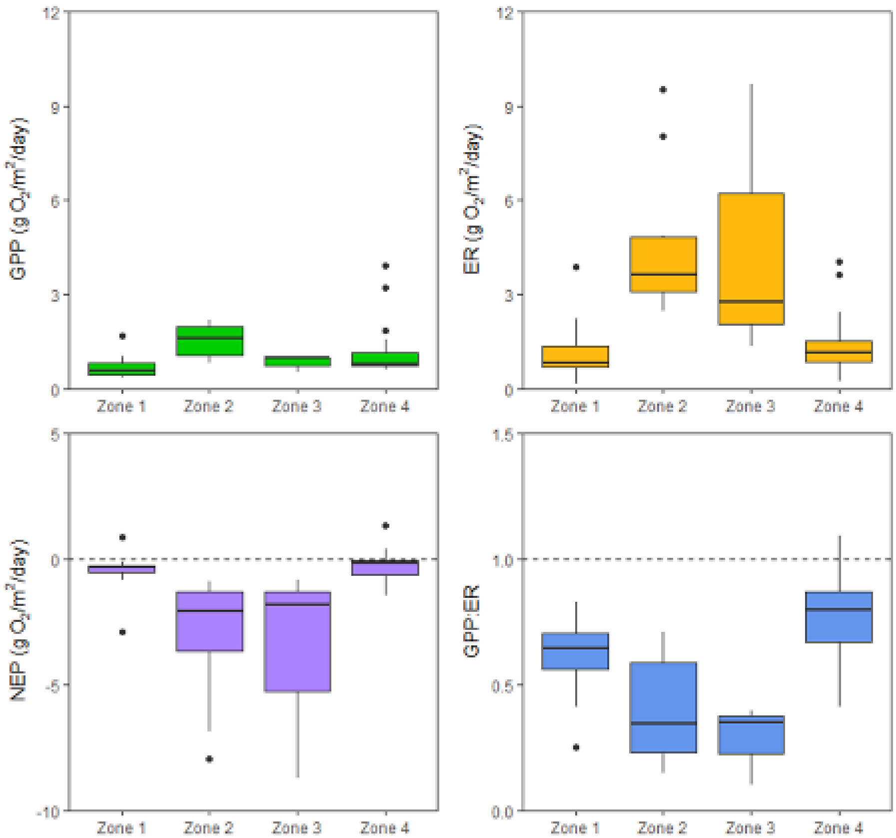


Figure 6.3 Watering action 1, winter 2019. Variation in daily rates for organic carbon production (GPP), consumption (ER), net production (NEP) and production: consumption Ratio (GGP:ER) are shown.

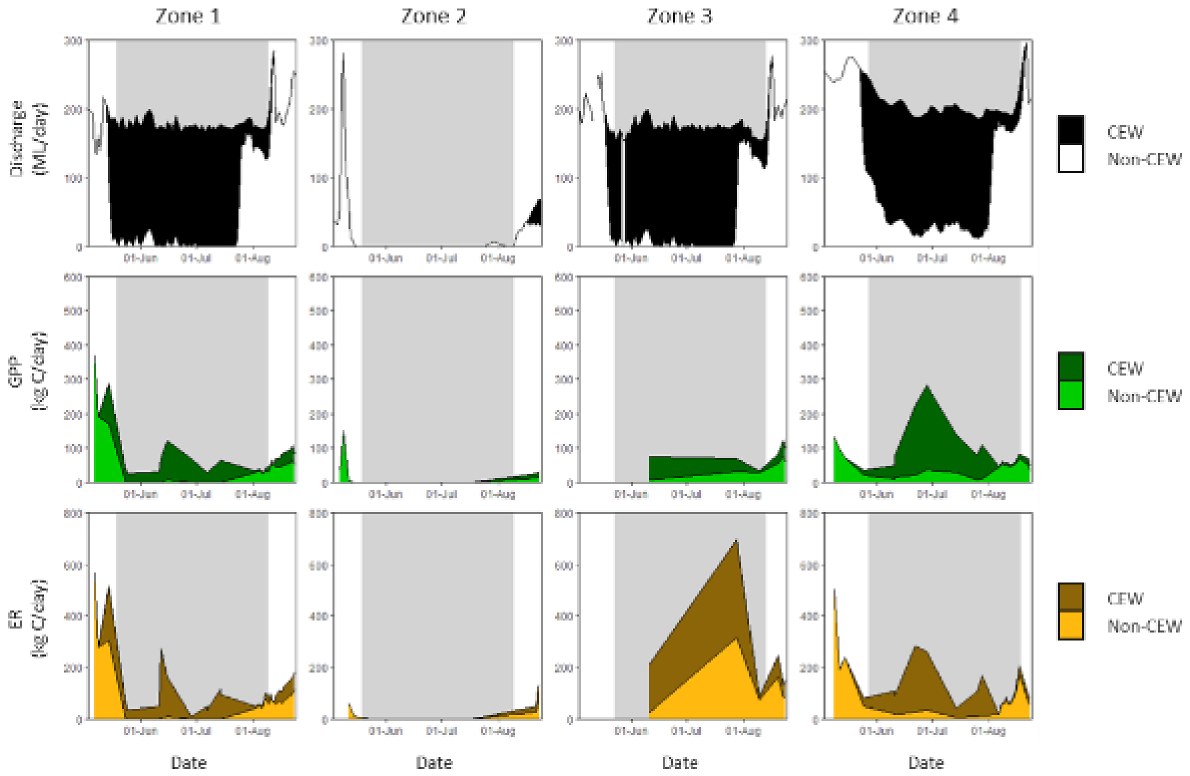


Figure 6.4 Plots of discharge (ML/day) and carbon production (GPP, kg C/day) and consumption (ER, kg C/day) during watering action 1 in winter 2019, showing the component attributed to Commonwealth environmental water. Duration of watering action 1 is shown by grey shaded area. Shaded bars are adjusted for travel time for zones 3 (4 days) and 4 (9 days).

Environmental watering action 3 in 2019: Median GPP rates were relatively consistent across sites during watering action 3, but a number of higher-productivity days (> 5 g $O_2/m^2/day$) also occurred at zones 1, 2 and 4. Median ER rates were slightly higher at zone 2, but zones 1 and 4 also had a number of high-ER days. Sites were still largely heterotrophic, although some net autotrophic days occurred particularly in zone 1 (Figure 6.5). There was an increase in overall carbon production and consumption across all zones in response to the CEW, and rates largely reflected the seasonal progression from spring into summer (Figure 6.6).

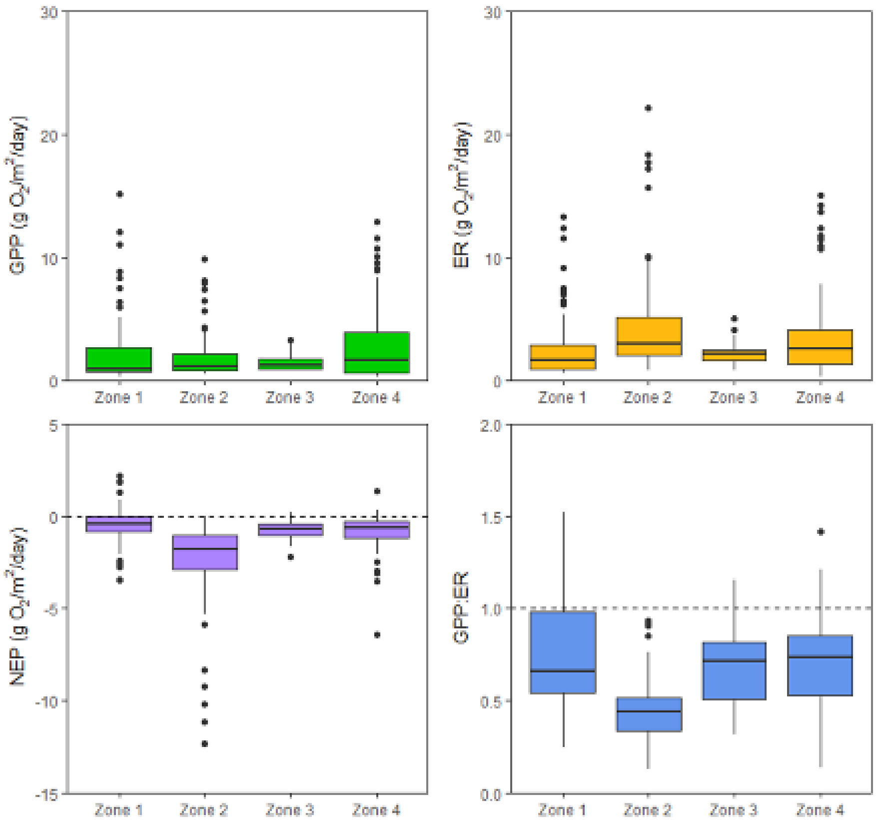


Figure 6.5 Watering action 3, 2019. Daily rates for organic carbon production (GPP), consumption (ER), net production (NEP) and production: consumption Ratio (GGP:ER) are shown.

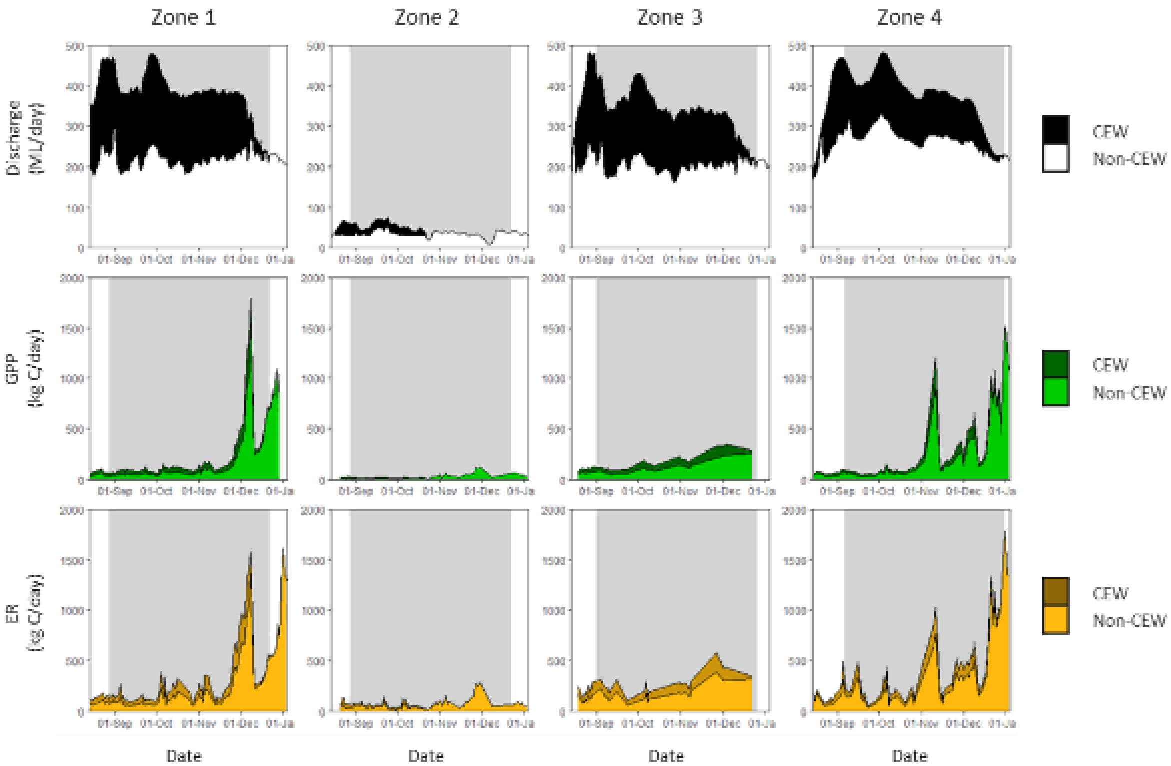


Figure 6.6 Plots of discharge (ML/day) and carbon production (GPP, kg C/day) and consumption (ER, kg C/day) during watering action 3 in 2019, showing the component attributed to Commonwealth environmental water. Duration of watering action 3 is shown by grey shaded area. Shaded bars are adjusted for travel time for zones 3 (4 days) and 4 (9 days).

Estimated contribution of Commonwealth environmental water to carbon production across all watering actions

The daily estimates of CEW/non-CEW derived production were averaged across the number of days for each watering action to determine the average daily additional production and consumption for each watering action, and the total additional carbon production and consumption over each watering action.

Both watering actions resulted in increased production (Figure 6.7) and consumption of carbon (Figure 6.8). Zone 2 was unable to be assessed in watering action 1 due to zero discharge. Overall C production and consumption during watering action 3 was higher than in watering action 1 due to (i) the longer period of the action (116 vs. 86 days) and (ii) higher overall rates of GPP (Figure 6.2) and ER. The exception may have been ER in zone 3, but as above these estimates are limited by low data availability at this site during watering action 1. The general trend largely reflects both the pulsed events that occurred in early summer and the generally increasing trend in GPP and ER rates from spring to summer. However, during watering action 1 the proportional contribution of CEW to total production was greater than in watering action 3. This parallels the greater proportional contribution of CEW to total discharge during winter low-flow periods.

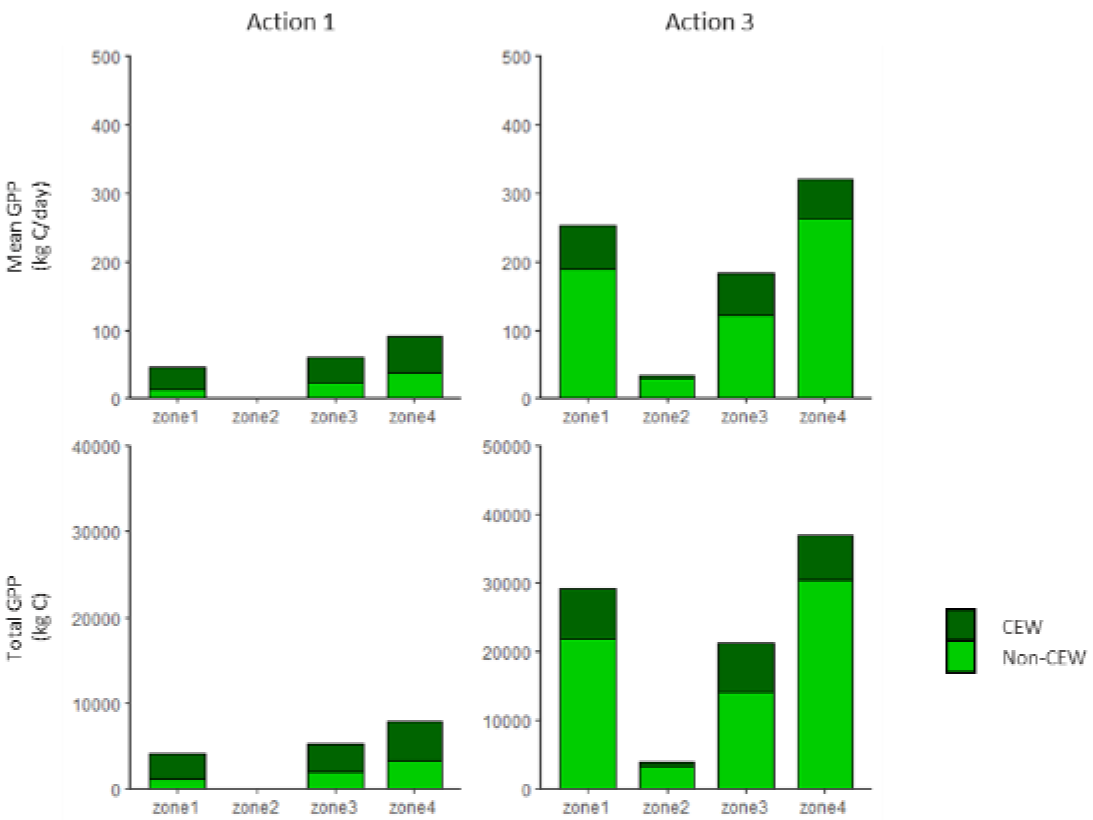


Figure 6.7 Top: The average daily additional production of carbon (kg C/day) during the two environmental watering actions. Bottom: The total additional production of carbon (kg) during the two watering actions. Light green is the production attributed to operational water (non CEW), and dark green indicates the production attributed to Commonwealth environmental water.

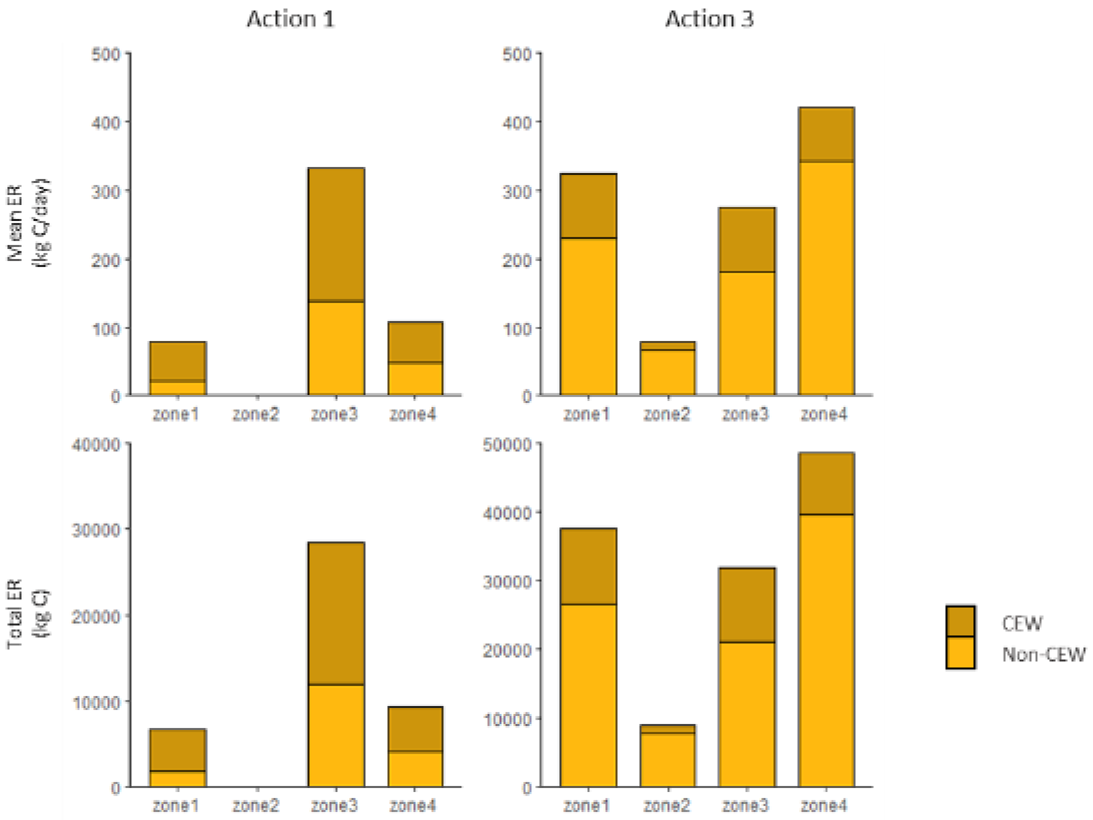


Figure 6.8 Top: The average daily additional consumption of carbon (kg C/day) during the two environmental watering actions. Bottom: The total additional consumption of carbon (kg) during the two watering actions. Light orange is the production attributed to operational water (non CEW), and dark orange indicates the production attributed to Commonwealth environmental water.

6.6 Discussion

What was the effect of Commonwealth environmental watering actions on rates of GPP, ER, and NPP?

In past reports (Watts *et al.*, 2017; Watts *et al.*, 2018) it was noted that the immediate effect of a significant flow increase was a substantial decrease in the rates of both GPP and ER. This is simply due to a dilution effect by the large increase in volume of water. Except in conditions of major phytoplankton growth (e.g. an algal bloom), much of the metabolism in the Edward/Kolety-Wakool system appears to be from biofilms and microbial communities growing on (and in the surface layers) of the sediment and also on hard substrates within the channel, such as logs and plant stems (Holland *et al.*, 2020). As the water level rises, the rate at which each photosynthetic or respiring organism is working (amount of oxygen produced or consumed) may not change but the output (change in oxygen concentration) is spread over a larger amount of water. Hence, solely on a volumetric basis, it can appear that GPP and ER have been suppressed by increasing discharge (less oxygen change *per litre of water*).

In 2019-20, conversion of volumetric rates (mg O_2/L/day) to areal rates (g O_2/m^2/day) was introduced to account for this dilution effect. Areal rates of GPP and ER, as well as the ratio between them, showed little change during Commonwealth environmental watering actions. Consequently, increases and decreases in flow likely had little effect on where production and consumption of carbon is occurring within the Edward/Kolety-Wakool system. As with previous years, the strongest pattern in rates of GPP and ER was a seasonal trend. In particular, rates of GPP are higher and pulses appear more frequently during warmer summer months, indicating that temperature and light are major drivers of GPP rates within the Edward/Kolety-Wakool system.

Return flows from the Southern Connected Flow in the Murray River influenced flows in the Edward/Kolety-Wakool system from 28 August to 9 September 2019, and 23 September to 1 October 2019. Return flows from Millewa Forest may have affected the water quality in the Edward/Kolety-Wakool system on these dates, and on later dates at sites further downstream. However, no substantial effect on stream metabolism was observed during these dates. Rates of GPP and ER in the Edward/Kolety-Wakool are likely to be relatively low by world standards, driven by low bioavailable phosphorus concentrations and low light penetration through the turbid water column (Watts *et al.*, 2019). Return flows from the Southern Connected Flow in 2019 were not associated with substantially increased filterable reactive phosphorus (FRP) or decreased turbidity (Section 5). In addition, the return flows did not occur during the summer period when rates of GPP and ER were not restricted by temperature or light. These appear the likely reasons why rates of GPP and ER also appeared to be unaffected by the return flows.

What did Commonwealth environmental water contribute to total GPP, ER, and NPP?

Overall, Commonwealth environmental water contributed significantly to total carbon production and consumption where water was delivered. Creating more 'food' at the base of the food web and more nutrients from ecosystem respiration (to generate this 'food') is a positive outcome of these watering actions, even though water remained within the defined stream channel.

The total additional production and consumption varied with (i) time of year (i.e. with season), (ii) the background flow (i.e. without CEW), and (iii) the volume of CEW being delivered. As above, season appears to be the strongest driver of overall rates, and is therefore also a strong influence on total carbon production and consumption. However, the proportional contribution of CEW to total production and consumption is higher during winter, when discharge is lower. This reflects the influence of channel hydraulics and channel shape. At smaller discharges, additional water volumes increase the surface area of the water and inundated channel bed, and therefore the area of the photic zone within which algal and plant growth occurs, proportionally more than at higher base discharge. The exception would occur if CEW delivery resulted in overbank flows which connected shallow anabranches and low-lying floodplains, greatly increasing the total wetted surface area where high rates of GPP could occur. Increasing the frequency and duration of such events could greatly increase overall rates of production in the system.

The method used to estimate total C production and consumption multiplies GPP and ER rates by discharge. Consequently, total C produced and consumed at zones 1, 2 and 3 during watering action 1 was estimated as being zero during zero-discharge periods, despite continued GPP and ER rates. Surface water would still been present at these times, but with zero flow occurring. This represents a limitation of the estimation method for zero-discharge days, where measuring the wetted area of the river (or remnant waterholes) might provide better estimates of total C production and consumption. Regardless, the strong contribution of CEW to GPP and ER at zone 4 (where no zero-discharge days occurred) indicates that the error induced by the estimation method is likely small.

Which aspect of Commonwealth environmental water delivery contributed most to productivity outcomes?

The median total contribution of Commonwealth environmental water to carbon production was higher during watering action 3 (6856 kg) than watering action 1 (3052 kg). As above, these results reflect the higher overall rates of GPP during summer and the greater probability that pulsed events (i.e., days with very high rates) will occur. However, delivery of Commonwealth environmental water had the greatest proportional effect during winter low-flow periods. Maintaining discharge and wetted area during these periods likely helps maintain zooplankton and other invertebrates that feed on phytoplankton and periphyton, and in turn this increases food availability for fish and other higher order consumers during periods in which food availability might otherwise be low.

It is still very important to note that although these small watering actions provided a beneficial outcome for the riverine ecosystem, it is highly probable that reconnecting backwaters and the floodplain to the river channel would result in much larger positive outcomes. At this stage there is too much uncertainty in the nominal flow category discharges to extend the analysis done here to the relatively small number of days with higher flows, but this may be achievable for future analyses. It is recommended that, when possible, consideration be given to providing a more variable flow regime in the Edward/Kolety-Wakool system in future years.

7 AQUATIC AND RIVERBANK VEGETATION

Authors: Robyn Watts, Sascha Healy, Nicole McCasker

Key findings	
Total species richness	There was an increase in the mean total species richness in each of the five monitored zones since the flood in 2016. The mean species richness has not yet recovered to the same levels as prior to the flood. The mean total number of taxa was consistently lower in zone 2, which has received minimal or no environmental water compared to the other zones. The exception was in 2018-19 when zone 2 received environmental water.
Richness of functional groups	Since 2017-18 there has been a gradual recovery of submerged taxa in all zones, but the total richness has not yet reached levels observed prior to the 2016 flood. In 2019-20 the maximum mean precent cover of submerged taxa increased (zones 1 and 8) or was maintained (zones 3 and 4) in zones that received environmental water, but reduced in zone 2 (upper Wakool River) that did not receive environmental water in 2019-20. Since the flood the number of amphibious taxa has increased in all zones. The mean total richness was higher in zones that received environmental water (zones 1, 3, 4 and 8) than in zone 2 that received no or minimal environmental water. However, zones 3 and 4 have not yet recovered to the same total richness observed prior to the flood. There were generally fewer amphibious taxa in zone 2 that received low or no environmental water. In 2018-19 there was a watering action in zone 2 and an increase in total and mean richness of amphibious taxa including spike rush and mudwort was recorded. However, in 2019-20 there was no watering action in zone 2 and the richness of amphibious taxa declined again.
Percent cover of functional groups	In 2019-20 there was a significant increase in the cover of *Chara* in the monitored hydrological zones that received environmental water (zones 1, 3, 4, 8), and the cover has returned to pre-flood levels in these zones. The change in cover of amphibious taxa since the 2016 flood has not been consistent among zones because there were different dominant taxa in different zones. Spiny mud grass (*Pseudoraphis spinescens)* was the most abundant taxa in zone 4 and has increased in percent cover in zone 4 such that it currently has a higher percent cover than was recorded prior to the flood. The common spikerush (*Eleocharis sp.)* was the dominant taxa in zone 8 (Colligen Creek) prior to the flood, but tolerated the flooding and has maintained similar mean percent cover across all years. In contrast, floating pondweed (*Potamogeton tricarinatus)* was the dominant amphibious taxa in zone 3 prior to the flood but significantly reduced in cover or was killed by the flood in 2016. It was recorded again for the first time in 2019-20 in zone 3 at low percent cover. Similarly, milfoil (*Myriophyllum spp)* was abundant in zones 1, 3 and 4 prior to the flood but was recorded at low percent cover in zones 1 and 3 in 2019-20.

7.1 Background

Riverbank vegetation and aquatic vegetation play an important role in the functioning of aquatic ecosystems, supporting riverine productivity and food webs and providing habitat for fish, invertebrates, frogs and birds (Roberts and Marston 2011).

Flow management and the water regime in a river system can affect the survival, growth and maintenance of adult plants and strongly influence aspects of reproductive cycles, including flowering, dispersal, germination and recruitment. Riverbank plant survival and growth is affected by the frequency and duration of inundation (Toner and Keddy 1997; Johansson and Nilsson 2002; Lowe et al. 2010). Frequent inundation can delay reproduction (Blom and Voesenek 1996), whilst long duration of inundation, such as can occur during floods or long periods of regulated flows, can reduce growth or survival of riverbank plants (Blom et al. 1994; Johansson and Nilsson 2002; Lowe et al. 2010). Favourable soil moisture and nutrient conditions created by a receding flood can encourage rapid recovery and root and shoot development. Many plants, including emergent macrophytes and riparian understorey herbs, often germinate on flood recessions (Nicol 2004; Roberts and Marston 2011). However, a high level of sediment deposition during periods of inundation can reduce the survival of some small herbaceous riverbank species (Lowe et al. 2010).

Riverbank and aquatic plants that occur within the channel and on the riverbank can be broadly classified into three functional groups that are defined by wetting and drying patterns. Submerged taxa occupy the wetted river channel, terrestrial taxa typically occupy the upper section of the riverbank, and amphibious taxa occupy both wet and dry parts of the riverbank and respond to, or tolerate, fluctuations in wetting and drying. Different aquatic macrophyte species have different watering requirements. For example, while it is critical that the submerged ribbon weed plants are re-flooded within three to four months to maintain existing plants (Roberts and Marston 2011), many amphibious taxa respond to and tolerate a broad range of wetting and drying regimes.

A long history of operational water delivery in the Edward/Kolety-Wakool system (section 4.1) combined with the prolonged millennium drought when flows in the Murray-Darling Basin were at record low levels (van Dijk 2013; Chiew et al. 2014), has had negative impacts on the riverbank and aquatic vegetation in the Edward/Kolety-Wakool system. Community members and landholders report there were beds of ribbon weed (*Valisineria australis*.) within the channels and other plants occurring on the banks of the Edward/Kolety-Wakool system prior to the drought. In 2010, after the break of the drought, submerged and amphibious plant taxa were largely absent throughout the system with the exception of the longer lived rush *Juncus* sp.

Environmental water has been delivered as base flows, freshes and recession flows in the Edward/Kolety-Wakool system since 2010, with one of the aims being to maintain the health of riparian and in-channel aquatic native vegetation communities and maintain ecosystem and population resilience through supporting ecological recovery and maintaining aquatic habitat (CEWO 2015). Environmental watering in this system is expected to increase lateral connectivity by increasing the area of river bank receiving periods of wetting and drying than under operational flows. This is expected to maintain the health of riparian and in-channel aquatic native vegetation and support ongoing recovery and re-establishment of native aquatic vegetation in this system.

The response of vegetation to environmental watering actions in 2019-20 was influenced and constrained by the condition and diversity of vegetation at the start of the watering year. In 2015-16 there was higher mean species richness in Yallakool Creek zone 1, Wakool River zone 3 and zone 4 that received the environmental base flow and fresh than in the upper Wakool River zone 2 that received none or very small volumes of environmental water (Watts et al. 2016). There was also a higher percent cover of riverbank aquatic taxa in zones 3 and 4 that have a history of environmental watering, compared to that in the Wakool River zone 2. However, in late 2016 there was a large unregulated flood event that had negative effects on the riverbank and aquatic vegetation in all zones by reducing the cover and richness of vegetation significantly (Watts et al. 2017b). In 2018-19 there was some evidence of slow recovery since the flood of 2016, however the total species richness and the percent cover of taxa was lower than prior to the 2016 flood (Watts et al. 2019) suggesting the aquatic riverbank vegetation is still recovering. Watts et al. (2019) also noted there was variation in responses of different taxa, with some tolerant taxa responding quickly after the flood while other less tolerant taxa were taking a longer-time to recover from the flood.

This section reports on the recovery of riverbank and aquatic vegetation in the Edward/Kolety-Wakool system in 2019-20 since the flood of late 2016.

7.2 Environmental watering actions for vegetation outcomes

Two Commonwealth environmental watering actions (and their sub-components) were delivered in the Edward/Kolety-Wakool system in 2019-20 (Table 7.1). The responses to these actions and antecedent actions over previous watering years is evaluated in this section.

Table 7.1 Commonwealth environmental watering actions in 2019-20 in the Edward/Kolety-Wakool River system and list of objectives targeting vegetation outcomes.

	Watering action	Dates	Zones	Objectives (from CEWO)
1	Winter base flow	15 May - 9 Aug 2019	1,3,4,8	For in-channel vegetation
3a	Winter/spring early fresh	28 Aug - 4 Sep 2019	1,3,4,8	To provide early season rise in river level to stimulate early growth of in-stream aquatic vegetation
3b	Early spring elevated base flow	5 - 22 Sep 2019	1,3,4,8	To provide inundation for aquatic vegetation growth
3c	Late spring fresh	23 Sep - 11 Oct 2019	1,3,4,8	No specific vegetation objectives
3d	Late spring elevated base flow	12 Oct - 30 Nov 2019	1,3,4,8	No specific vegetation objectives
3e	Recession	1 Dec – 22 Dec 2019	1,3,4,8	Slow recessions for instream water plants

7.3 Selected Area evaluation questions

Long-term evaluation questions

- *What has Commonwealth environmental water contributed to the recovery (measured through species richness, plant cover and recruitment) of riverbank and aquatic vegetation in Yallakool Creek and the middle and upper Wakool River that have been impacted by operational flows and drought and how do those responses vary over time?*
- *How do vegetation responses to Commonwealth environmental water delivery vary among hydrological zones?*

Short-term evaluation questions

- *What did Commonwealth environmental water contribute to the percent cover of riverbank and aquatic vegetation?*
- *What did Commonwealth environmental water contribute to the diversity of riverbank and aquatic vegetation taxa?*

7.4 Methods

Monitoring design and field sampling

Four sites in each of five hydrological zones (Yallakool Creek, Wakool River zone 2, Wakool River zone 3, Wakool River zone 4 and Colligen Creek zone 8) were surveyed. Sites were initially established in late 2014 in areas where grazing impacts were minimal or absent, and were located a minimum of two kilometres apart. Monitoring was undertaken once per month from August 2019 to May 2020. At each site six permanent 20 m long transects were established in 2014 parallel with the river channel. Star pickets were installed at each end of the permanent transect. The lowest transect on the riverbank was labelled as transect 0 and the other five transects labelled consecutively up to transect 5 highest on the river bank. The transects were surveyed so they were 25 cm apart in vertical height, with the five transects thus covering 1.25 m of vertical height of the bank. Transects zero and one were generally in the water at base operational flows, and the other four transects further up the riverbank have the potential to be inundated during environmental watering or during unregulated flows.

Vegetation was assessed using the line point intercept method along transects. At each of the transects on each sampling date a 20 m tape measure was laid out running horizontally along the riverbank between two star pickets that had been installed at a known height of riverbank. The taxa at each 50 cm point quadrat along the 20 m transect (40 points on each transect) were recorded. Plants and macroscopic algae (e.g. Charophytes) were identified to species where possible, but if the plants were very small and without seeds or flowers to enable correct identification they were identified as far as possible. Plants were identified using the Flora of New South Wales Volumes 1–4 (Harden 1992, 1993, 2000, 2002) and keys and descriptions from PlantNet (RBGDT, 2019) and information from field guides (Sainty and Jacobs 2003, Cunningham et al. 1992). If no vegetation was present at a point, then that point was recorded as bare ground, leaf litter or log/tree trunk. When the transects were in the water the tape measure was laid at the water's edge and a flexible fibreglass pole held from the tape out to the water surface to locate the point on the transect for recording data. Photo-points were established in 2014 at each site and photos were taken on every sampling event.

Data analysis

Each taxa was classified into one of three broad functional categories using a range of sources including Brock and Casanova (1997), Casanova (2011) and Roberts and Marston (2011). Although there are some limitations of using water plant functional groups to classify taxa, the approach of using three functional categories is sound for common taxa that can be related to hydrological information on wetting and drying regimes. The three functional categories were:

Submerged - taxa that have adaptations for living submerged in water. These plants grow to, but do not emerge from, the surface of the water.

Amphibious - taxa that tolerate wetting and drying and taxa that respond to water level fluctuations

Terrestrial - taxa that typically occur in damp or dry habitats.

Total species richness was calculated for each site in each zone for each month. The percent cover was calculated for each transect for each month. To compare cover of vegetation across the six years of the LTIM/MER program (2014-2020), the month when the maximum cover occurred across the months of October to May was identified for each taxa. The period from October to May was selected because it is the main growing season for these plants.

7.5 Results

Total species richness and cover

There has been an increase in the mean total species richness in each of the five monitored zones since the unregulated flood in 2016 (Figure 7.1). The mean species richness in zones 1, 2, 3, and 4 has not yet recovered to the same levels as prior to the flood. The mean total number of taxa was consistently lower in zone 2, which has received minimal or no environmental water compared to the other zones. The exception was in 2018-19 when zone 2 received environmental water.

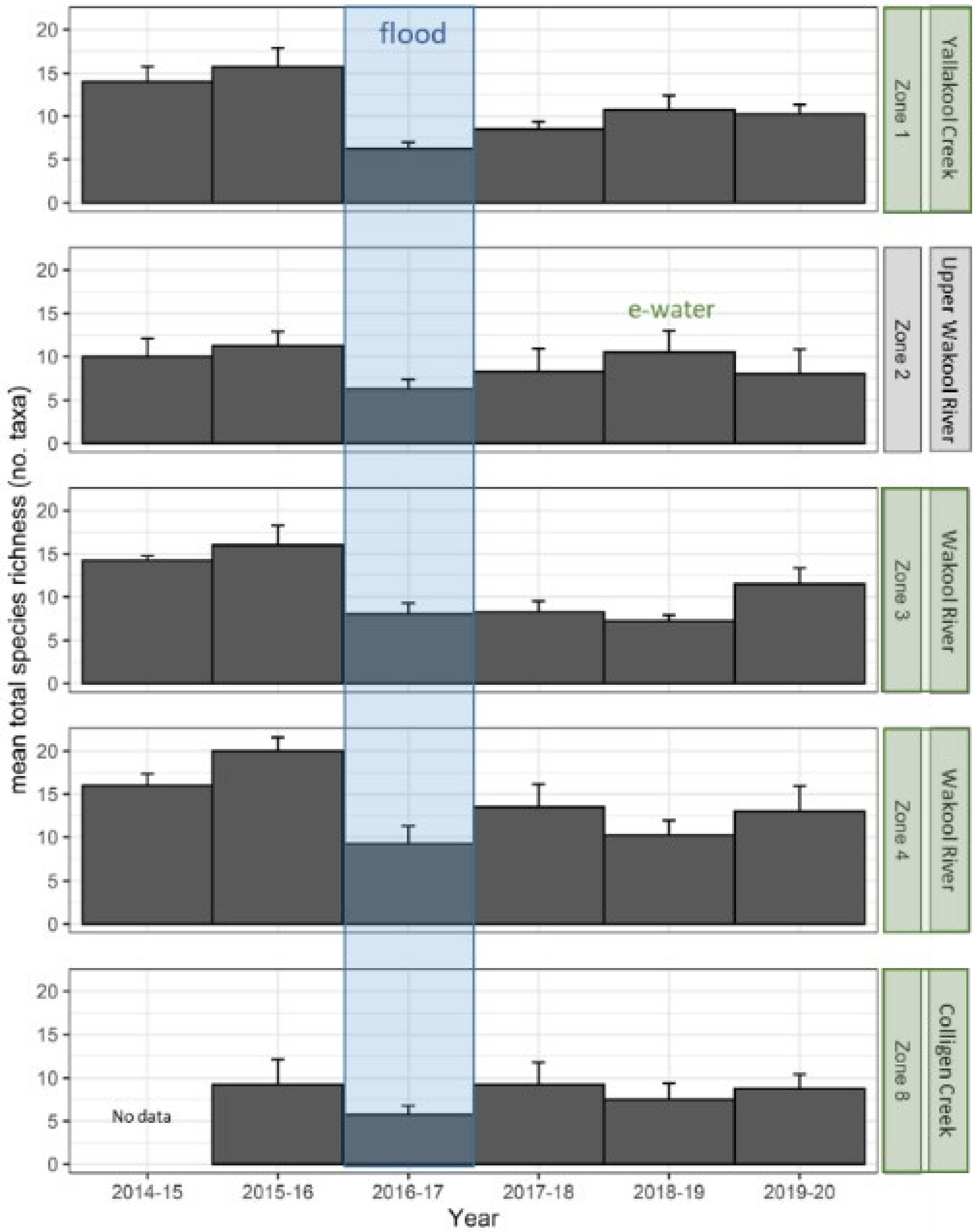


Figure 7.1 Mean total richness of vegetation taxa monitored monthly in five zones in the Edward/Kolety-Wakool system between 2014 and 2020. Blue shading indicates the unregulated flood in 2016-17 water year. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

A large percentage of taxa across the five hydrological zones were native taxa (Figure 7.2). A greater proportion of native taxa were negatively impacted by the 2016 flood. There was a significant reduction in percent cover of native taxa following the 2016 flood (Figure 7.3), particularly in zones 3 and 4. There has been a gradual increase in species richness following the flood in all zones (Figure 7.2). There has also been a slight increase in percent cover of native taxa over the subsequent years, however the maximum mean percent cover is still considerably lower than that observed in 2015-16 prior to the flood (Figure 7.3).

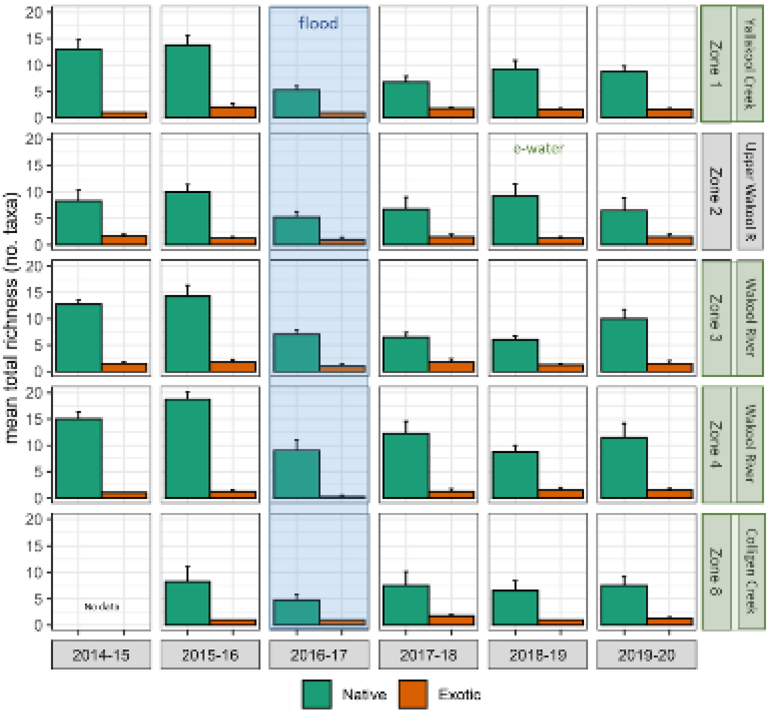


Figure 7.2 Mean richness of native and exotic vegetation taxa monitored monthly in five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Blue shading indicates the unregulated flood in 2016-17 water year. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

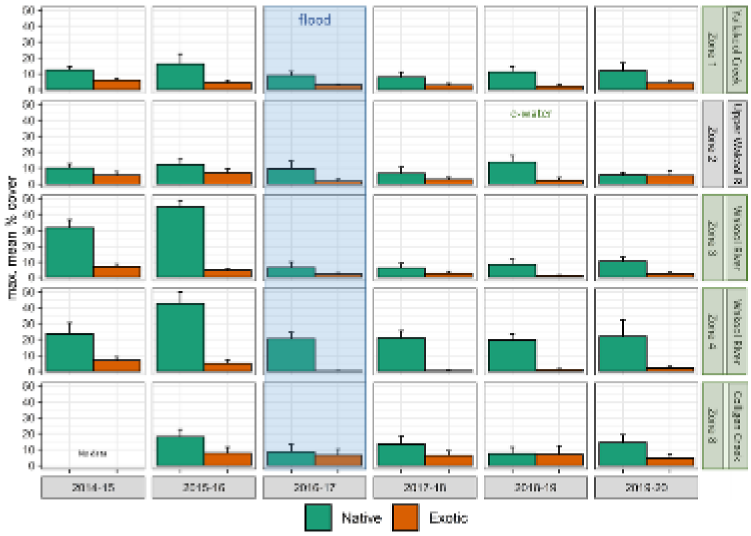


Figure 7.3 Mean percent cover of native and exotic vegetation taxa monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Blue shading indicates the unregulated flood in 2016-17. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

Richness and cover of submerged taxa

Following the flood in 2016 there was a reduction in mean total richness of submerged taxa in all zones. Since 2017-18 there has been a recovery of submerged taxa in all zones (Figure 7.4), but the total richness has not yet reached levels observed prior to the flood. In 2019-20 the maximum mean precent cover of submerged taxa increased (zones 1 and 8) or was maintained (zones 3 and 4) in zones that received environmental water, but reduced in zone 2 (Upper Wakool River) that did not receive environmental water in 2019-20 (Figure 7.5).

There is a seasonal pattern in the presence of Chara, with highest cover observed between September and December. In 2018-19 Chara was present in all five study zones following environmental watering actions, but the percent cover of this taxa was low. In 2019-20 there was a significant increase in the cover of *Chara* in all hydrological zones that received environmental water (Figures 7.6, 7.7). This increase was substantial, such that the percent cover of Chara in zones 1, 3, 4 and 8 had returned to levels observed prior to the flood. There was a small amount of Chara present in zone 2 in 2018-19 following an environmental watering action, however in 2019-20 there was no environmental water delivered to zone 2 and Chara was absent (Figures 7.6, 7.7).

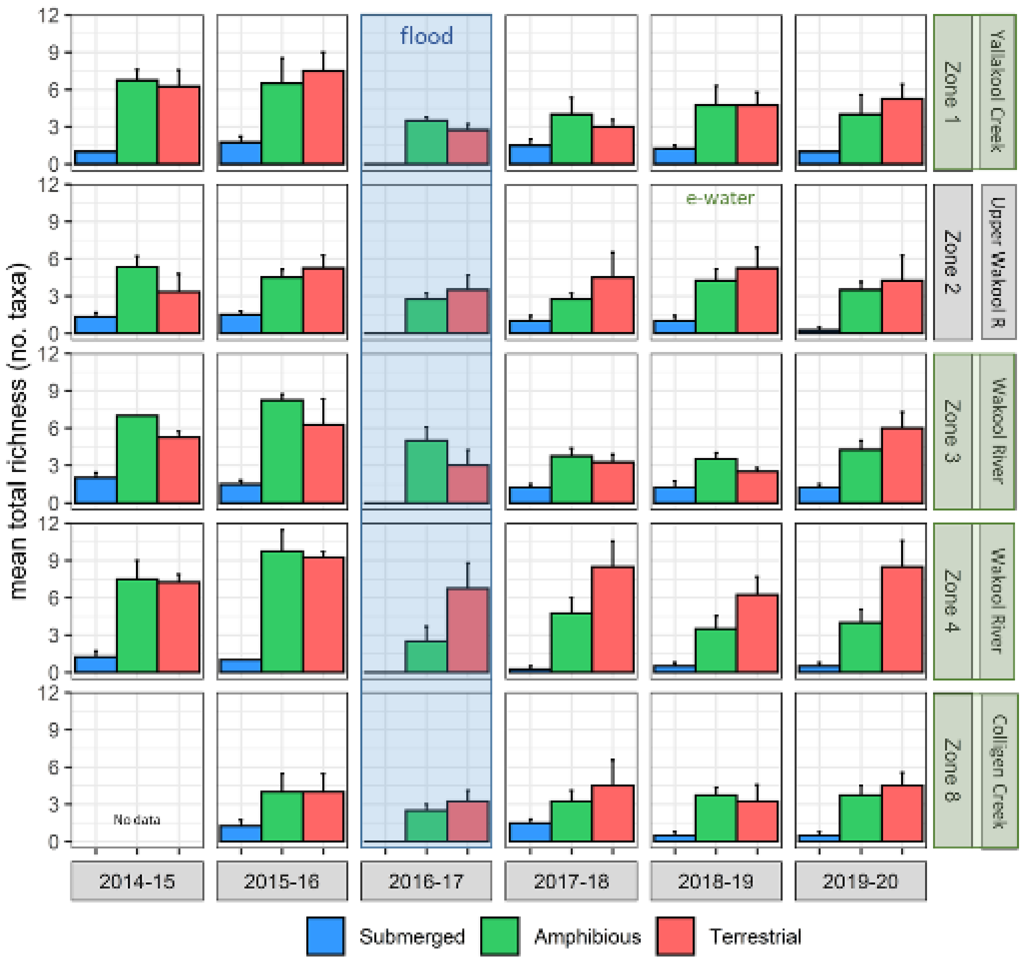


Figure 7.4 Mean total richness of vegetation taxa monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Taxa were classified as submerged, amphibious or terrestrial. Blue shading indicates the unregulated flood in 2016-17. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

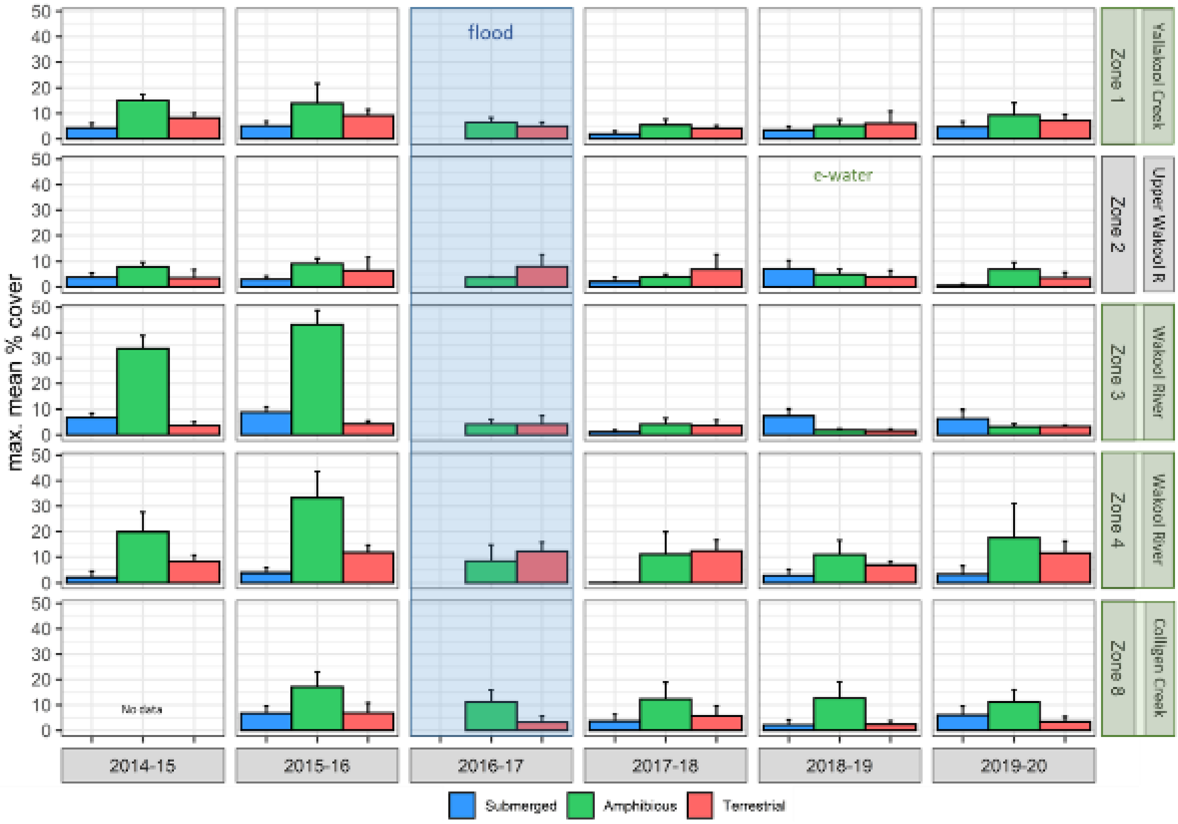


Figure 7.5 Maximum mean percent cover of vegetation taxa monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Taxa were classified as submerged, amphibious or terrestrial. Blue shading indicates the unregulated flood in 2016-17. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

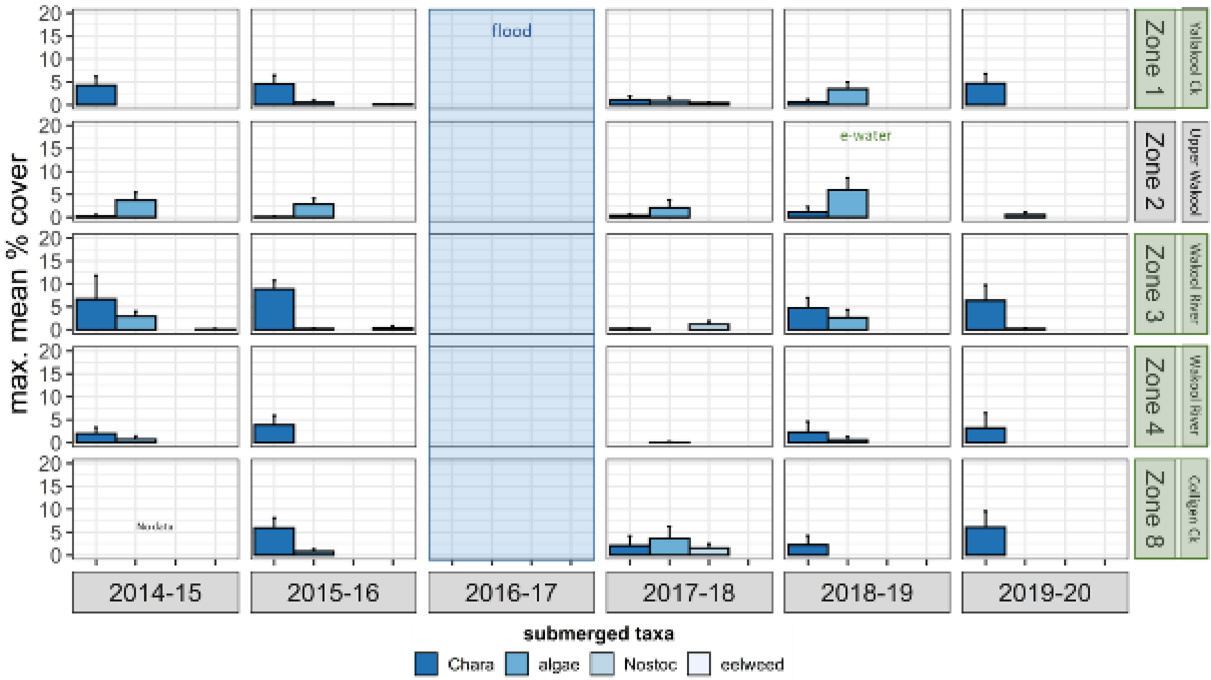


Figure 7.6 Mean percent cover of four submerged vegetation taxa monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

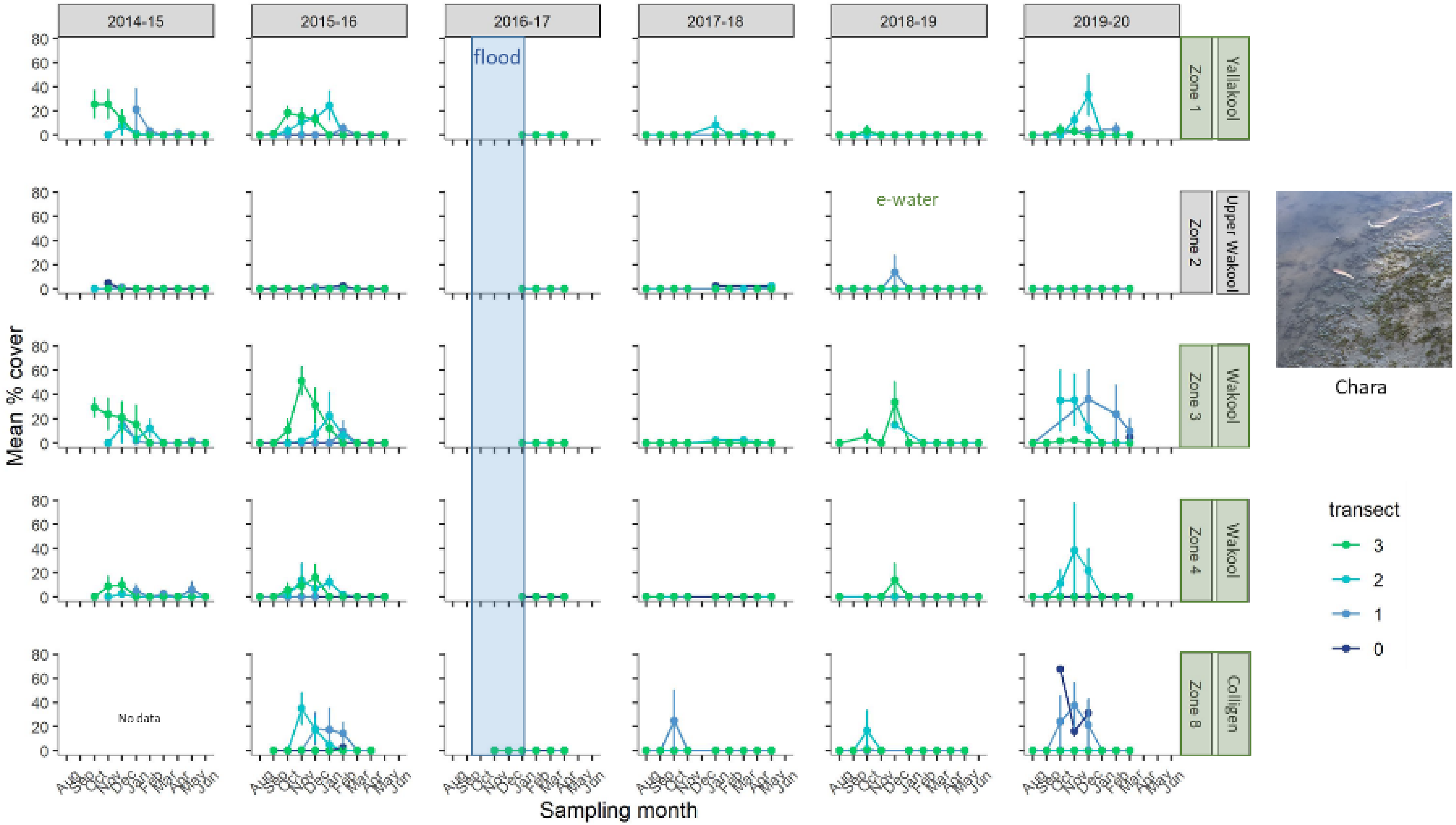


Figure 7.7 Mean percent cover of *Chara* monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Transect zero is lowest on the riverbank and transects are labelled consecutively up to transect 5 highest on the river bank. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

Richness and cover of amphibious taxa

Following the flood in 2016 there was a reduction in mean total richness and percent cover of amphibious taxa in all zones (Figures 7.4, 7.5). The reduction in percent cover was considerable in zones 3 and 4. Since the flood the number of amphibious taxa has increased in all zones (Figure 7.4). The mean total richness was higher in zones that received environmental water (zone 1, 3, 4 and 8) than in zone 2 that received no or minimal environmental water. However, zones 3 and 4 have not yet recovered to the same total richness observed prior to the flood.

While there has been an increase in the maximum mean percent cover of amphibious taxa in zones 1, 2 and 4 since the flood (Figure 7.5), there has not been the same increase of percent cover of amphibious taxa observed in zones 3 and 8, despite the delivery of environmental water to those zones. The reason for this is because the dominant taxa in different zones respond differently to inundation. Thus, the vegetation response to the flood and environmental watering actions has not been consistent among zones.

Spiny mud grass (*Pseudoraphis spinescens)* was the most abundant taxa in zone 4 (Figure 7.8) prior to the flood. This species has recovered well since the flood and increased in percent cover in zone 4 such that it currently has a higher percent cover than was recorded prior to the flood (Figures 7.8, 7.9). The rush (*Juncus sp*.) was also a common taxa in all zones prior to the flood (Figure 7.8). This rush reduced in percent cover during the flood but tolerated the flood and has recovered in all zones regardless of whether there have been environmental watering actions (zones 1, 3,4 and 8) or no environmental water (zone 2). Similarly, common spikerush (*Eleocharis sp.)* was the dominant taxa in zone 8 (Colligen Creek) prior to the flood, but tolerated the flooding and has maintained similar mean percent cover across all years (Figure 7.8).

In contrast, several other amphibious taxa were negatively impacted by the flood. Floating pondweed (*Potamogeton tricarinatus)* was previously the dominant amphibious taxa in zone 3 prior to the flood (Figure 7.8) but significantly reduced in cover or was killed by the flood in 2016 (Figure 7.8). It was absent from all zones after the flood and was not recorded in 2017-18 or 2018-19. It was recorded again for the first time in 2019-20 in zone 3 at low percent cover (Figure 7.10). Similarly, milfoil (*Myriophyllum spp)* was abundant in zones 1, 3 and 4 prior to the flood and was absent in 2018-19 but was recorded at low percent cover in zones 1 and 3 in 2019-20 (Figure 7.11). Small plants of these species have been observed outside the monitored transects, suggesting there is the possibility of the recovery of these species following future environmental watering actions.

Between 2015 and 2018 there were generally fewer amphibious taxa in zone 2 that received very low or no environmental watering actions compared to the other zones that regularly received environmental water. However, in 2018-19 there was a watering action in zone 2 with water delivered from the MIL Wakool escape between October 2018 and February 2019. In response to this flow there was an increase in total and mean richness with taxa including spike rush and mudwort recorded (Figure 7.8). However, in 2019-20 there was no watering action in zone 2 and the richness of amphibious taxa declined again.

Richness and cover of terrestrial taxa

Following the flood in 2016 there was a reduction in the mean total richness of terrestrial taxa in all zones (Figures 7.4), but the change in cover was variable. Indeed in some zones there was an increase in percent cover of terrestrial taxa (Figure 7.5). For example, common sneeze weed (*Centipeda cunninghamii)* (Figure 7.13) increased in cover in zones 1, 2 and 3 after the flood, especially at transects higher up on the bank (Figure 7.13) that are not usually inundated during operational flows or environmental actions. Most other terrestrial taxa did not show much change.

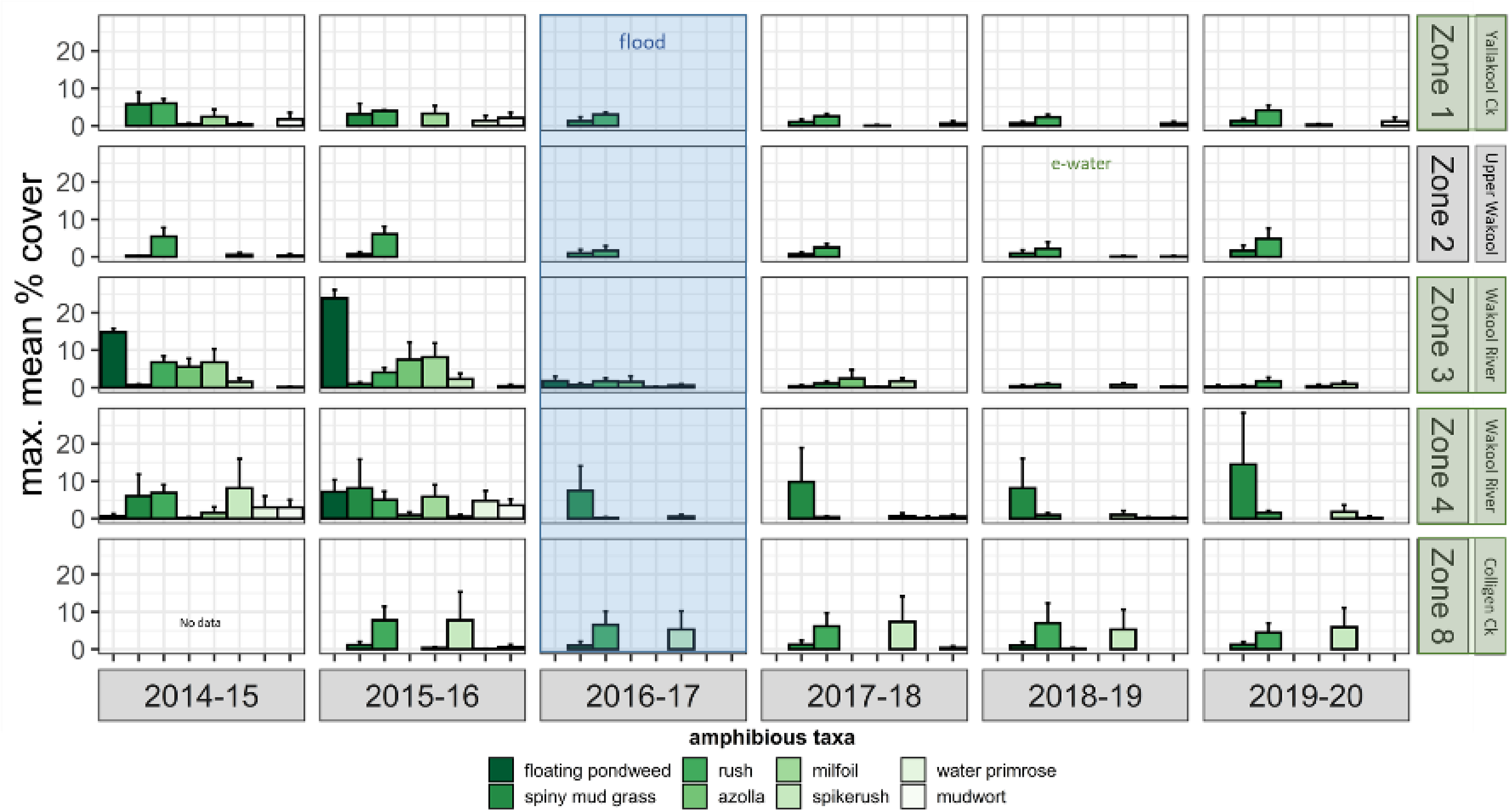


Figure 7.8 Mean percent cover of the eight most abundant amphibious vegetation taxa monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

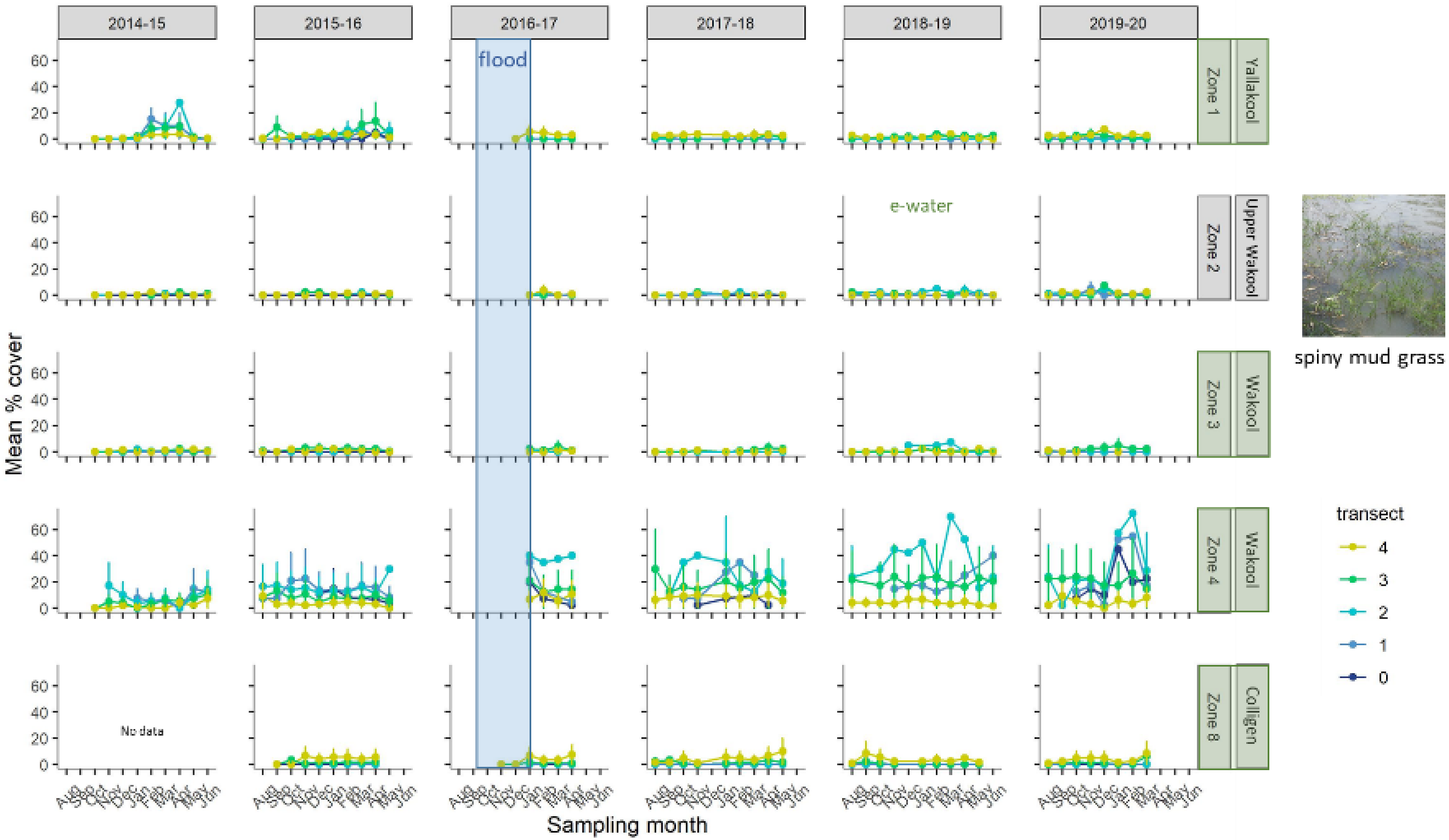


Figure 7.9 Mean percent cover of spiny mud grass (*Pseudoraphis spinescens*) monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Transect zero is lowest on the riverbank and transects are labelled consecutively up to transect 5 highest on the river bank. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

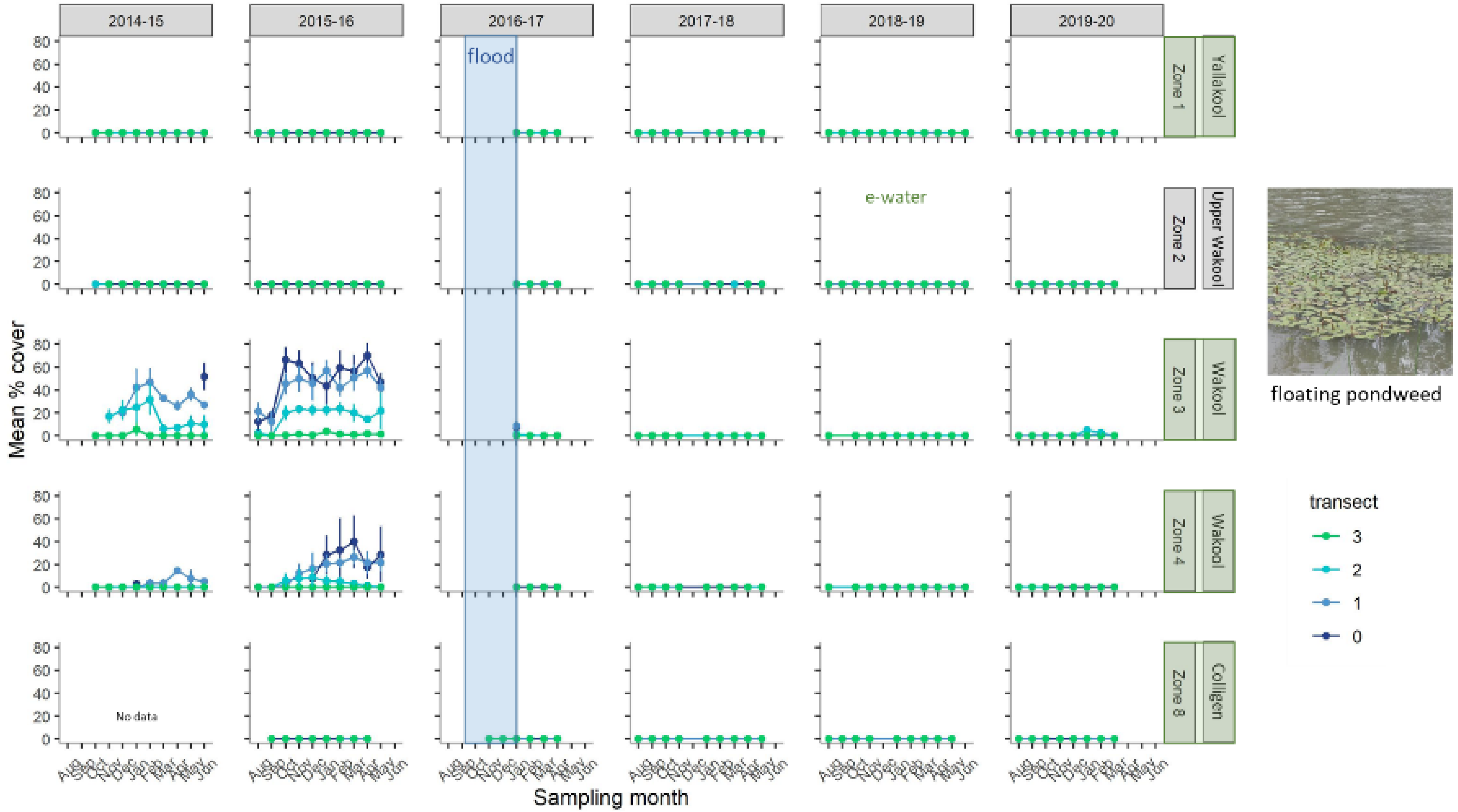


Figure 7.10 Mean percent cover of floating pondweed (*Potamogeton tricarinatus*) monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Transect zero is lowest on the riverbank and transects are labelled consecutively up to transect 5 highest on the river bank. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

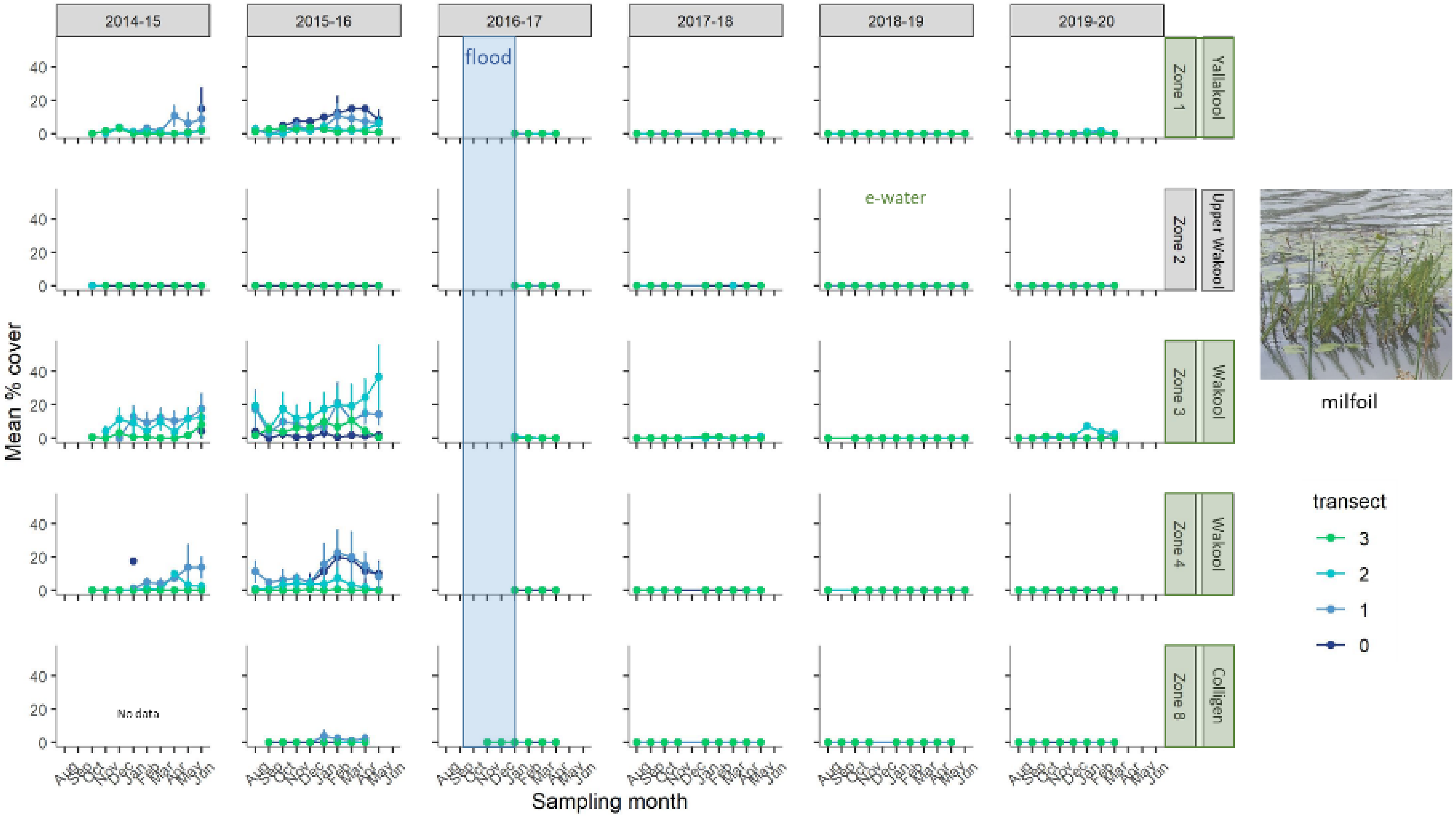


Figure 7.11 Mean percent cover of milfoil (*Myriophyllum spp*) monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Transect zero is lowest on the riverbank and transects are labelled consecutively up to transect 5 highest on the river bank. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

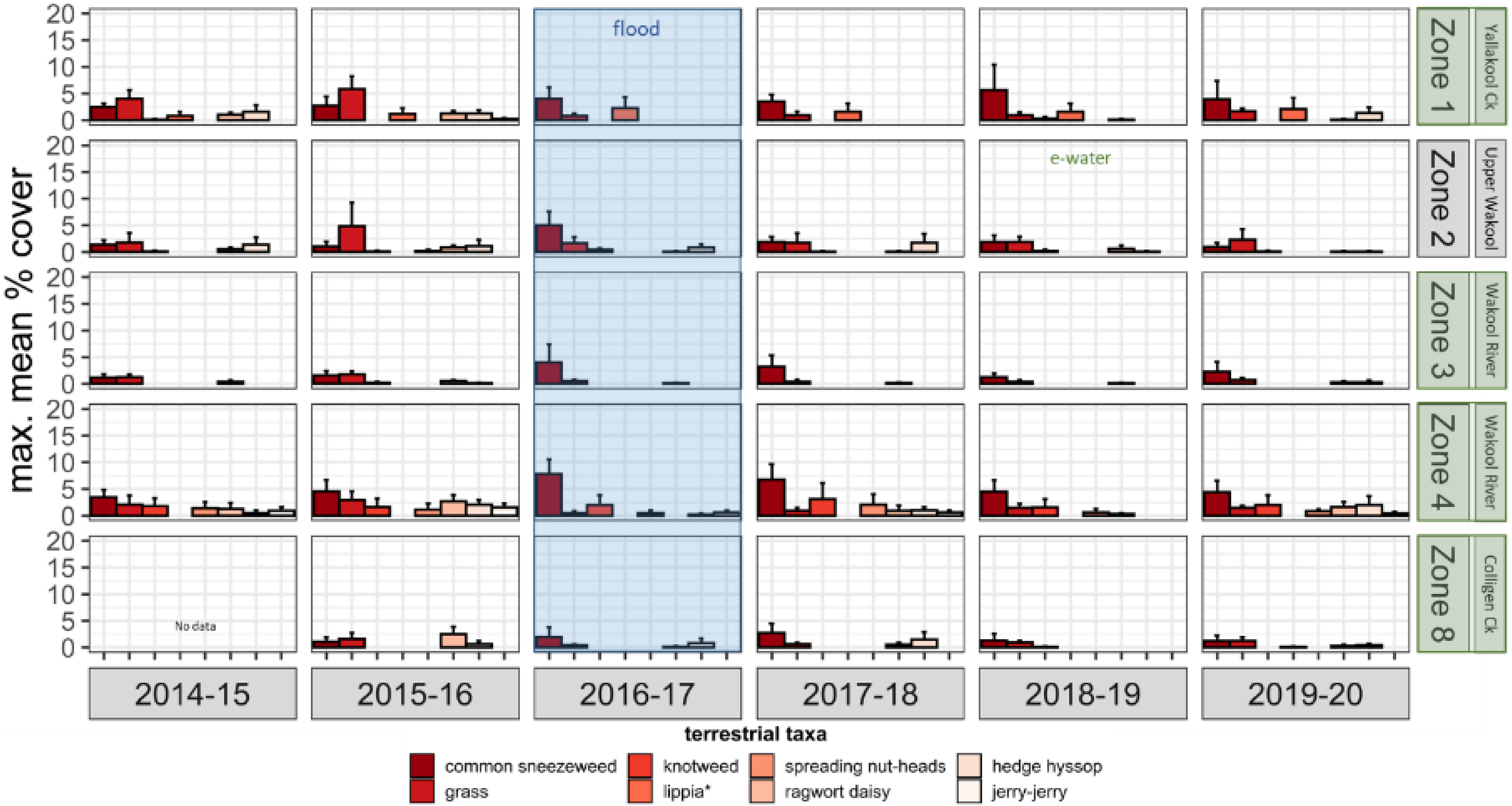


Figure 7.12 Mean percent cover of the eight most abundant terrestrial vegetation taxa monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated

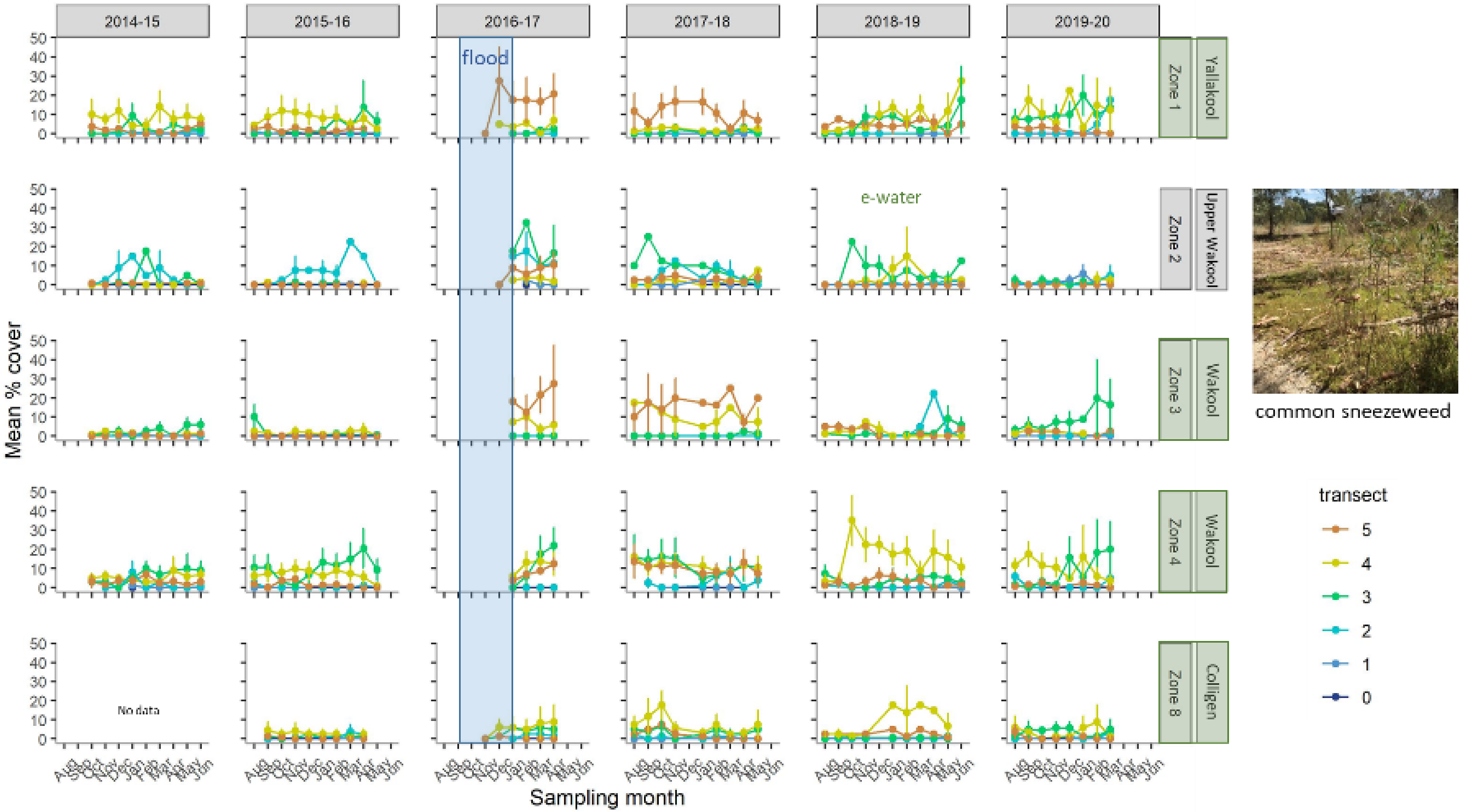


Figure 7.13 Mean percent cover of common sneeze weed (*Centipeda cunninghamii*) monitored monthly across five hydrological zones in the Edward/Kolety-Wakool system between 2014 and 2020. Transect zero is lowest on the riverbank and transects are labelled consecutively up to transect 5 highest on the river bank. Green shading indicates that zones 1, 3, 4 and 8 received environmental water each year. Zone 2 received minimal no environmental water, with the exception being in 2018-19, as indicated.

7.6 Discussion

Riverbank and aquatic plants in the Edward/Kolety-Wakool system continue to recover following the reduction in mean species richness and mean cover that occurred following the unregulated flood in 2016.

Since the flood in 2016 there has been evidence of recovery of submerged and amphibious taxa in 2017-18 and again in 2018-19 (Watts et al. 2018, 2019). In 2019-20 there was further evidence signs of recovery such as an increase in the number of amphibious taxa. However, there was considerable variability in the recovery of different taxa and the response to environmental watering actions has not been consistent among zones. The reason for this is because the dominant taxa in different zones have characteristics that enable them to respond differently to inundation. Amphibious taxa such as floating pondweed and milfoil that were more common in zone 3 are slowly recovering. Some plants of these taxa have been observed outside the monitored transects at several other reaches, suggesting there is the possibility of the recovery of these species following future environmental watering actions. In contrast, other more tolerant taxa such as Chara, rush (*Juncus sp.)* and common spike rush have recovered more quickly following the 2016 flood. This variability in responses can make it difficult to assess the contribution of environmental water in the recovery of vegetation.

The potential for environmental water to promote recovery of vegetation is evident by comparing the response of taxa in zone 2 in 2018-19 and 2019-20. In most years zone 2 receives none or very minimal environmental water and this zone has lower taxa richness than other zones. However in 2018-19 zone 2 received environmental water released from the Wakool escape from Mulwala canal. This resulted in an increase in mean total species richness of amphibious taxa in the upper Wakool River zone 2 and increased cover of terrestrial taxa. Watts et al (2019) suggested this was likely to be in response to the higher flows and increased variability in this river, and particularly increased wetted area of riverbank that is not usually experienced in this system during operational flows. In 2019-20 zone 2 did not receive any environmental water and the richness and cover of submerged and amphibious taxa reduced relative to the previous year. These observations suggest that environmental water is contributing to the recovery of submerged and amphibious vegetation in this system. The results also support the hypothesis that future delivery of environmental water to zone 2 would result in better environmental outcomes.

These observations from 2019-20 combined with previous LTIM/MER results (Watts et al. 2015, 2016, 2017, 2018, 2019) suggest that late winter/early spring freshes that inundate slackwater, in-channel benches or low lying areas of riverbank within the channel can trigger emergence of river bank vegetation. Following the recession of flows, these damp banks provide ideal conditions for plant growth prior to the onset of hotter weather in summer that can quickly dry out the river banks. Further freshes delivered after the initial event that re-wet these areas can provide suitable conditions for amphibious plants to grow and survive the warmer conditions over the summer.

The floods in 2016 decreased the richness and cover of submerged and amphibious taxa throughout the Edward/Kolety-Wakool system. The reduction in the cover of submerged taxa and amphibious taxa may have been due to extreme physical disturbance experienced during the flood which can restrict access to atmospheric carbon dioxide and oxygen, causing anoxic soil conditions and depleted soil biota (Campbell et al. 2019). Some of the sites had overbank flows for over 1 month during late 2016 and most riverbank transects were underwater for 4 to 5 months and higher turbidity levels with values ranging from ~50 to 300 NTU were observed during this period (Figure 5.4). A reduced light climate during the 2016 flood would have potentially prevented submerged and amphibious plants from photosynthesising. Likewise, in a controlled experiment Doyle and Smart (2001) found that higher turbidity levels significantly affected *Vallisneria americana* in terms of producing less leaf production and biomass and causing a higher mortality rate of plants. In the years since the flood the turbidity in this Edward/Kolety-Wakool study sites were generally above the ANZECC (2000) trigger level and in the range ~40 to150 NTU (section 5). This limitation on light penetration may offer, at least in part, a hypothesis as to why the recovery of submerged and amphibious taxa is slow.

On the recession of the flood, some plants were observed to have died and rotted during the long period of inundation. These observations are consistent with findings of previous studies that long duration of inundation, such as can occur during floods or long periods of regulated flows, can reduce growth or survival of riverbank plants (Blom et al. 1994; Johansson and Nilsson 2002; Lowe et al. 2010). The risks to recovery of the submerged and amphibious riverbank plants include disturbance by carp, disturbance by pigs when rhizomes become exposed, damage to riverbanks and reduction of establishing vegetation by stock, and damage from frost if the regulators and system is shut down during the winter.

Long-term evaluation questions

What has Commonwealth environmental water contributed to the recovery (measured through species richness, plant cover and recruitment) of riverbank and aquatic vegetation in Yallakool Creek and the mid and upper Wakool River that have been impacted by operational flows and drought and how do those responses vary over time?

How do vegetation responses to Commonwealth environmental water delivery vary among hydrological zones?

Riverbank and aquatic vegetation in the Edward/Kolety-Wakool system was considerably impacted by the large unregulated flood in spring 2016. Since 2017 there is evidence that riverbank and aquatic vegetation is recovering.

There is evidence that Commonwealth environmental watering actions has contributed to this recovery. Spring freshes have increased opportunities for germination and follow-up freshes contribute to growth and survival. The winter watering action in 2017 would have prevented loss from frost and aided the recovery of vegetation.

In previous years the species richness and cover of vegetation was lower in the upper Wakool River zone 2 (received minimal or no environmental water) than in zones 1, 3 and 4 that had received environmental water. In 2018-19 a pulse of environmental water was delivered to zone 2 in September 2018 during the 800 ML/d flow trial and this was followed by a period of operational flows from the MIL Wakool escape between October 2018 and February 2019. These actions resulted in an increase in total and mean richness of vegetation taxa in zone 2, demonstrating a clear response to environmental watering.

Despite the increase in the total species richness, the mean species richness in zones 1, 3 and 4 has not yet recovered to the same levels as prior to the 2016 flood. Some amphibious taxa such as floating pondweed and milfoil that had high percent cover prior to the flood were considerably reduced in cover or were killed during flood in 2016. In 2019-20, three years after the flood, there re signs that these taxa are beginning to recover. Small plants of these species were observed outside the formal transects in 2018-19 (Watts et al. 2019) and were observed within transects this year in 2019-20. This suggests there is the possibility of the recovery of these species over the next years that can be supported by environmental watering.

Short-term evaluation questions

What did Commonwealth environmental water contribute to the diversity and percent cover of riverbank and aquatic vegetation taxa?

Evidence of responses to individual watering actions include observations of germinating plants on river banks following recession of flows. Observed germination following winter or early spring flows appears to provide better opportunity for seedlings to establish and get roots deeper into the soil prior to hotter summer weather that dries out riverbanks. These observations suggest that late winter/early spring freshes that inundate slackwater, in-channel benches or low lying areas of riverbank within the channel can have positive outcomes on the germination of river bank vegetation. Following the recession of flows, the damp banks provided ideal conditions for plant growth prior to the onset of hotter weather in summer that can quickly dry out the river banks. The best outcome is when there are subsequent freshes (environmental actions or operational flows) that re-wet these areas and provide ongoing conditions that are suitable for amphibious plants to grow and survive the warmer conditions over the summer.

Recommendation for aquatic and riverbank vegetation outcomes

Recommendation: Deliver series of freshes to all rivers in all major tributaries of the Edward/Kolety-Wakool system to increase the wetted area of the bank. Late winter/early spring freshes that inundate slackwater, in-channel benches or low lying areas of riverbank within the channel will trigger emergence of river bank vegetation. Following the recession of flows, these damp banks provide ideal conditions for plants to establish and grow prior to the onset of hotter weather in summer that can quickly dry out the river banks.

Recommendation: Undertake actions that improve the aquatic and riverbank vegetation outcomes in the Upper Wakool River. Deliver larger freshes with increased variability to enable riverbank vegetation to establish and be maintained.

Recommendation: Prevent negative impacts of a-seasonal cease-to-flow events by delivering winter base flows to promote temporal availability and continuity of instream habitat for aquatic vegetation. This will have minimise damage from damage from frost and livestock if the system is shut down during the winter, and result in positive benefits for the survival and maintenance of aquatic and riverbank vegetation.

Recommendation: Consider developing communication products and contribute to engagement programs in collaboration with other agencies (e.g. Local Land Services) to support projects that reduce risks to recovery and maintenance of aquatic and riverbank plants by carp, pigs and livestock. Disturbance of the riverbank caused by carp, pigs and livestock has a high potential to undo the positive outcomes of environmental watering actions.

8 FISH

Authors: Nicole McCasker, Jason Thiem, John Trethewie, Daniel Wright

Key findings		
Movement	Movement of golden perch and silver perch	No discernible differences were observed in the scale of the movements of golden perch or silver perch during the 2019 winter watering event, although we note that sample sizes were low. Movements during winter are typically localised for both species in this system based on previous observed and modelled data, although modelling based on previous water delivery years (2017 and 2018) indicates that CEW deliveries in winter result in a discernible increase in the frequency of movement of golden perch, silver perch and Murray cod, but that it is most pronounced in silver perch. Occupation of Yallakool Creek zone 1 by golden perch was enabled during winter watering in 2019 in comparison to winter 2018 (no watering), indicating that increased habitat was both available and utilised during the watering event.
Spawning	Periodic species	Significantly more bony herring larvae were found in some study rivers that received environmental water compared to the Upper Wakool River, which did not receive environmental water. Despite the highly connected Murray River Channel flow that influenced the Edward/Kolety River, Wakool River and Yallakool Creek, there was no evidence of golden or silver perch spawning recorded.
	Opportunistic species	Significantly more Australian smelt larvae were found in all study rivers that received environmental water compared to the Upper Wakool River which did not receive environmental water.
Recruitment	Murray cod, silver perch and golden perch recruitment	Murray cod YOY recruits were detected in zones 3 and 4 for first time since 2015-16. Murray cod 1+ recruits were at their highest relative abundance since surveys began, although slower growth rates were observed compared to the previous year. Silver perch 1+ recruits were present at a low relative abundance and YOY recruits were not detected. No golden perch 1+ or YOY recruits were detected.
Adults	Adult fish populations	Eight native species and two alien fish species were captured during fish community sampling. Both flathead gudgeon and Eastern gambusia were absent, although these were in low and/or variable abundance in previous years. Limited-to-no carp recruitment was observed in 2020, and the adult population exhibited decreased relative abundance and biomass. The golden perch population continues to exhibit no recruitment, and is predominantly comprised of large adults. The population is ageing but stable. Murray cod relative abundance and biomass continue to increase following fish kills in 2016. Bony herring were present at the highest relative abundance observed in the program, reflecting a strong spawning and recruitment year. Typical annual fluctuations were observed in small bodied generalist species.

8.1 Background

The Edward/Kolety-Wakool River system is recognized as a priority area for fish diversity in the Murray-Darling Basin and is part of the threatened 'aquatic ecological community in the natural drainage system of the lower Murray River catchment' in New South Wales (*NSW Fisheries Management Act 1994*). Outcomes for fish have been a main focus of environmental water delivery in the Edward/Kolety-Wakool system. Historically, the Edward/Kolety-Wakool system had diverse fish communities and supported extensive commercial and recreational fisheries (Rowland 1998). Twenty two native freshwater fish species are thought to have historically occupied the lowland region of the central Murray valley (Table 8.1), including the recently described obscure galaxias (*Galaxias oliros*). Fourteen of these native species still occur within the system. Fish remain a key environmental asset valued by the broader Edward/Kolety-Wakool community.

The overarching principle that underpins the monitoring and evaluation of Commonwealth environmental water for the Edward/Kolety-Wakool Selected Area is that we are taking an ecosystem approach to evaluate Commonwealth environmental watering. A suite of questions and indicators have been selected that all have clear linkages to other components of the Monitoring, Evaluation and Research Plan (Figure 8.1). The Edward/Kolety-Wakool Monitoring , Evaluation and Research Plan (Watts et al. 2019a) has a strong emphasis on the response of fish populations to Commonwealth environmental watering, and includes components directly assessing fish movement, reproduction, recruitment and adult populations. In addition, many of the other indicators evaluated in this report (such as hydrology, water quality, stream metabolism and aquatic vegetation) are likely to have indirect influence on fish population dynamics, and thus a key goal of the long-term intervention monitoring in the Edward/Kolety-Wakool Selected Area is to improve our understanding and interpretation of these interdependences.

Key processes that ultimately shape adult fish populations (movement, spawning, recruitment and growth) have been monitored and evaluated in response to the contribution of Commonwealth environmental water. Monitoring of these key elements are complementary, allowing us to assess contributions of environmental water to the key population processes that structure fish assemblages in the Edward/Kolety-Wakool (Figure 8.1). The responses measured across these key fish indicators will be used in a multiple lines of evidence approach to evaluate competing hypotheses about underlying mechanisms driving or limiting the outcomes from environmental water delivery. For example, if watering achieves increases in production and fish spawning, but not recruitment, it may be possible to identify potential bottlenecks and strategies for overcoming those limitations as part of an adaptive management cycle. Each of the fish indicators being monitored in the Edward/Kolety - Wakool system is described below.

In section 8.6 we bring together our results across the movement, spawning, recruitment and adult sampling to provide an overview of how the fish community in the Edward/Kolety-Wakool responded to watering events and Edward/Kolety-Wakool hydrological conditions in general.

Table 8.1 Fish species of Edward/Kolety - Wakool River system (recorded and expected). Recorded and alien species are those that have been sampled in the region since 2010, and expected native species are species that were historically likely to have been in the lowland central Murray region. Asterisks highlight if local spawning has been detected since LTIM and MER monitoring commenced in 2014. [1]Indicates species have been recorded in the Edward/Kolety Wakool system, but outside the LTIM focal study zones.

Common name	species name	spawning detected 2014-19
Native species – recorded		
Australian smelt	*Retropinna semoni*	*
carp gudgeon	*Hypseleotris* spp.	*
flathead gudgeon	*Philypnodon grandiceps*	*
Murray cod	*Maccullochella peelii*	*
Murray River rainbowfish	*Melanotaenia fluviatilis*	*
unspecked hardyhead	*Craterocephalus stercusmascarum fulvus*	*
obscure galaxias	*Galaxias oliros*	*
river blackfish	*Gadopsis marmoratus*	*
silver perch	*Bidyanus bidyanus*	*
bony herring	*Nematolosa erebi*	*
golden perch	*Macquaria ambigua*	
trout cod[1]	*Maccullochella macquariensis*	
dwarf flathead gudgeon	*Philypnodon macrostomus*	
freshwater catfish[1]	*Tandanus tandanus*	*
Native species – expected		
Agassiz's glassfish (olive perchlet)	*Ambassis agassizii*	
flathead galaxias	*Galaxias rostratus*	
Macquarie perch	*Macquaria australasica*	
mountain galaxias	*Galaxias olidus*	
Murray hardyhead	*Craterocephalus fluviatilis*	
shorthead lamprey	*Mordacia mordax*	
southern purple spotted gudgeon	*Mogurnda adspersa*	
southern pygmy perch	*Nannoperca australis*	
Alien species – recorded		
common carp	*Cyrpinus carpio*	*
eastern gambusia	*Gambusia holbrooki*	*
oriental weatherloach	*Misgurnus anguillicaudatus*	*
redfin perch	*Perca fluviatilis*	*
goldfish	*Carrassius auratus*	

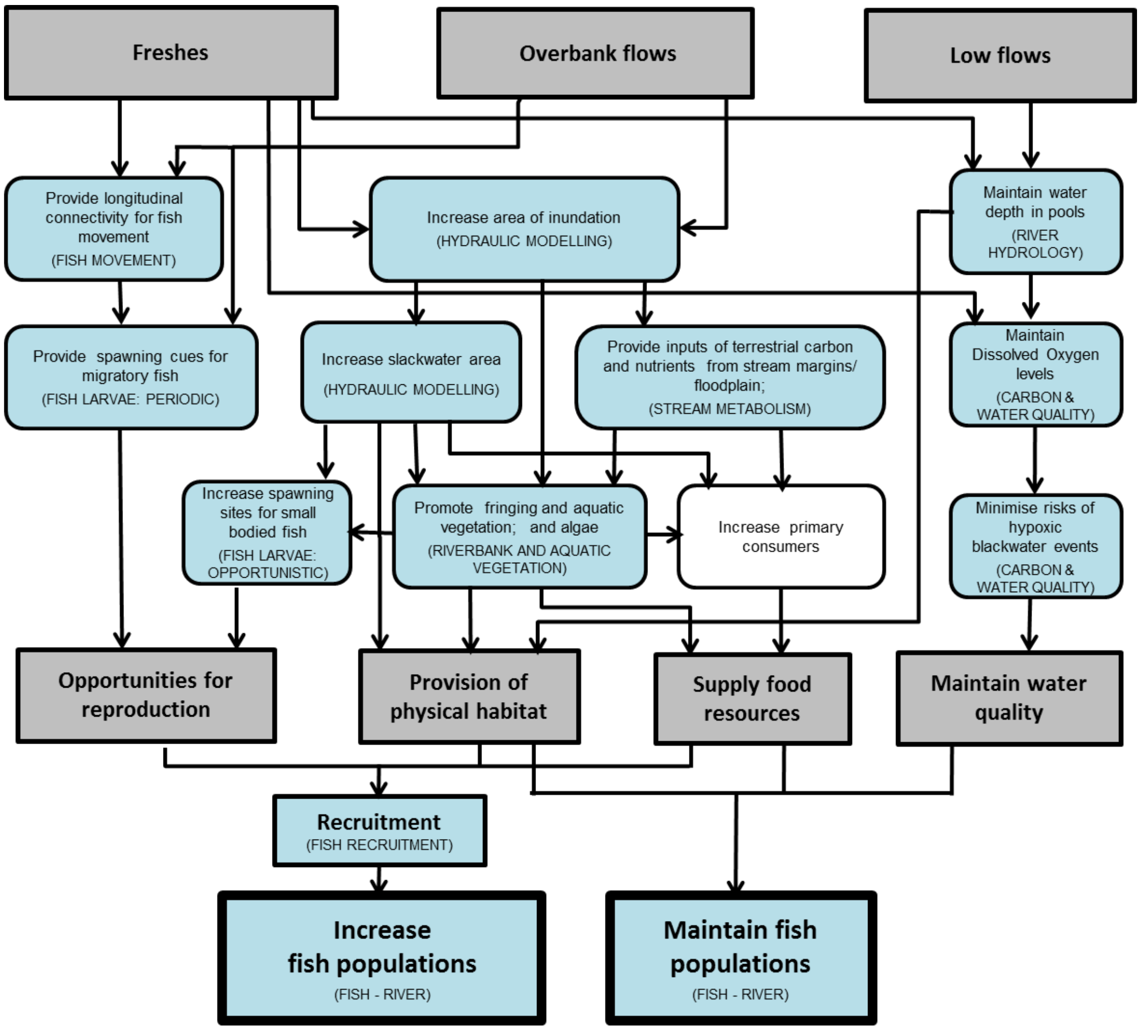


Figure 8.1 Conceptual diagram illustrating the linkages between different types of environmental watering (freshes, overbank flows, low flows) to fish populations via key ecological processes. Key ecological processes that are being monitored as part of the Edward/Kolety-Wakool Monitoring, Evaluation and Research Plan are highlighted in blue.

Fish movement

We used acoustic telemetry methods for investigating broad-scale and fine-scale fish movement of golden perch adults. This information can be used to quantify large scale dispersal, including movements to and from refuge habitats, and serves as a useful additional line of evidence to infer successful reproduction (e.g. Thiem et al. 2013, Walsh et al. 2013). Specifically, the movement response of golden perch already fitted with active acoustic transmitters was monitored from May 2019-September 2019 in response to a winter watering event. More general information in the context of fish movement data collections during LTIM are presented in this report and can also be found in Thiem et al. (2020). Monitoring of fish movement studies will not continue as part of the Edward/Kolety-Wakool MER Plan after 2019, although the data generated through LTIM in this region will be integrated into the basin scale MER research project.

Fish spawning and reproduction

Monitoring the diversity and abundance of fish eggs and larvae across the spring-summer spawning period is used to identify which fish species have successfully spawned, and under what hydraulic and temperature conditions. This information provides will allow us to develop ecologically meaningful flow-spawning relationships for the Edward/Kolety-Wakool fish assemblage and will assist in future planning of environmental water delivery for fish population outcomes.

Recruitment of Murray cod, silver perch and golden perch

Relationships among early life-history growth and recruitment ultimately determine the abundance of many marine fish populations (Pepin et al. 2015), but much less is known about how these factors contribute to populations of freshwater species. It is well established that many species of fish in the Murray-Darling basin do not require over-bank flows, or changes in water level to indicate spawning (Humphries et al. 1999), but nonetheless *recruitment* of all species may be affected by disruption to the natural flow regime, and environmental flows may be able to address this. Fish recruitment monitoring was developed specifically for the Edward/Kolety-Wakool system in order to target juvenile Murray cod, silver perch and golden perch. This monitoring enables comparison of juvenile growth rates among zones of the Edward/Kolety-Wakool and is used to determine recruitment variation of these species among years, in response to environmental watering.

Adult fish community

Evaluation of the adult fish community to Commonwealth environmental watering is being undertaken in the Edward/Kolety-Wakool River system. This work will allow us to determine long-term trajectories in the fish community assemblage in response to Commonwealth environmental watering, and to assess if movement, spawning and recruitment ultimately lead to positive responses (condition, biomass, abundance, diversity) in the adult fish community both within and outside of the MER focal area. It is anticipated that changes to the fish community will occur over longer time scales, and as such a broad-scale monitoring program of the fish community will be undertaken in year three of the MER program (2021-2022). Additionally, annual fish community censuses are undertaken within a single focal zone (Wakool River, zone 3) to provide data for Basin-scale evaluation of fish communities (see https://www.environment.gov.au/water/cewo/publications/cewo-ltim-basin-evaluation-plan) and these data are incorporated into our Selected Area evaluation, where relevant.

8.2 Environmental watering actions

The CEWO's overarching objective for environmental watering for fish populations in the Edward/Kolety-Wakool River system was to provide flows to "support habitat (including longitudinal connectivity and bench inundation), food sources and promote increase movement/dispersal, recruitment and survival/condition of native fish" (CEWO 2019). Off-channel assets such as the Edward/Kolety-Wakool Forests (e.g. Werai State Forest, Neimur State Forest) were also a key target for CEWO watering actions for improving fish outcomes, particularly for floodplain specialists, however these are not monitored as part of the Flow-MER program. There are three Commonwealth environmental watering actions in the Edward/Kolety-Wakool system in 2019-20 that were evaluated by the fish monitoring program of Flow-MER (Table 8.2).

Table 8.2 Commonwealth environmental watering actions in 2019-20 in the Edward/Kolety -Wakool River system that had objectives targeting native fish outcomes. Watering actions assessed by the MER monitoring program are highlighted in grey.

	Watering action	Dates	Rivers	Objectives (from CEWO)
1	Winter base flow	15 May - 9 Aug 2019	Yallakool Creek, mid- and lower Wakool River	For native fish condition and movement Provide refuge habitat during irrigation shut-down period
3a	Winter/spring early fresh	28 Aug - 4 Sep 2019	Yallakool Creek, mid- and lower Wakool River	To provide early season rise in river level to contribute to pre-spawning condition of native fish
				Contribute to spawning in early spawning native fish
3b	Early spring elevated base flow	5 - 22 Sep 2019	Yallakool Creek, mid- and lower Wakool River	To maintain nesting habitat for Murray Cod
3c	Late spring fresh	23 Sep - 11 Oct 2019	Yallakool Creek, mid and lower Wakool River	To promote silver perch spawning To influence and encourage fish movement, coordinated with wider Murray River actions to maximise benefit To assist with dispersal of larvae and juveniles of a number of fish species
3d	Late spring elevated base flow	12 Oct - 30 Nov 2019	Yallakool Creek, mid- and lower Wakool River	To influence and encourage fish movement, may be coordinated with wider Murray River actions to maximise benefit To also assist with dispersal of larvae and juveniles of a number of fish species

8.3 Selected Area evaluation questions

Data from the Edward/Kolety-Wakool system is being evaluated at the Selected Area scale and will further contribute to Basin scale evaluation. Basin-scale evaluation involves the integration of multiple datasets from a number of different catchments (Hale et al. 2014), and this will be undertaken by University of Canberra/CSIRO and evaluated in a separate report.

This is the first year of reporting for the MER project. Much of the work reported here is a continuation of the monitoring undertaken during LTIM program (2014-19). The short and long term Selected Area evaluation questions, as outlined in the Monitoring, Evaluation and Research Plan for the Edward/Kolety-Wakool system (Watts et al. 2019) are outlined in Table 8.3. This report will evaluate environmental water against the short-term questions, with long-term evaluation questions being further assessed in 2022.

Table 8.3 Selected Area evaluation questions relating to the effect of Commonwealth environmental water on Edward/Kolety-Wakool fish populations relevant to this report.

Monitoring component	Selected Area-scale short term evaluation questions
Fish movement	• Does Commonwealth environmental water facilitate longitudinal connectivity for periodic species during winter?
Fish spawning and reproduction	• What did CEW water contribute to the spawning of 'opportunistic' species? • What did CEW contribute to spawning in 'flow-dependent' spawning species?
Recruitment and growth of young of year	• What did CEW contribute to native fish recruitment to the first year of life? • What did CEW contribute to native fish growth rates during the first year of life?
Adult fish population demographics	• Does CEW contribute to the maintenance or enhancement of fish condition in the Edward/Kolety-Wakool river system? • Does CEW contribute to the recovery of fish communities following negative conditions within the Edward/Kolety-Wakool river system

8.4 Methods

8.4.1 *Fish movement*

A total of 71 acoustic receivers (VEMCO VR2W) were installed in the Edward/Kolety-Wakool system in August 2015. Of these, 51 constituted the fine-scale acoustic receiver array (Figure 8.2) of ~6 km receiver spacing and 20 additional receivers were placed at key entry/exit points and major junctions within the wider Edward/Kolety-Wakool system to monitor any potential emigration out of the system. The installation of these receivers was specifically supported by the local community and undertaken by funds received by Murray Local Land Services through the National Landcare Programme. A total of 79 golden perch, 21 Murray cod and 43 silver perch have been fitted with telemetry tags between August 2015 and September 2017. Acoustic tag implantation procedures followed those outlined by Hale et al. (2014). Here we report on overall movement trends following 4+ years of data collection as well as specific movements in response to watering events in winter 2019. Sample sizes of tagged fish were low in 2019-20 as a result of battery life expiration and emigration from the study reach. Therefore we were careful to use descriptive statistics when sample sizes are low (e.g. using remaining fish during the winter 2019 period to determine the effects of a watering action) and inferential statistics for populations when sample sizes are high and sufficient (e.g. using all tagged fish over the entire period to develop relationships between flow and movement probability).

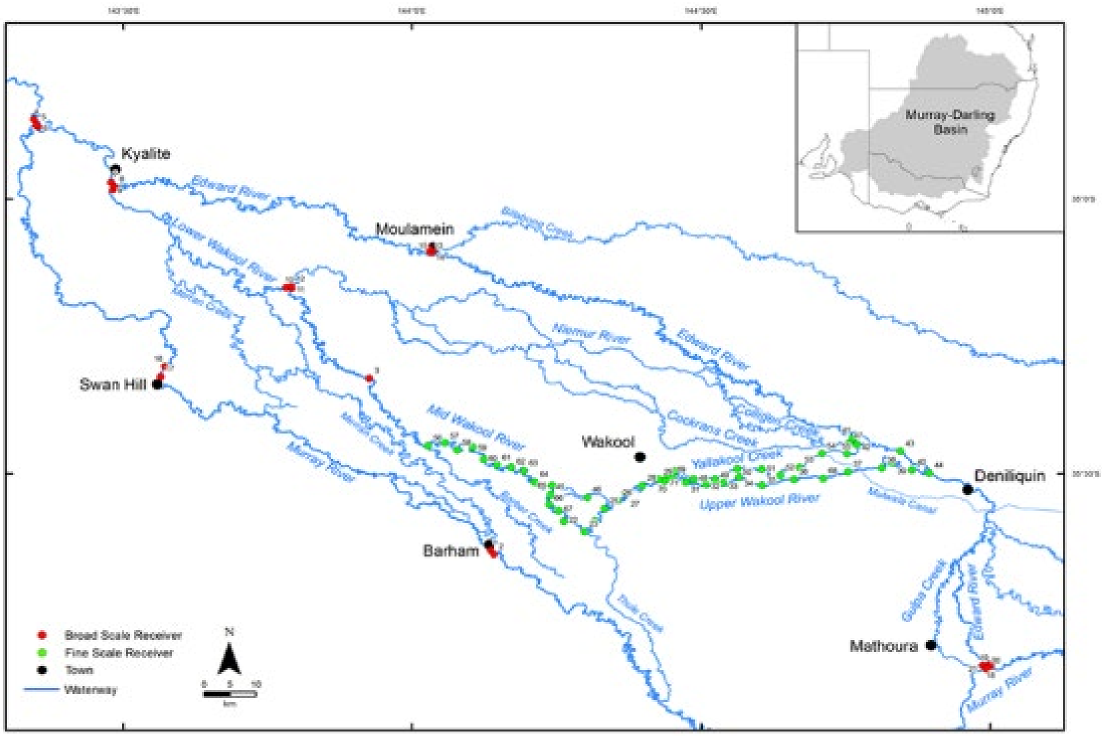


Figure 8.2 Location of acoustic telemetry receivers (green dots) moored in the Edward/Kolety-Wakool system to determine movements of acoustically tagged golden perch and silver perch. Red dots indicate the 20 additional receivers placed at key entry/exit points and major junctions to monitor any potential emigration out of the system. The installation of these receivers was supported by the local community and undertaken by funds received by Murray Local Land Services through the National Landcare Programme.

Acoustic receiver downloads are undertaken quarterly (Figure 8.3). Downloaded acoustic tag detection data and meta-data are uploaded into a custom SQL database. Data are subsequently screened and all duplicates, false detections and orphan tags quarantined prior to storage. Individual movements of fish were recreated over time to determine 1) location within the Edward/Kolety-Wakool system at any given time and, 2) timing and distance of movements. As receivers were spaced at ~6 km intervals, this represents the minimum distance of movements within the receiver array and detection on multiple receivers is required to determine location and direction of movement. Individual fish were assigned a location based on their previous location until any new location (i.e. detection at a new/different location) was determined. Where a new location was not determined (i.e. an individual was never detected again), individual records were truncated to the last verified detection location and date. This data may represent emigration from the acoustic array (and hence the entire Edward/Kolety-Wakool system), an individual between two receivers and not moving, tag failure (battery expiration) or mortality.

We used generalised additive mixed models (GAMM) with a binomial distribution to model the probability of movement for each of the three species in response to river flows and time of year. We aggregated daily river flows (ML day^{-1}) to a five day time step, as mean daily flow for each five day period. We classified individuals as having moved during a 5 day time step when they were tracked at a receiver that was different from their last known location (i.e. moved a minimum of ~6 km). A random intercept was included in each of the models to account for the fact that individual fish were considered a random draw from the overall population with potentially different tendencies toward movement. Simulations were then run for each calendar year for which there were adequate data (2017 and 2018) for each species to visualise movement responses to varying flows at different times of year. The contribution of river flows with and without CEW was used as a visualisation tool, and represents simulated data generated from GAMM probabilities. The period of flooding in late 2016 was not included in the simulations as the flood magnitude overwhelms the response signal (meaning that comparison among years is unrealistic) and is outside the bounds of any water deliveries and general regulated conditions within the system.

Figure 8.3 Clockwise from left: An acoustic receiver ready for deployment and an acoustic tag for scale, downloading information from tagged fish passing an acoustic receiver and, an anaesthetised silver perch undergoing surgical implantation of an acoustic tag.

8.4.2 *Fish spawning*

Fish larvae and eggs were sampled fortnightly within the Edward/Kolety - Wakool Selected Area from 9 September 2019 – 5 March 2020 (13 sampling trips). A combination of modified quatrefoil light traps and drift nets were used in all four study zones; Yallakool Creek (Zone 1), Upper Wakool River (Zone 2), Mid Wakool River upstream of Thule Creek (Zone 3), and Mid Wakool River downstream of Thule Creek (Zone 4).

As part of the routine fish larval sampling for the Edward/Kolety-Wakool Selected Area (Category 3), three modified quatrefoil light traps were deployed overnight at five sites within the four study zones each trip. The occurrence of fish larvae throughout a given river reach is patchy, and so to account for this, the three light traps deployed were pooled to create one composite light trap sample per site.

Drift nets were also used for sampling larvae (Category 1 and 3 methods), albeit over a shorter period than that of the light trap surveys. Drift nets are used in addition to the light traps as they are more effective in detecting eggs and early-stage larvae of flow-dependent spawning species, such as golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*). Cat 3 drift net methods consisted of drift nets deployed fortnightly for six sampling trips from 8 October– 20 December 2019. Here, three drift nets were deployed overnight at one site in each of the four study zones. The volume of water filtered by the nets was calculated using Oceanic® flow meters positioned at the mouth of each net. Volume sampled by the net was estimated as:

$$\pi r^2 \cdot v \cdot t,$$

where *r* is radius in metres, *v* is mean velocity in m/s, and *t* is time set in seconds. Drift net samples were also collected fortnightly from the 23 September – 20 December 2019 from Wakool River US Thule Creek Zone 3 (n=7 sampling trips), for Category 1 Basin Matter Analysis, however this data is not reported on here. Drift nets were deployed in the late afternoon, and retrieved the following morning. Up on retrieval, drift nets were rinsed down and entire samples preserved in 70% ethanol, and returned to the laboratory for processing.

All eggs and fish larvae collected in light trap and drift net samples were identified to species according to Serafini and Humphries (2004), and enumerated. Carp gudgeon larvae were identified to genus level (*Hypseleotris* spp.) only. Genetic analyses undertaken for all eggs collected, and as well on a sample of early-caught cod larvae, since Murray cod and trout cod larvae have similar morphological features and cannot be easily distinguished visually. Consequently, a subsample of larvae comprising of possible Murray cod or trout cod were submitted to the Australian Genome Research Facility (AGRF). Nucleic acid extraction and subsequent verification of species assignment was based on dual direction sequencing following PCR amplification. Results of the PCR amplification revealed all larvae to be Murray cod, and thus, from here on, we consider all cod larvae collected in the study zone to be Murray cod. The developmental stage of each individual was recorded as egg, larvae, or juvenile/adult, according to classifications of Serafini and Humphries (2004). Only the trends in abundances of eggs and larvae are reported.

To address the short-term selected area evaluation questions relevant to spawning and reproduction, we tested to see if the total abundance of larvae (as an indication of magnitude of spawning across a season) varied significantly between the four study zones. We used generalised linear models to test differences in larval catch for individual species where 'zone' (zone 1, zone 2, zone 3, zone 4) was treated as a categorical, fixed effect. We hypothesised that the total production of fish larvae across the 2019-20 spawning season would be significantly higher in the study zones that received environmental watering actions (zone 1,3 and 4) compared to zone 2 which did not receive any environmental water. Larval and eggs counts collected from light traps and drift nets were pooled for this analysis and restricted to the species were more than 50 individuals were collected.

The distribution of larval counts were non-Gaussian and highly skewed, so a Gamma distribution with a log-link function was used for all statistical models. Statistical analyses were carried out using the freeware R (version 3.3.2, R core team 2020). F-tests were used to test the significance of the zone. P-values of <0.05 were used to determine the significance of each test. When significant differences were indicated, pairwise comparisons were undertaken to determine differences in estimated marginal means between the zones using the package 'emmeans' (Length 2020, v.1.5.0).

8.4.3 *Fish recruitment*

Four sites were sampled in each of four river zones within the Edward/Kolety-Wakool system: Yallakool Creek Zone 1, Wakool River Zone 2, Wakool River Zone 3 and Wakool River Zone 4. Each of the 16 sites were sampled once in a randomly selected order between February and March for six years: 2014-15; 2015-16; 2016-17; 2017-18, 2018-19 and 2019-20.

Three sampling methods including backpack electrofishing, standardised angling and baited set-lines were undertaken to sample recruits of Murray cod, golden perch and silver perch at each of the 16 sites. In 2019-20 boat electrofishing was added as sampling method for the sites that were too deep to effectively sample with backpack electrofishing. A sub-sample of less than 25 Murray cod per zone were euthanized and frozen to determine the age and growth rate of recruits, while all other fish were released alive excluding carp which were euthanized.

Backpack electrofishing, using a 12 V DC battery with a Smith-Root LR-20 unit was undertaken at sites 1, 4 and 5 in Zone 2 by an operator and one person equipped with a 5 mm mesh dip-net. Each of these sites was sampled for a minimum of 3000 seconds of backpack-on electrofishing time. All other sites were sampled using a Smith-Root 2.5 GPP boat-mounted electrofishing unit for a minimum of 1400 seconds of electrofishing time. Presence of non-target species was recorded at each site, while total length measurements and counts were made for all individuals of the three target species. Standardised angling was carried out by two anglers with the specific aim of targeting young silver perch and golden perch. Standardised angling at each site consisted of two anglers fishing on the bank for two hours. Angling gear was matched to the specifications commonly used by local fisherman with worms and cheese used as bait on size 10 circle hooks. Species and length were recorded for all individuals caught.

Ten set-lines, each with a 3-10 m (100 lb) monofilament main-line and two 0.5-1.5 m (4 lb) leaders were set at each site. Lines were set, baited with worms and cheese and hauled hourly during day-light hours for 5-7 hours at each site. Hook type and bait matched those in the standardised angling section. Species and length were recorded for all individuals caught.

To determine the annual age of 1+ recruits and daily age of YOY, sagittal otoliths were extracted, embedded in a polyester resin and sectioned in the transverse plane to approximately 100 μm thick and mounted on a microscope slide. Final age estimates were based on samples with matching age readings from three reads.

Recruitment catch per unit effort (CPUE; number of recruits per 10 000 s of sampling) of YOY and 1+ Murray cod and 1+ silver perch were calculated from catch and effort data from backpack electrofishing, set-lines and angling.

8.4.4 *Adult fish community*

System-wide fish community surveys were undertaken as part of LTIM in year 1 (2014-15) and year 5 (2018-19) of the program (Watts et al. 2014). As part of the continuation of this monitoring in MER, the next system wide fish community survey will take place in 2021-22. In the absence of fish community data for the current monitoring year we present Category 1 fish community standardised survey data from the mid Wakool River - zone 3. Standardised sampling was undertaken in June–July 2020, and each site was sampled once using a suite of passive and active gears including boat-electrofishing (n=32 operations, each consisting of 90 seconds ‘on-time’), unbaited bait traps (n=10) and small fyke nets (n=10) (Hale et al. 2014). All captures (fish and other non-target taxa) were identified to species level and released onsite. Where large catches of particular species occurred, a sub-sample of individuals was measured and examined for each gear type. The sub-sampling procedure consisted of firstly measuring all individuals in each operation until at least 50 individuals had been measured in total. The remainder of individuals in that operation were also measured, although any individuals of that species from subsequent operations of that gear type were only counted.

8.5 Results

8.5.1 *Fish movement*

General observations

A total of 78 golden perch, 21 Murray cod and 42 silver perch contributed movement data from August 2015 until September 2019 (Figure 8.4). Given the mortality and emigration of all three species associated with the flooding and subsequent hypoxia within the system in late 2016, additional tagging of golden perch and silver perch was undertaken in 2017 (Figure 8.4).
Outside of periods of flooding, movements of golden perch and Murray cod were generally over 10’s of kilometres and movements of silver perch over 100’s of kilometres (Figures 8.5-8.7). Emigration was only observed during flooding in 2016, coinciding with maximum daily movements of 50.7 km for golden perch, 33.0 km for Murray cod and 48.1 km for silver perch. Increased movement was generally observed in spring and summer for all three species, including outside of 2016 flooding (Figure 8.8.).

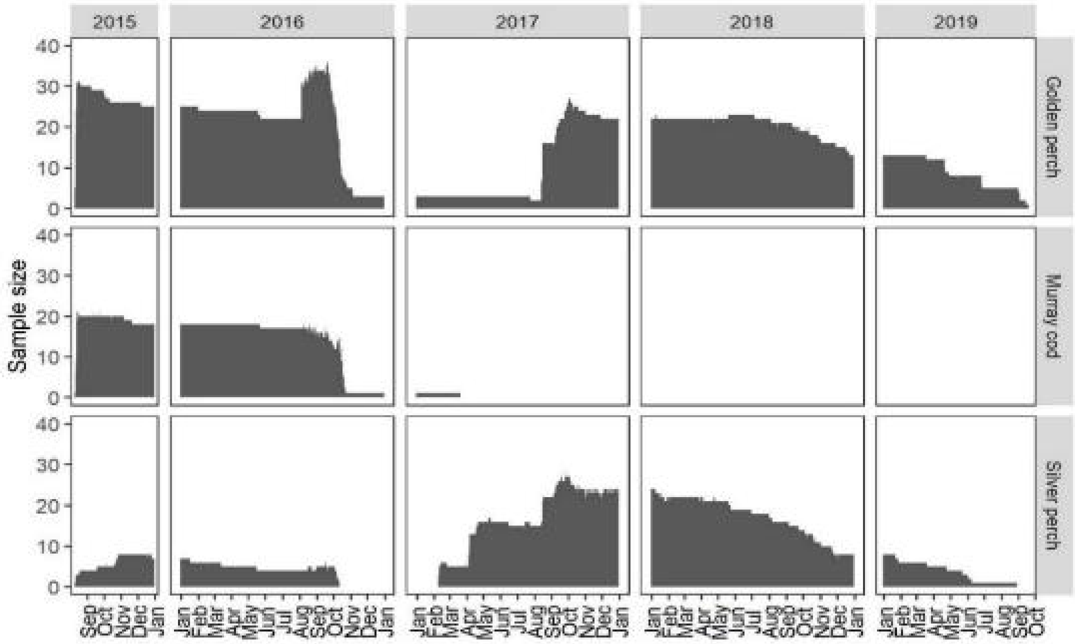


Figure 8.4 Sample sizes of golden perch, Murray cod and silver perch fitted with acoustic tags and contributing to fish movement data on any given day in the Edward/Kolety-Wakool system. Note that individual records are truncated to the last valid detection on an acoustic receiver, and after this period individuals may have either left the array, may occupy a position between two receivers, or may be considered a mortality.

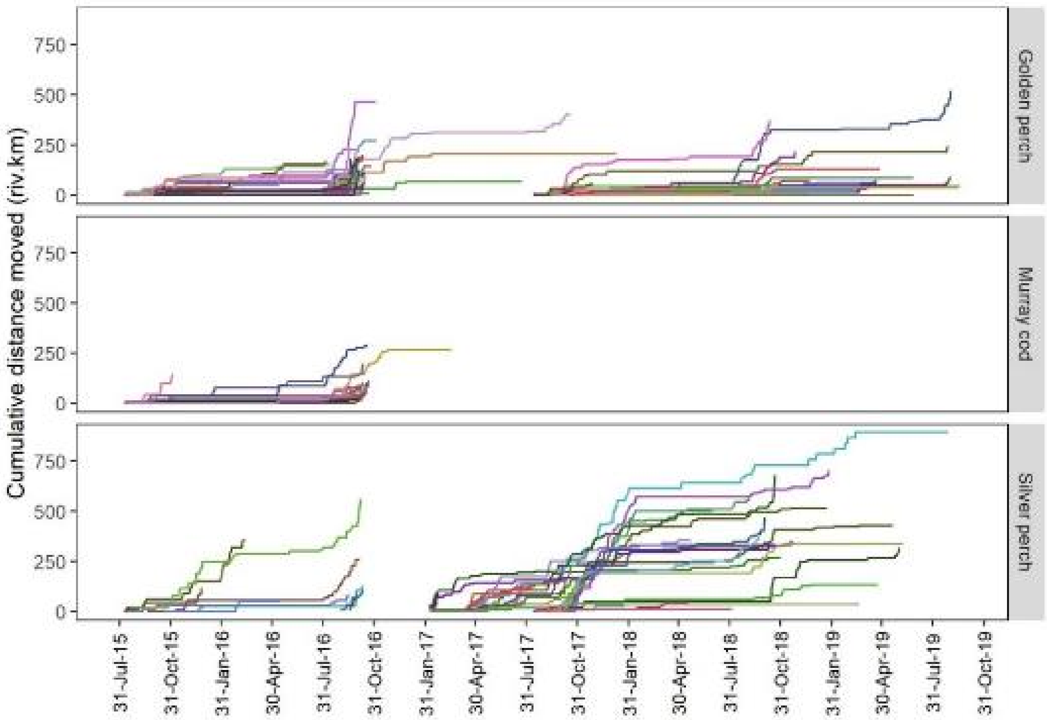


Figure 8.5 Cumulative daily distance moved (irrespective of direction) of acoustically tagged golden perch, Murray cod and silver perch in the Edward/Kolety-Wakool system between August 2015 and September 2019. Different lines represent different tagged individuals and 0 km represents the first detection of an individual fish. Note that when the individual line finishes this represents the last detection of this individual fish within the acoustic array.

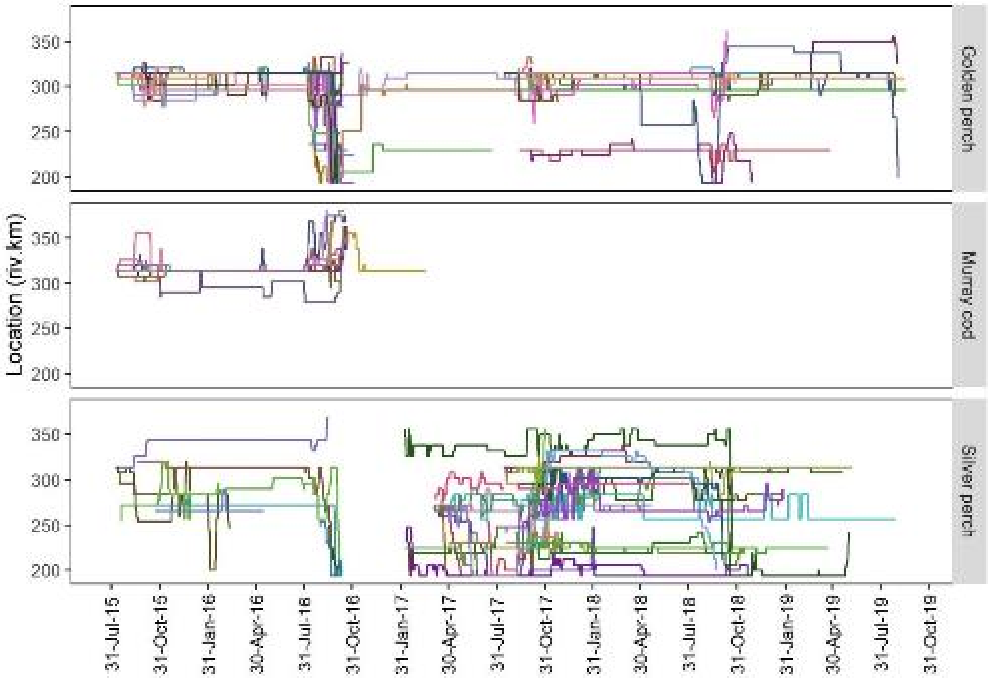


Figure 8.6 Daily locations of acoustically tagged golden perch, Murray cod and silver perch in the Edward/Kolety-Wakool system from August 2015 to September 2019. Different coloured lines represent different tagged individuals and the km value represents the location (distance in km from the junction of the Wakool and Murray rivers).

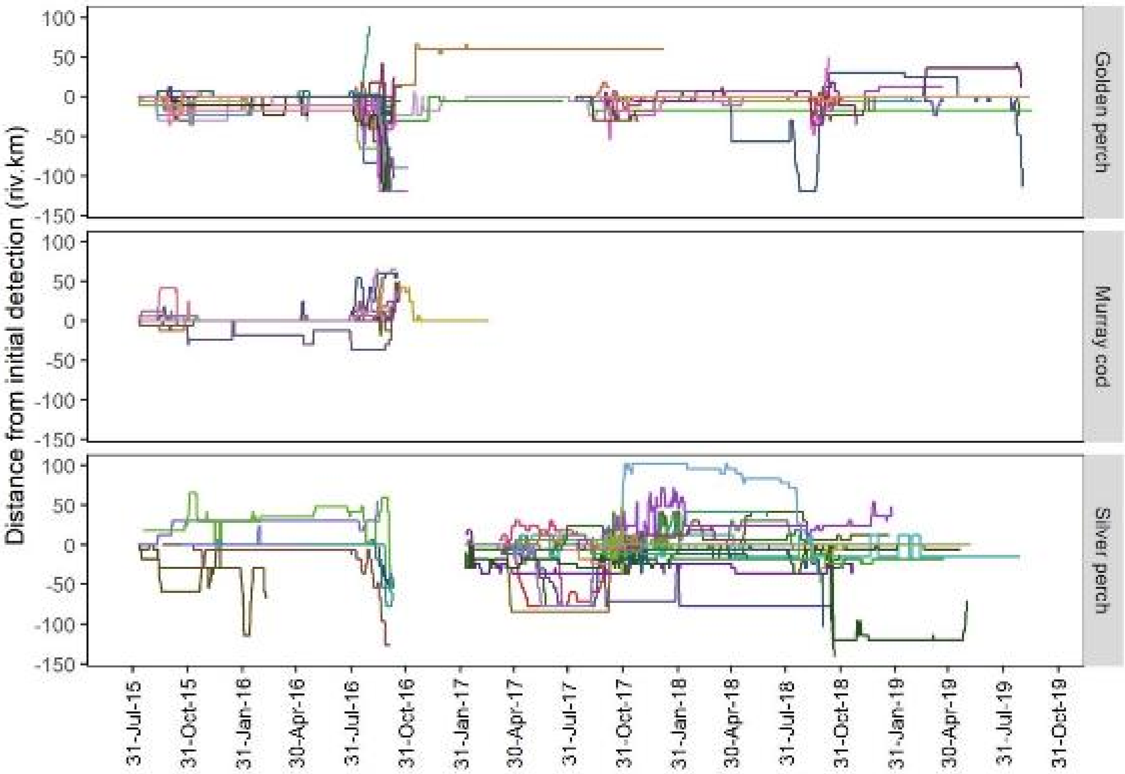


Figure 8.7 Daily locations of acoustically tagged golden perch, Murray cod and silver perch in the Edward/Kolety-Wakool system from August 2015 to September 2019. Different coloured lines represent different tagged individuals and 0 km represents the initial detection (i.e. first location).

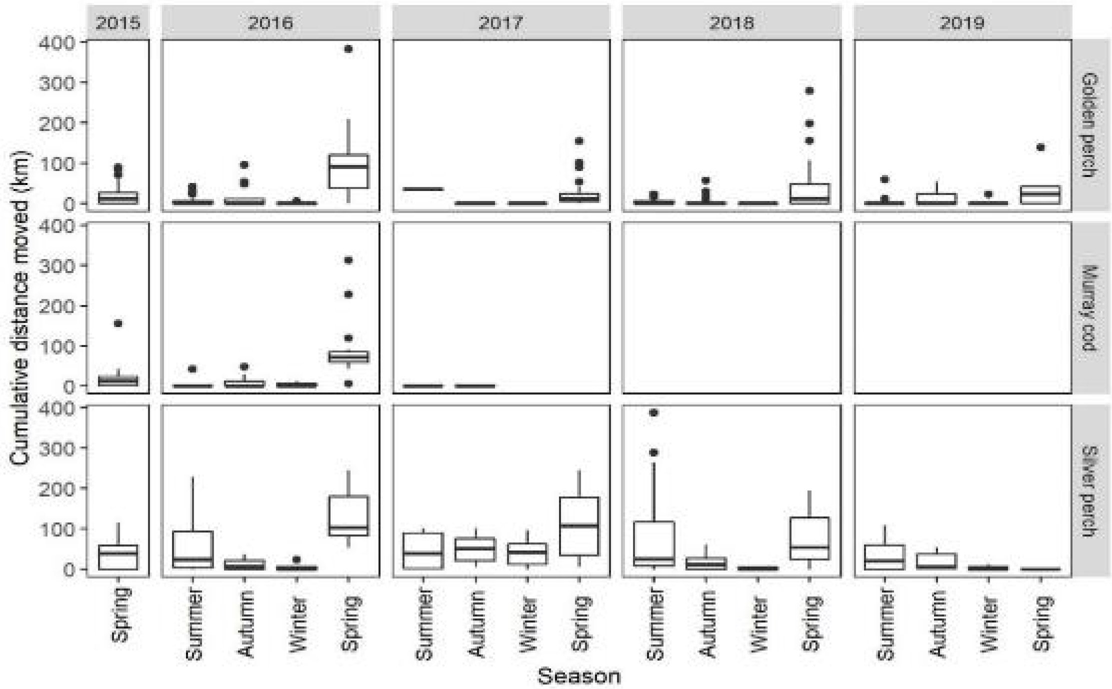


Figure 8.8 Seasonal cumulative movements of golden perch, Murray cod and silver perch in the Edward/Kolety-Wakool system for the duration of the study. Data are represented as median, 25th and 75th percentiles (box) and 5th and 95th percentiles (whisker).

Winter watering action

From 1 August 2017 to 31 August 2019, a two-year period in which a watering action took place in winter 2019, a total of 28 golden perch and 29 silver perch contributed to movement data (Figure 8.4). Silver perch mostly resided in LTIM zones 3 and 4 in 2018–2019 (between August 2018 and August 2019) including during Action 1 – winter base flow in 2019, switching from all zones in 2017–2018 (between August 2017 and August 2018) (Figure 8.9). Golden perch rather resided predominantly in zones 1, 3 and 4 in 2018–2019 during Action 1, which was an expansion compared to only zones 3 and 4 in 2017–2018 (Figure 8.9). Of the 18 silver perch recorded in 2018–2019, 0 (0%) were in Yallakool Creek (zone 1) and 2 (11%) were in the upper Wakool River (zone 2). In 2017–2018, 5 (17%) and 4 (14%) of the 29 silver perch were detected in zones 1 and 2, respectively. For golden perch, 3 (14%) of 22 individuals were recorded in zones 1 and 2 in 2018–2019, while 2 (7%) of 28 individuals were in zones 1 and 2 in 2017–2018 (Figure 8.9).

The proportions of moving fish and the distances moved differed slightly between winter 2019 when Action 1 occurred and the previous winter in 2018 (Figure 8.9). For golden perch, 3 (38%) of 8 individuals moved in winter 2019 compared to 5 (22%) of 23 individuals in winter 2018 (Figure 8.9). Although, only 1 (33%) of 3 silver perch moved in winter 2019, whereas 9 (47%) of 19 silver perch moved in winter 2018. The Action 1 - winter base flow in 2019 resulted in silver perch movements (median and 5^{th} – 95^{th} percentiles) of 0.0 km (0.0–10.8 km) compared to 0.0 (0.0–5.8) km in winter 2018. In contrast, golden perch moved 0.0 (0.0–15.1) km in winter 2019 relative to 0.0 (0.0–0.0) km in winter 2018 (Figure 8.10). Modelled data indicates a measurable benefit, in terms of fish movement, as a result of previous winter water delivery (2017) compared to no winter water delivery (2018) with silver perch responding more than golden perch and Murray cod to the watering action (Figure 8.11).

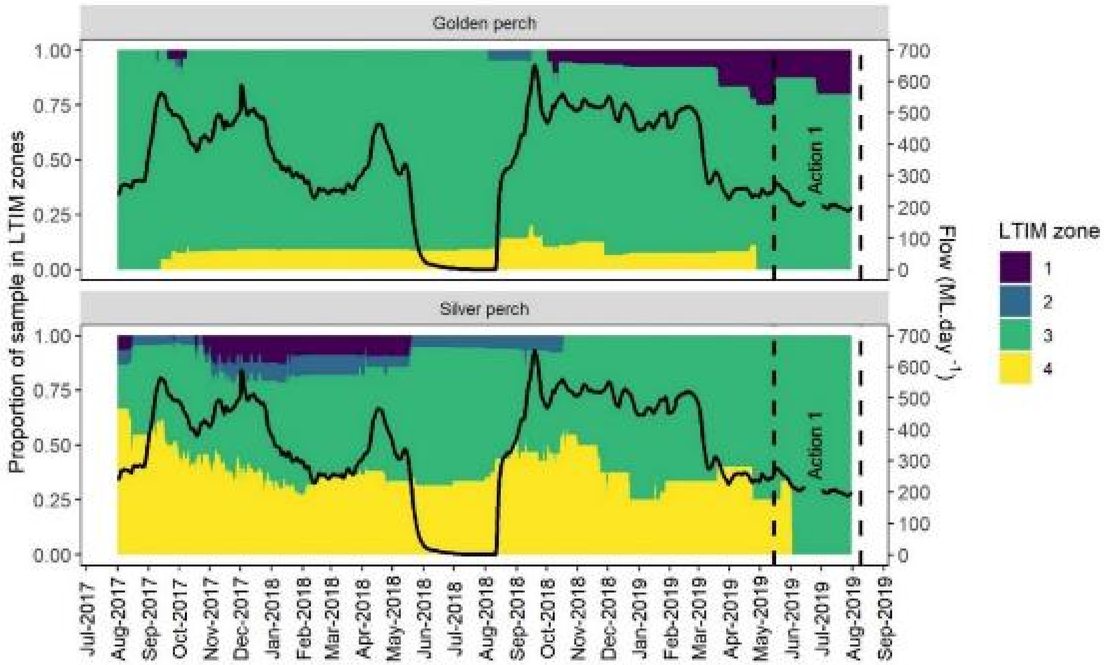


Figure 8.9 Proportionate daily location of acoustically tagged golden perch and silver perch within each LTIM focal zone from August 2017 until September 2019. Daily discharge is also plotted (black line) to help explain fish movements between zones. The period between dashed lines represents Action 1 - winter base flow from 15 May – 9 August 2019.

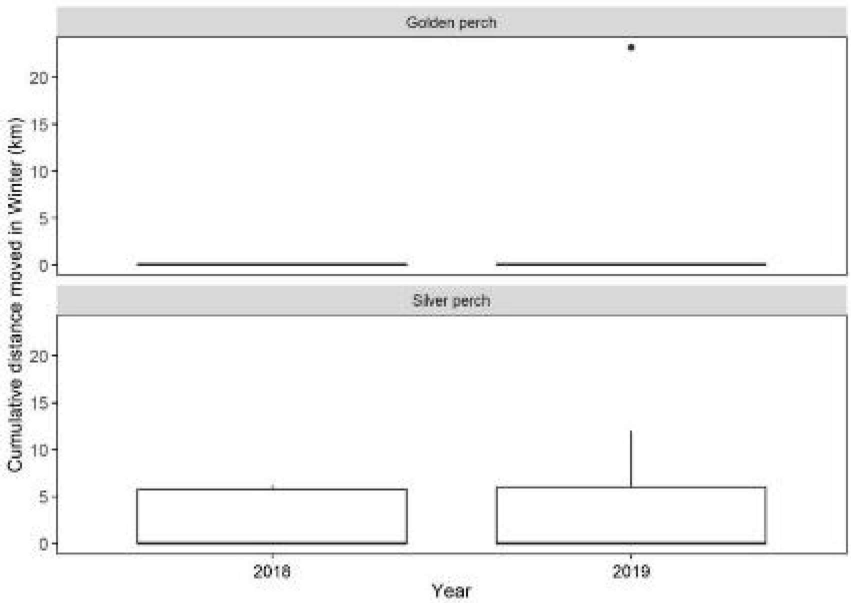


Figure 8.10 Cumulative movements of golden perch and silver perch in the Edward/Kolety-Wakool system in winter 2018 compared to winter 2019 during the Commonwealth environmental watering action. Data are represented as median, 25th and 75th percentiles (box) and 5th and 95th percentiles (whisker).

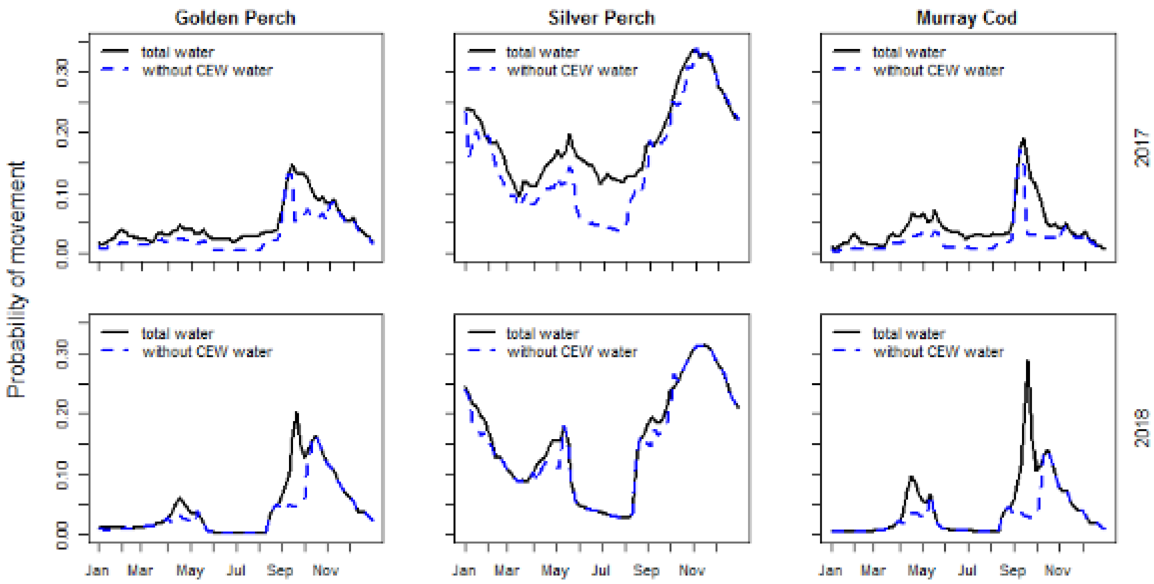


Figure 8.11. Modelled probability of golden perch, silver perch and Murray cod movement, as determined by acoustic tracking, in relation to flow events with and without CEW in 2017 and 2018 in the Edward/Kolety-Wakool river system.

8.5.2 *Fish spawning*

A total of 3,052 fish eggs and larvae, representing nine species, were collected in the 2019-20 monitoring year from a combination of light traps (n=2,744) and drift nets (n=308) (Table 8.4). The total number of larvae collected in 2019-20 compares with previous monitored years characterised by within-channel flows conditions (LTIM: 2014-15= 4249, 2015-16= 3418, 2016-18 = 4428 and 2018-19 = 3,509).

Overall, eight of the nine fish species collected as larvae in 2019-20 were native. Carp gudgeon larvae were the most numerically abundant and widespread larvae collected (*Hypseleotris* spp., n=2,343, 78% of total catch). Found across all four of the focal zones, over half of the carp gudgeon larvae (63%) were collected from the Mid. Wakool River – Zone 3. Murray cod (*Maccullochella peelii*, n=440 larvae, n=2 eggs) and Australian smelt (*Retropinna semoni*, n=191 larvae, n=8 eggs) larvae were the next most abundant, and detected in each of the four study zones (Table 8.4). Other less commonly collected larvae were those of flathead gudgeon (*Philypnodon grandiceps*, n=20), bony herring (*Nematalosa erebi*, n=11), obscure galaxias (*Galaxias oliros*, n=3), river blackfish (*Gadopsis marmoratus,* n=9), and unspecked hardyhead (*Craterocephalus stercumsacarum fulvus*, n=1). Carp (*Cyprinus carpio*) was the only introduce species captured as larvae in the 2019-20 spawning period, and similarly to other non-flood years, only collected in very small numbers (n=2).

Table 8.4. Total abundance of fish larvae sampled using light traps (LT) and drift nets (DN) in the four study zones of the Edward/Kolety-Wakool River system in spring/summer 2019-20. Eggs are denoted by subscript e. Fish species listed are those known to occur in the Edward/Kolety-Wakool river system. To date, trout cod have not been detected in the four study zones, however they are known to be present in the wider Edward/Kolety Wakool Selected Area.

Common name	**Yallakool Ck Z1**		**Wakool R Z2**		**Wakool R Z3**		**Wakool R Z4**		**Total**	
	LT	DN	LT	DN	LT	DN	LT	DN	LT	DN
Native										
Australian smelt	72	-	2	8_e	96	-	44	-	214	(8_e)
carp gudgeon	34	-	503	60	1495	-	251	-	2283	60
flathead gudgeon	-	-	-	-	-	-	18	-	18	-
dwarf flathead gudgeon*	-	-	-	-	-	-	-	-	-	-
unspecked hardyhead	-	-	-	-	1	-	-	-	1	-
Murray River rainbowfish	-	-	-	-	-	-	-	-	-	-
obscure galaxias	2	-	-	-	-	1	-	-	2	1
bony herring	-	-	-	-	2	-	9	-	11	-
silver perch	-	--	-	-	-	-	-	-	-	-
golden perch	-	-	-	-	-	-	-	-	-	-
freshwater catfish	-	-	-	-	-	-	-	-	-	-
river blackfish	-	-	4	4	1	-	-	-	5	4
Murray cod	25	2 +2_e	117	231	56	-	8	1	206	234 (2_e)
Introduced										
gambusia	-	-	-	-	-	-	-	-	-	-
oriental weatherloach	-	-	-	-	-	-	-	-	-	-
redfin perch	-	-	-	-	-	-	-	-	-	-
carp	-	-	-	-	1	-	1	-	2	-
goldfish	-	-	-	-	-	-	-	-	-	-

Periodic 'flow-cued' species

Drift net sampling, aimed at detecting a response of golden and silver perch spawning to CEW environmental water delivery, commenced early October. The first sampling event coincided with the start of Watering Action 3c (late spring fresh). The late spring fresh watering action, aimed at promoting silver perch spawning (CEWO 2019), saw local discharge increase from 350 ML/day to 410 ML/day in the Mid Wakool zone 3, and returning to back 350 ML/day by early October. The water temperature at this time had reached 17°C for the first time in the season. Neither silver perch or golden perch eggs or larvae were detected; the water temperature may have not been warm enough at the time of the environmental water delivery to illicit a spawning response in silver perch.

In contrast, bony herring larvae were collected in 2019-20 for the fourth consecutive year. Appearing between Jan-Feb, bony herring larvae were found in the lower Wakool River in zones 3 and 4. The spatial appearance of bony herring larvae in Zones 3 and 4 is consistent with previous years monitoring, where they have not been found in either Yallakool Creek (zone 1) or the upper Wakool River (zone 2) (Figure 8.12, Figure 8.13).

There was limited evidence of carp spawning in the Edward/Kolety-Wakool focal zones in 2019-20 (n=2). The low levels of carp spawning in this current monitoring year are similar to trends observed in previous years when flows have remained in channel, including 2014-15, 215-16, 2017-18 and 2018-19 (Figure 12, Figure 8.13a).

Equilibrium species

Late-staged Murray cod larvae appeared throughout the Edward/Kolety-Wakool Selected Area from 4 Nov - 19 Dec 2019. Numbers of Murray cod larvae were the lowest detected since 2016-17 (Figure 8.13). Murray cod larvae were most abundant in the upper Wakool River (zone 2) compared to zones 1 (Yallakool Creek), zone 3 (Wakool River us Thule Creek) and zone 4 (Wakool River us Barbers Creek), despite the fact that discharge was lowest in this zone (50-60 ML/day during spawning and nesting period) (Figure 8.12). In the 6 years of monitoring, the abundance of Murray cod larval was greatest in 2018-19, with the majority of larvae coming from upper Wakool River (zone 2). Discharge in the upper Wakool River is typically operated at low base flows of 50-100 ML/d, however in 2018-19 zone 2 received flows up 200 ML/day from October 2018 through to January 2019. Zone 2 is structurally complex, woody-containing high loads of wood debris. We have previously hypothesised that spring flows that allow the recolonization and nesting in zone 2 by Murray cod would be beneficial for spawning. The lower catch of Murray cod larvae in this current watering year, when flows in the zone 2 were characterised by a return to baseflows, provides further evidence that delivery of environmental water during spring/summer will have a positive and disproportionate effect to Murray cod nesting and spawning when delivered to the upper Wakool River compared to Yallakool Creek. River blackfish larvae were detected in the Edward/Kolety-Wakool Selected area from 21 Oct – 22 Nov 2019 (Figure 8.12). Typically only detected in the upper Wakool River (zone 2), river blackfish larvae were recorded for the first time in 2018-19 in the Yallkaool River (zone 1). Of note, was that in 2019-20 we have detected river blackfish larvae in the Wakool River upstream of Thule Creek (zone 3) for the first time, an indication that the distribution and range of river blackfish may be slowly expanding throughout the study zones.

Opportunistic species

Larval abundance of small-bodied opportunistic species was numerically dominated by carp gudgeon and Australian smelt in 2019-20. Carp gudgeon larvae were present throughout much of the study period, and all study zones, appearing from early September 2019 through to early March 2020 (Figure 8.12). Australian smelt, also found in all study zones, appeared from early September to early December 2019. Flathead gudgeon larvae, were detected in zone 4 and zone 1 between mid – December 2019 and 9 January 2020 and obscure galaxias larvae was detected only in Yallakool Creek (zone 1) in late September 2019 (Figure 8.12).

Targeted watering actions for spawning outcomes in 2019-20

It was hypothesised that the abundance of fish larvae across the 2019-20 spawning season would be significantly higher in the study zones that received an environmental watering action (zones 1, 3 and 4) compared to zone 2 which did not receive any environmental water. Statistical analyses revealed variation in individual fish species responses to environmental water delivery. For bony herring, a 'periodic' flow dependent species, a significant difference in abundance of fish larvae/eggs across zones was detected. We observed significantly more larvae collected in zones 3 and 4 than zone 2, although no difference in larval abundance was observed between zones 1 and 2 (Table 8.5, Figure 8.13a).

Trends in larval abundance across zones the equilibrium species whose spawning is less reliant on flow-patterns. Watering action 3b delivered an early spring elevated based flow from 5-22 September 2019 in Yallakool Creek (zone1) and mid Wakool River (zones 3 and 4) 'to maintain nesting habitat for Murray cod'. While we detected a significant difference in larval abundance across zones for both Murray cod and River blackfish, there were significantly more larvae for both species found in Upper Wakool (Zone 2), which did not receive environmental water, compare to the zones that did. (Table 8.5, Figure 8.13b). This is consistent with previous years where greater numbers of Murray cod larvae are associated with the structurally complex Upper Wakool River (Zone 2). In 2018-2019 the greatest number of Murray cod larvae were collected to date from the Edward/Kolety-Wakool River system (Figure 8.13b). 2018-19 was the first year that the Upper Wakool River had been a target for environmental flow delivery, and the resultant record numbers of Murray cod larvae recorded indicate the watering action had significant outcomes for Murray cod nesting and spawning. Based on the results from this current year, there is little evidence to suggest that Watering action 3b had a measurable impact on Murray cod nesting and spawning, and we continue to advocate for the delivery of environmental water in the Upper Wakool for future years.

Small-bodied opportunistic fish showed a diversity of responses to environmental watering actions (Table 8.5, Figure 8.13c). Watering action 3a was an early spring fresh delivered 28 Aug – 4 September, aimed at contributing to spawning in early spawning native fish. Australian smelt, which is a pelagic, early spawning species, was found to respond positively towards to the conditions provided by environmental flows: with significantly more larvae found in all zones (zones 1,3 and 4) that received environmental watering actions compared those that did not (zone 2). There was no other outcomes detected in other opportunistic fish. For flathead gudgeon, only zone 4 had greater numbers of larvae than zone 2, and for carp gudgeon, none of the zones that received environmental water had greater numbers of larvae than zone 2. Numbers of obscure galaxias and Murray River Rainbowfish were too low for any statistical comparisons across zones for 2019-20.

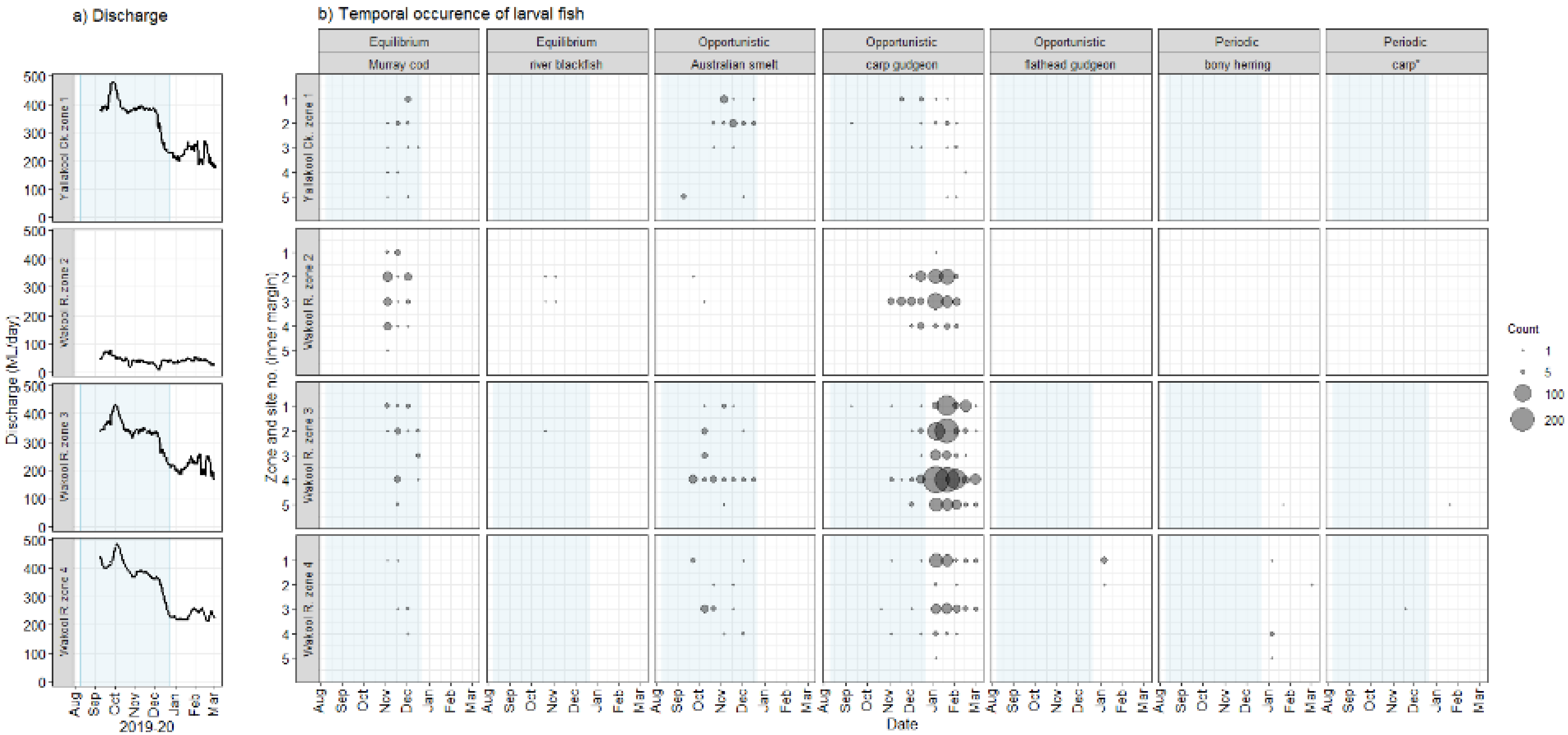


Figure 8.12. a) Discharge and b) Bubble plots depictings temporal occurrence of larval fish in the four study zones (from top row to bottom): Yallakool Creek – zone 1, Wakool River - zone 2, Wakool River - zone 3, and Wakool River - zone 4 during the 2019-20 spawning period. Bubble size (count) represents relative abundance of larvae collected in light traps (n=3) at each study site. Blue bars indicate the timing of watering actions 2 and 3.

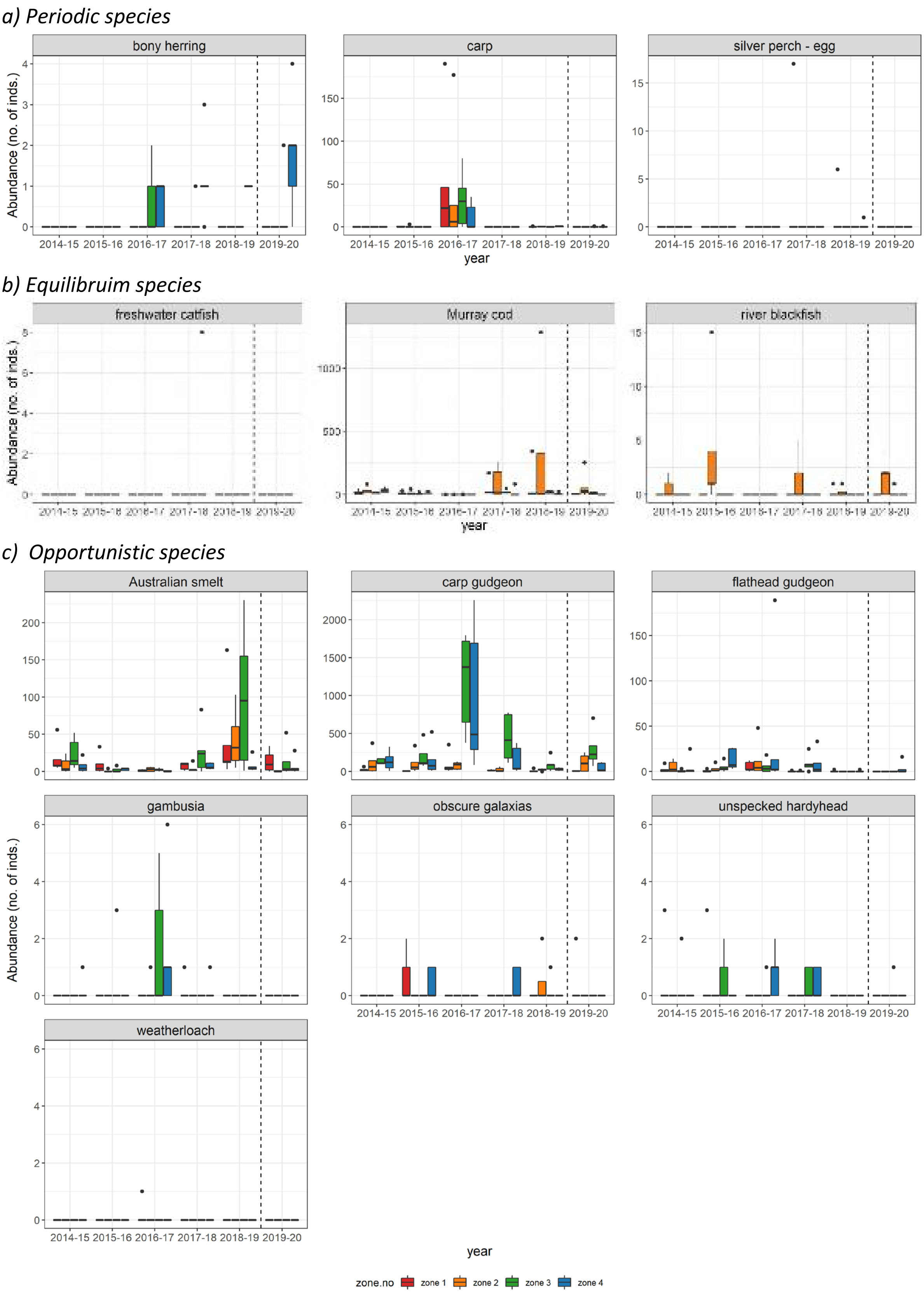


Figure 8.13 Abundance of larval fish collected across years and the four MER study zones for *a)* Periodic, *b)* Equilibrium and *c)* Opportunistic fish species in the Edward/Kolety-Wakool Selected Area, 2014-2019. Dashed vertical line denotes 2019-20 data, for which statistical comparisons of larval abundance between zones were made (Table 5).

Table 8.5 Results of GLMs that tested the effect of zone on mean annual catch of fish larvae (light traps and drift net catch combined) in 2019-20. Models were run only on species with n>50. *P* values <0.05 used to determine significance. Significance codes: ***<0.001, **<0.01, *<0.05. CEW prediction: 'yes' all zones that received CEW (zones 1,3 and 4) had higher abundances of fish eggs/larvae than zones that did not (zone 2), 'partial', some but not all zones that received CEW had higher larvae/egg abundances than zone 2, and 'no', none of the zones that received CEW had higher larvae/egg abundances than zone 2.

Fish species	d.f	F statistic	*P* value	significance	CEWO prediction (zone 2 < zone 1,3,4)
periodic species					
bony herring	3,16	10.267	<0.001	***	partial
equilibrium species					
Murray cod (n=3,866)	3	11.767	<0.001	***	no
river blackfish	3,16	12.063	<0.001	***	no
opportunistic species					
Australian smelt	3,16	3.810	<0.030	*	yes
carp gudgeon (n=22,212)	3,16	9.774	<0.0001	***	no
flathead gudgeon	3,16	20.944	<0.001	***	partial

8.5.3 *Fish recruitment*

A total of nine native fish species and five alien species were sampled between 2014-15 and 2019-20 as part of fish recruitment monitoring. Murray cod (*Maccullochella peelii*) young-of-year (YOY) and 1+ recruits were detected in all zones for the first time since 2015-16 (Table 8.6). One silver perch (*Bidyanus bidyanus*) age-class 1 (1+) recruit was in detected in zone 4 (Table 8.6). Golden perch (*Macquaria ambigua*) recruits have not been detected during any year since surveys began.

Table 8.6 Number of young-of-year (YOY), age class 1 (1+) recruits and older juveniles or adults (JA) of the three target species sampled in recruitment and growth monitoring in the Edward/Kolety-Wakool system for 2014-15 through 2019-20.

Zone	2014-15			2015-16			2016-17			2017-18			2018-19			2019-20		
	YOY	1+	JA	YOY	1+	JA	YOY	1+	JA	YOY	1+	JA	YOY	1+	JA	YOY	1+	JA
Murray cod																		
Zone 1	5	15	17	2	8	1	-	-	-	2	-	4	5	2	1	4	15	8
Zone 2	5	11	11	9	16	19	-	-	-	6	1	2	2	6	4	5	11	8
Zone 3	3	14	13	8	9	16	-	-	-	-	-	-	-	2	-	4	12	17
Zone 4	7	6	14	5	17	11	-	-	-	-	-	-	-	-	-	1	10	5
Silver perch																		
Zone 1	-	-	7	-	1	5	-	-	12	-	-	2	-	-	1	-	-	4
Zone 2	-	-	2	-	-	3	-	-	3	-	-	1	-	-	-	-	-	1
Zone 3	-	-	6	-	4	9	-	-	13	-	-	9	-	7	1	-	-	6
Zone 4	-	1	1	5	15	14	-	-	7	-	-	14	-	3	4	-	1	14
Golden perch																		
Zone 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zone 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zone 3	-	-	1	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Zone 4	-	-	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-

Murray cod

A total of 14 YOY and 48 1+ Murray cod recruits were detected in 2019-20 sampling (Table 8.6). This represents the highest number of both classes of recruits since 2015-16 prior to the blackwater event in 2016 (Figures 8.14 and 8.15). Some of the apparent increased abundance of 1+ recruits may be attributable to the change in methods to from backpack electrofishing to boat electrofishing at most sites, however the CPUE in zone two where three out of the 4 sites where sampled with backpack electrofishing was comparable to 2015-16 (Figure 8.15).

Boat electrofishing resulted in an increase in the capture older juvenile and adult Murray cod (Table 8.6), which provided an overall snapshot of the current population structure in the system. It is clear from Figure 8.16 that the population is currently dominated by recent year classes less than 400 mm in length.

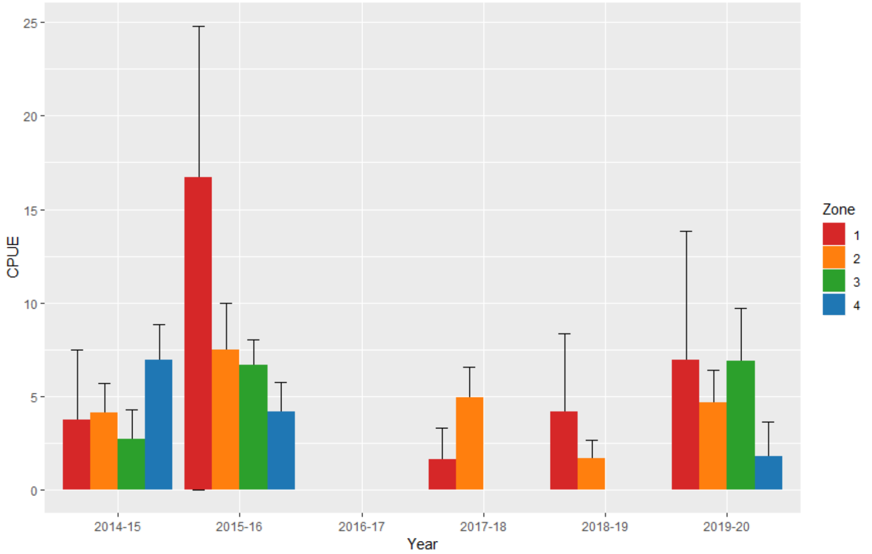


Figure 8.14 Mean (+SE) catch per unit effort (CPUE; number of fish caught per 10 000 seconds of electrofishing) of YOY Murray cod in the Edward/Kolety-Wakool LTIM/MER zones from 2019-20.

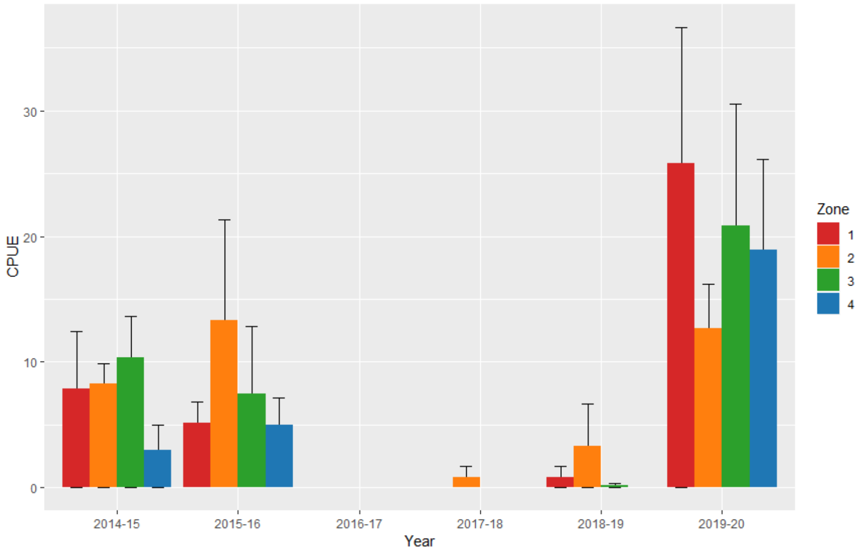


Figure 8.15 Mean (+SE) catch per unit effort (CPUE; number of fish caught per 10 000 seconds of sampling time) of Murray cod among the Edward/Kolety-Wakool LTIM/MER zones using electrofishing, setlines and angling form 2014-15 to 2019-20.

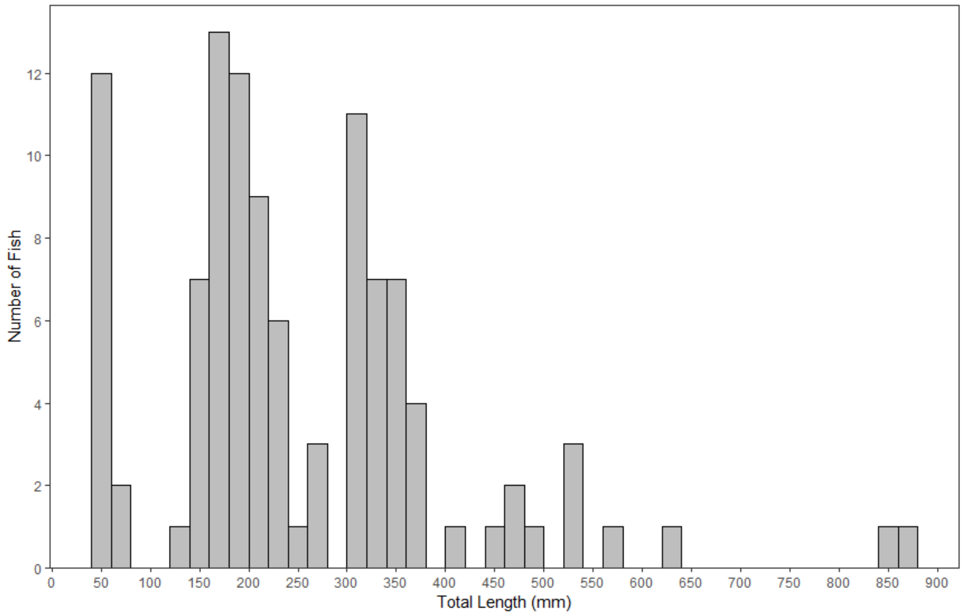


Figure 8.16 Length frequencies of Murray cod captured using backpack and boat electrofishing, angling and setlines in 2019-20.

Growth of Murray cod

Growth per day (mm) of YOY Murray cod was similar to previous years except for 2017-18 which were the first cohort following the flood in 2016 (Figure 8.17). This trend is followed by the 1+ recruits in 2019-20 generally growing slower than in 2018-19 (the cohort spawned in 2017-18), with the exception of recruits in zone 4 that displayed similar growth rates to those in 2018-19 from other zones (Figure 8.18).

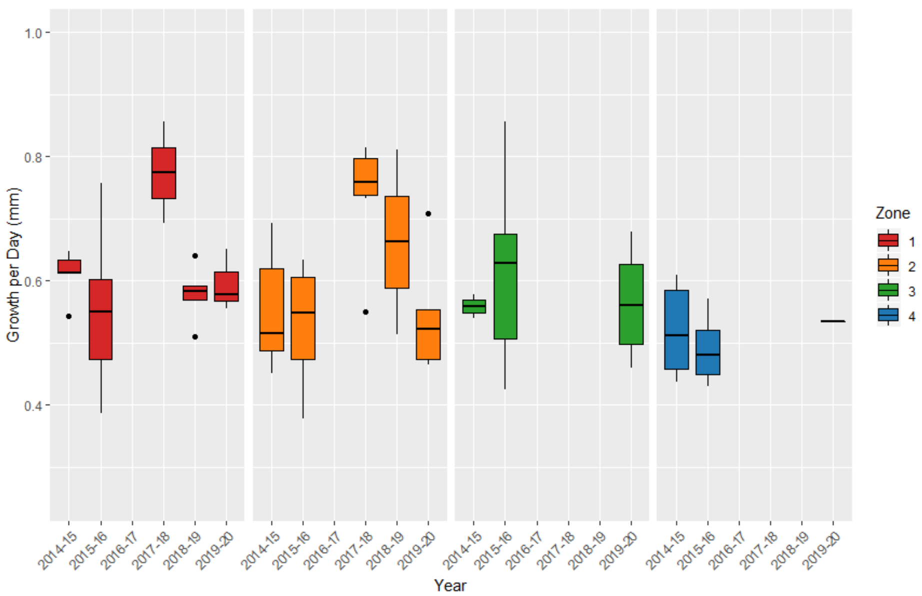


Figure 8.17 Boxplots of the annual growth rates (mm per year) of YOY Murray cod in each zone between 2014-15 and 2018-19. Number of individuals (n) per zone: 2014-15 n = 5, 5, 3, 7; 2015-16 n = 20, 9, 8, 5; 2016-17 n = 0, 0, 0, 0; 2017-18 n = 2, 6, 0, 0; 2018-19 n = 5, 2, 0, 0; 2019-20 n = 4, 5, 4, 1.

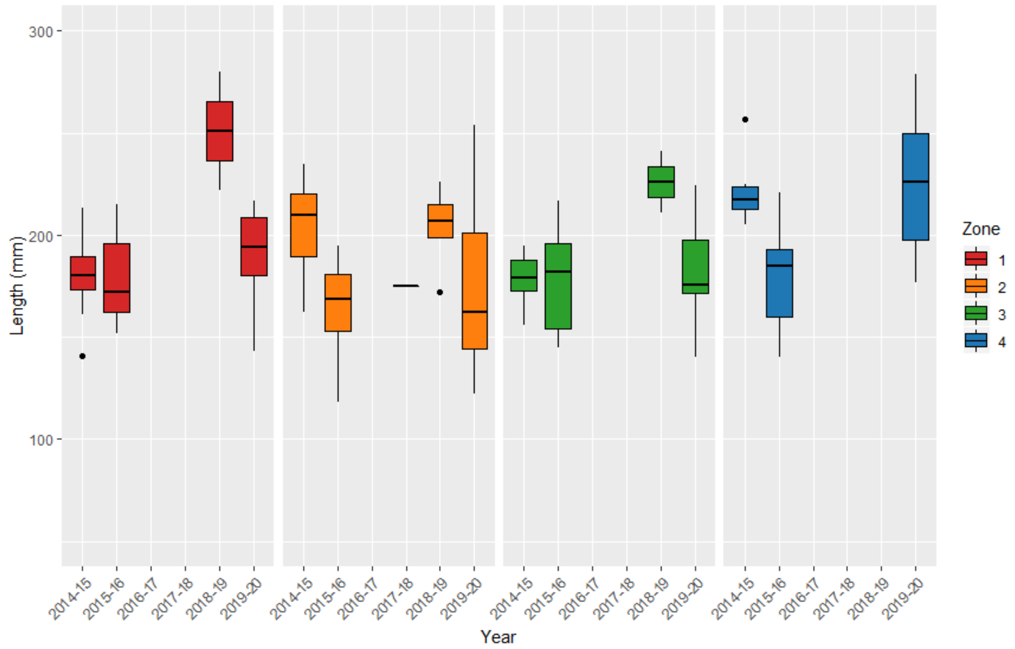


Figure 8.18 Boxplots of the annual length-at-age (mm) for 1+ Murray cod in each zone between 2014-15 and 2018-19. Number of individuals (n) per zone: 2014-15 n = 15, 11, 14, 6; 2015-16 n = 8, 16, 9, 17; 2016-17 n = 0, 0, 0, 0; 2017-18 n = 0, 1, 0, 0; 2018-19 n = 2, 6, 2, 0; 2019-20 n = 15, 11, 12, 10.

Silver perch

Twenty-six silver perch were captured using setlines and angling in 2019-20, ranging from 101 mm to 370 mm. No silver perch were retained for ageing in 2019-20 due to concerns over threatened species survival during drought conditions. Determination of age class recruits was done using length data from fish previously aged on this project.

Only one fish was a likely 1+ recruit captured in zone 4 (Figure 8.19), with all other fish either other juveniles or adults. Figure 8.20 shows the current population structure in system dominated by fish between 200 and 300 mm.

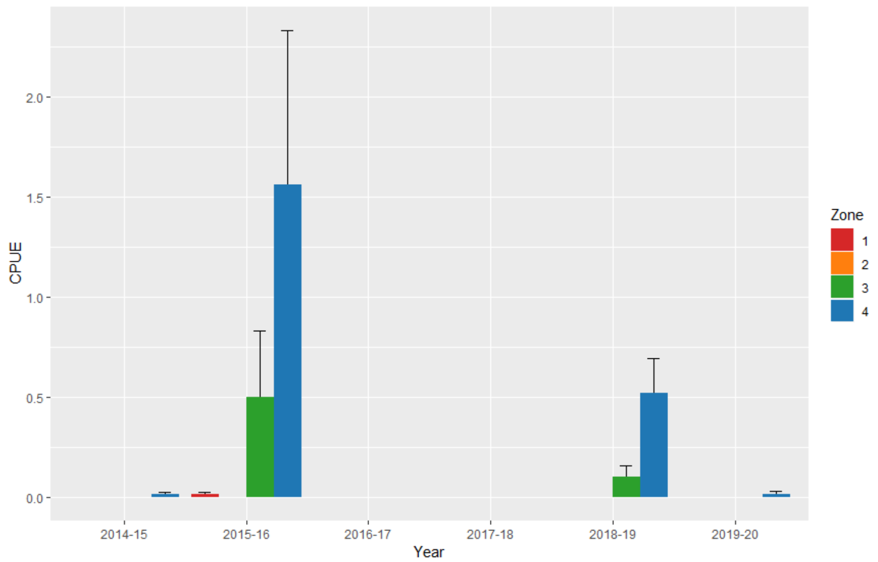


Figure 8.19 Mean (+ SE) CPUE of 1+ silver perch in the Edward/Kolety-Wakool LTIM zones using setlines and angling (number of fish per 10 000 seconds of sampling) between 2014-15 and 2019-20.

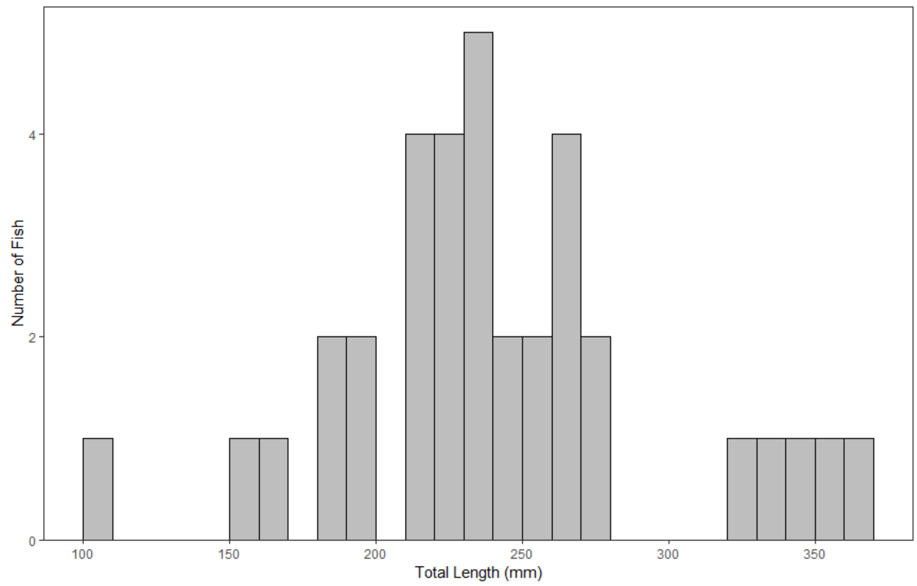


Figure 8.20 Length frequencies of silver perch captured using setlines and angling in 2019-20.

8.5.4 Adult fish community

Basin scale - Category 1 fish community sampling (undertaken only in zone 3) identified eight native fish species and two alien species in 2020 (Table 8.7). Overall, standardised catch of Murray cod and golden perch remained substantially lower through 2017–2020 compared with 2015 and 2016 (Table 8.8, Figure 8.21). Although, Murray cod numbers have risen from 12 in 2017 to 66 in 2020. Flathead gudgeon were not captured in 2020 but were only captured at low abundances when previously present in 2016 and 2015 (Table 8.7, Figure 8.21).

There were significant differences in the abundance of the fish assemblage between sampling years in zone 3 (Pseudo-$F_{5,54}$ = 10.246, $p < 0.001$). Pair-wise differences were found between all pairs of years ($t > 4.46$, $p < 0.05$), except 2015 and 2016 ($t = 2.127$, $p = 0.840$). Differences between 2020 and 2019 ($t = 9.747$, $p = 0.015$) were driven by a higher abundance of bony herring and carp gudgeon in 2020 (contribution to dissimilarity between groups of 20.5 and 11.8%, respectively) and a lower abundance of goldfish in 2020 (11.9%).

Length-frequency distributions (Figure 8.22) indicated that bony herring captured in 2020 were significantly smaller than those captured in 2015–2019 ($p < 0.001$), and numerous new recruits were captured.

Golden perch captured in 2020 were significantly larger than those captured in 2019 ($p = 0.009$), 2017 ($p = 0.024$), 2016 ($p < 0.001$) and 2015 ($p < 0.001$), but of a similar size structure compared to those in 2018 ($p = 0.372$). New recruits of this species have not been captured during six years of sampling (Figure 8.22).

Common carp new recruits were captured in 2020, but overall fish were significantly larger in 2020 compared with 2019 ($p < 0.001$) due to a lower proportionate abundance of new recruits in 2020. Even so fish were larger still in 2016 ($p < 0.001$) and 2015 ($p < 0.001$) when compared with the size structure in 2020 (Figure 8.22).

Murray cod new recruits were captured in 2020 (Figure 8.20-Figure.8.21). Overall the sampled population was significantly smaller in 2020 compared to 2017 ($p < 0.001$), 2018 ($p < 0.001$) and 2019 ($p = 0.049$) (indicating a higher proportion of smaller size classes including new recruits), but of larger size in 2020 than in 2015 ($p < 0.001$) and 2016 ($p < 0.001$) (Figure 8.22).

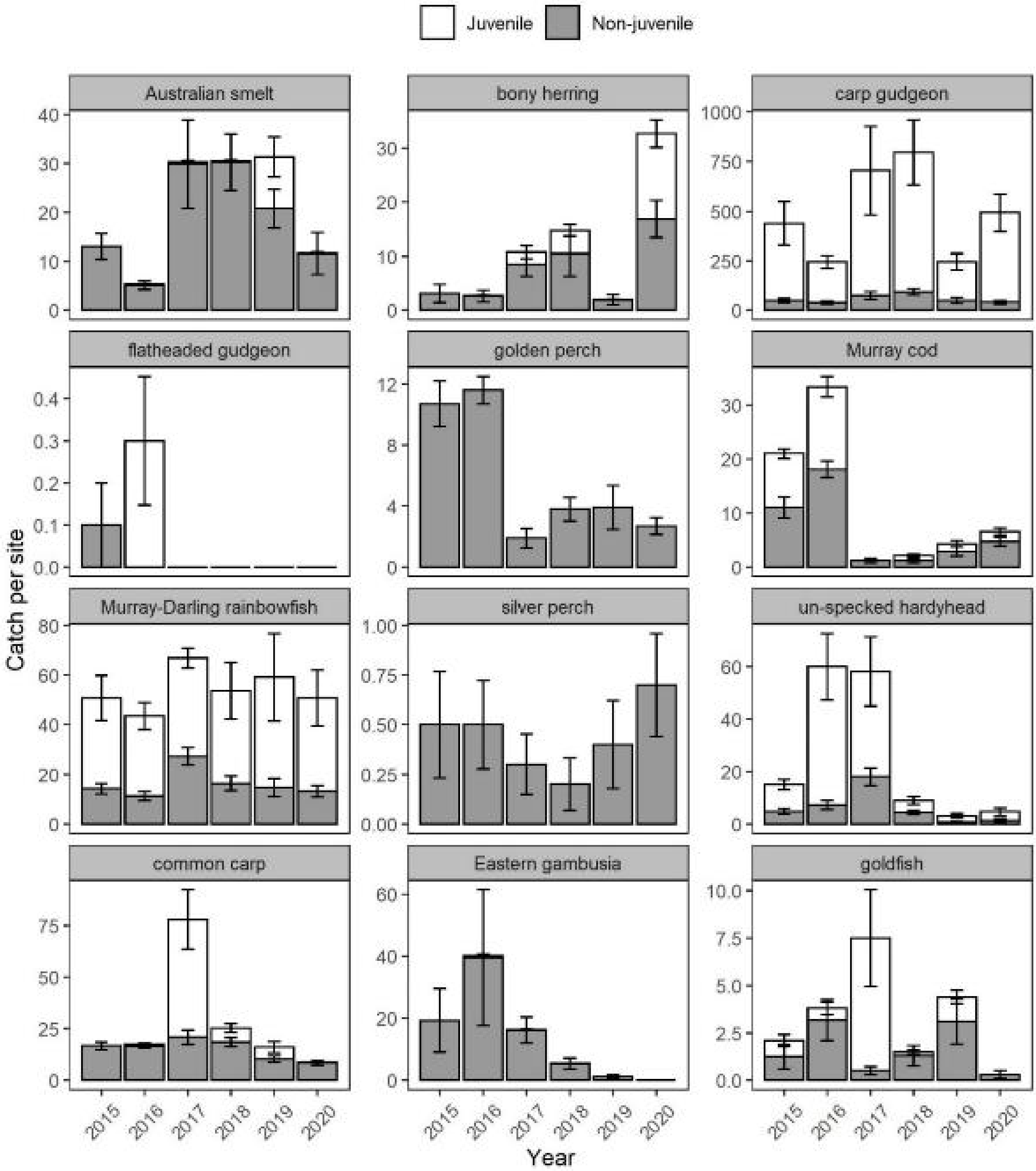


Figure 8.21 Catch per site (number of fish; mean ± SE) for each fish species within the Edward/Kolety-Wakool river system target reach, sampled from 2015–2020. Cumulative stacked bars separate the catch of juveniles (white bars) and non-juveniles (grey bars).

Table 8.7 Summary of fish captured during annual Category 1 standardised sampling from 2015–2020 in the Edward/Kolety-Wakool LTIM project. BE = boat electrofishing, SFN = small fyke net and BT = bait trap.
8

Fish species	2015				2016				2017				2018				2019				2020			
	BE	SFN	BT	**Total**	BE	SFN	BT	**Total**	BE	SFN	BT	**Total**	BE	SFN	BT	**Total**	BE	SFN	BT	**Total**	BE	SFN	BT	**Total**
native species																								
Australian smelt	129	2	-	**131**	52	1	-	**53**	293	10	-	**303**	301	4	-	**305**	287	26	-	**313**	73	45	-	**118**
bony herring	31	-	-	**31**	27	-	-	**27**	108	-	-	**108**	148	-	-	**148**	20	-	-	**20**	320	5	1	**326**
carp gudgeon	47	4302	51	**4400**	68	2367	15	**2450**	165	6814	66	**7045**	52	7804	98	**7954**	23	2396	38	**2457**	4	4873	57	**4934**
flathead gudgeon	-	-	1	**1**	-	-	3	**3**	-	-	-	**0**	-	-	-	**0**	-	-	-	**0**	-	-	-	**0**
golden perch	107	-	-	**107**	116	-	-	**116**	19	-	-	**19**	38	-	-	**38**	39	-	-	**39**	27	-	-	**27**
Murray cod	210	-	-	**210**	333	1	-	**334**	12	-	-	**12**	21	-	-	**21**	43	-	-	**43**	66	-	-	**66**
Murray-Darling rainbowfish	339	168	-	**507**	353	77	5	**435**	650	19	-	**669**	518	19	-	**537**	508	83	-	**591**	424	83	-	**507**
silver perch	5	-	-	**5**	5	-	-	**5**	3	-	-	**3**	2	-	-	**2**	4	-	-	**4**	7	-	-	**7**
unspecked hardyhead	86	64	-	**150**	565	35	-	**600**	510	72	-	**582**	82	7	-	**89**	22	9	-	**31**	22	25	-	**47**
alien species																								
common carp	167	-	-	**167**	176	-	-	**176**	735	40	3	**778**	251	1	-	**252**	160	1	-	**161**	89	-	-	**89**
Eastern gambusia	18	175	-	**193**	36	366	1	**403**	31	125	8	**164**	2	53	-	**55**	2	10	-	**12**	-	-	-	**0**
goldfish	21	-	-	**21**	38	-	-	**38**	73	2	-	**75**	15	-	-	**15**	44	-	-	**44**	3	-	-	**3**

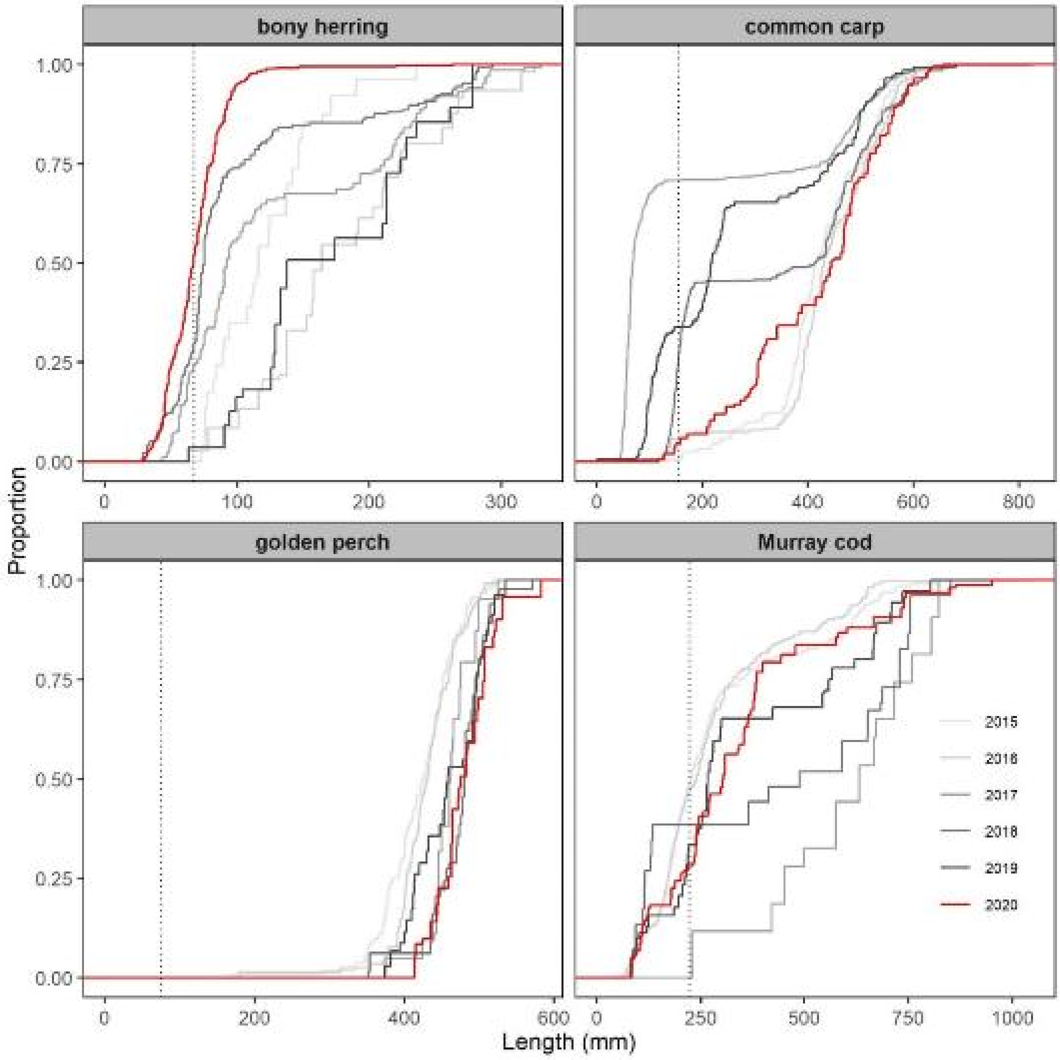


Figure 8.22 Cumulative length-frequency histograms of the four most common large-bodied species captured during Category 1 sampling in the Edward/Kolety-Wakool LTIM project in 2015–2020. The dashed line indicates approximate length at one year of age and annual sample sizes are provided in Table 8.7 for each respective species and sampling year.

8.6 Discussion

Here, we bring together our results from movement, spawning, recruitment and adult fish community monitoring to provide an overview of how the fish community in the Edward/Kolety-Wakool has responded to targeted watering events and the broader hydrological conditions of 2019-20. A summary of the species of larvae, recruits and adults present in the system in 2019-20 is provided in Table 8.8. Using these multiple lines of evidence, we provide a summary on how fish responded to each of the watering actions delivered in 2019-20, and provide recommendations for future water delivery.

In 2016-17 the Edward/Kolety-Wakool River fish community was heavily impacted by hypoxic blackwater and associated fish kills. These followed numerous fish kills in the preceding 6 years and prior to LTIM. Since this time, LTIM and Flow-MER fish monitoring have identified a gradual recovery of the fish community.

Promisingly, adults of most species have since been captured in the system, and regular spawning and recruitment through to the juvenile stage has been observed for numerous species (Table 8.9, Watts et al. 2019). Of the fifteen native fish species that have been recorded in the Edward/Kolety-Wakool Selected Area since LTIM commenced in 2014, eleven were detected as either eggs/larvae, recruits or adults in 2019-20 (Table 8.8).

Table 8.8 Multiple lines of evidence: a summary of 2019-20 fish monitoring results in the Edward/Kolety-Wakool Selected Area, of the species known to occur in the area prior to 2019. For the 2019-20 sampling season – ticks denote the presence of larvae/eggs (indicating successful spawning), recruits (indicating successful recruitment) and adults. ^ denotes introduced species. [1] Indicates species have been recorded in the Edward/Kolety Wakool system, but outside the LTIM and Flow-MER focal study zones. [2] indicates species have been recorded in the focal areas as larvae, but not adults.

2014-2019	2019-20		
Fish species	Larvae/eggs	Recruits	Adults
periodic species			
bony herring	✓	✓	✓
golden perch			✓
silver perch		✓	✓
common carp ^	✓		✓
goldfish ^			✓
redfin ^			
equilibrium species			
Murray cod	✓	✓	✓
river blackfish	✓		
freshwater catfish[2]			
trout cod[1]			
opportunistic species			
Australian smelt	✓		✓
carp gudgeon	✓		✓
Murray River rainbowfish		✓	✓
flathead gudgeon	✓		
unspecked hardyhead	✓	✓	✓
obscure galaxias	✓		
dwarf flathead gudgeon[2]			
gambusia ^			
oriental weatherloach ^			

Targeted watering actions in 2019-20

The CEWO's overarching objective for environmental watering for fish populations in the Edward/Kolety-Wakool River system was to provide flows to "support habitat (including longitudinal connectivity and bench inundation), food sources and promote increase movement/dispersal, recruitment and survival/condition of native fish" (CEWO 2019). Three Commonwealth environmental watering actions in the Edward/Kolety-Wakool system in 2019-20 that were evaluated by the fish monitoring program of Flow-MER (Table 8.9).

Table 8.9 Monitored outcomes of the fish-focussed CEWO watering actions in 2019-20. Note: watering actions that could not be monitored by the Flow-MER program are not listed here (e.g. watering action 3d).

	Watering action	Dates	Rivers	Flow objectives	Monitored outcomes
1	Winter base flow	15 May - 9 Aug 2019	Yallakool Creek, mid- and lower Wakool River	For native fish condition and movement	Occupation of Yallakool Creek - zone 1 by golden perch was enabled during winter watering in 2019 in comparison to winter 2018 (no watering), indicating that increased habitat was both available and utilised during the watering event.
				Provide refuge habitat during irrigation shut-down period	Strongest cohort of Murray 1+ found detected this year. Winter flows may have provided important over-wintering refuge habitat for the record number of larval and juvenile YOY recorded in 2018-19.
3a	Winter/ spring early fresh	28 Aug - 4 Sep 2019	Yallakool Creek, mid- and lower Wakool River	Contribute to spawning in early spawning native fish	Greater numbers of Australian smelt found in reaches where environmental water was delivered.
3b	Early spring elevated base flow	5 - 22 Sep 2019	Yallakool Creek, mid- and lower Wakool River	To maintain nesting habitat for Murray Cod	The abundance of Murray cod was not significantly higher in zones receiving environmental water compared to those that did not. Environmental water may have a great outcome in zone 2 due to the complex habitat structure in this reach. We saw positive evidence of Murray cod abundance in zone 2 in response to 2018-19 environmental flows. (sensu 2018-19).
3c	Late spring fresh	23 Sep - 11 Oct 2019	Yallakool Creek, mid- and lower Wakool River	To promote silver perch spawning	No silver perch spawning response detected. Temperatures may have been too low for the flow that was delivered.

Watering action 1: Winter base flow

From 15 May – 9 August 2019, a winter base flow was delivered through Yallakool Creek, the mid- Wakool River and the lower Wakool River. Typically, winter flows cease in many creek and river reaches of the Edward/Kolety River system, as well as other regulated rivers in the Murray Darling Basin, due to lack of irrigation demand for water at this time (Stuart et al. 2019; Tonkin et al. 2020). The provision of a winter base flows can provide flow connectivity through the complex array of creeks, anabranches and distributary channels throughout the selected area. It can also provide retention of local habitat for native fish, that may either otherwise have to move out during this winter period, or be confined to suboptimal overwintering habitat where competition and predation risk increases as populations are forced to contract and concentrate into the limited available refuge (Stuart et al. 2019; Tonkin et al. 2020). Indeed, 2019-20 movement monitoring data revealed that the occupation of Yallakool Creek by golden perch was enabled during winter watering in 2019 in comparison to winter 2018 (no watering), indicating that increase habitat was both available, and utilised during the winter watering action.

No discernible differences were observed in the scale of the movements of golden perch or silver perch during the 2019 winter watering action, although we note that sample sizes were low. Movements during winter are typically localised for both species in this system based on previous observed and modelled data, although modelling based on previous water delivery years (2017 and 2018) indicate that CEW deliveries in winter increase the movement of golden perch, silver perch and Murray cod, and movement is most pronounced in silver perch.

The 2019 winter base flows likely contributed to positive outcomes for juvenile Murray cod. In spring 2018 a record number of Murray cod larvae were recorded as a result of a spring watering action delivered to the upper Wakool River (Watts et al. 2019). In this current monitoring year, we recorded a record number of 1+ Murray cod juveniles, and we hypothesise that the subsequent preceding winter base flow in 2019 provided critical overwinter refuge habitat for these YOY, increasing local retention and survival. The ability to provide both springtime elevated base flows in the Upper Wakool River, followed by winter base flows to prevent unnatural cease-to-flow events will have positive outcomes for the recovery of Murray cod in the Edward/Kolety-Wakool Selected area.

Watering action 3: Sustained spring fresh and elevated base flows

Despite the longitudinal extent of watering action 3, which coincided with the broader Murray River Channel flow, we did not detect silver perch or golden perch spawning in the monitored areas of the Edward/Kolety-Wakool River system. Additional sampling in the Edward/Kolety River targeting golden and silver perch spawning took place for the first time in 2019-20 (Section 11), however we did not detected any silver or golden perch eggs/larvae. Although catches of adult silver perch are back to pre-2016 fish kills levels, detectable spawning events have not yet been recorded for in the selected area. For the flow delivered in 2019-20, water temperature may have been too low, or critical water velocities not met. The presence of 1+ silver perch in the Selected Area does however indicate that successful spawning and recruitment of the species is occurring in the southern Basin, but most likely at a much broader geographic scale than the Edward/Kolety Selected Area itself (see Tonkin et al. 2019). The life cycle of golden and silver perch is considered to require unimpeded flowing water habitats encompassing at least 100's of kilometres, and therefore maintaining connectivity to the nearby Mid-Murray River to ensure bi-directional movement of juveniles and adults of both species will continue to help support recovery (Thiem et al. 2017). Future planning of environmental water delivery could consider adaptive use of water to coincide with high Murray River flows to maximise attraction/immigration of upstream migrating juvenile golden perch and silver perch in late summer. The probability of silver perch moving into and then staying in other more upstream tributaries of the Murray River (Goulburn and Campaspe rivers) is elevated in March-May (Koster et al. 2020), so delivering attraction flows in the Edward/Kolety-Wakool river system at this time or before (e.g. January-March) may be optimal for this more downstream tributary.

Two native fish species, Australian smelt and bony herring had a positive spawning response to environmental water delivery. Australian smelt are an early spawning species, and greater numbers of larvae were found in all study reaches where environmental water was delivered. Although we note this species is tolerant to river regulation (Gehrke and Harris 2001). A similar result occurred in 2018-19 where Australian smelt larvae were found in greater abundances in reaches that received environmental water during winter/early spring compared to those that did not. Bony Herring larvae were found in greater numbers in reaches that received environmental water, and juvenile and adult CPUE in 2019-20 was the highest collected to date.

Flow recommendation for fish outcomes

Recommendation: Deliver elevated base flows to the Upper Wakool River from September-December to maximise nesting and spawning opportunities for Murray cod. Record catches of larvae have been recorded when this type of watering action is delivered. This type of flow delivery should be supported with subsequent winter base flows throughout the Selected Area to maximise retention and survival of YOY in the region.

Recommendation: Prevent negative impacts of a-seasonal cease-to-flow events by delivering winter base flows to promote temporal availability and continuity of instream habitat for fish. Evidence from 2019-20 monitoring indicates this has positive benefits for the survival and local retention of juvenile fish.

Recommendation: In years with high water availability, consider a late spring/early summer pulse, immediately after Murray cod larvae have left the nest, to support food resources for Murray cod larvae while at the same time providing opportunities for spawning to occur in silver perch and golden perch.

Recommendation: Consider adaptive use of water to coincide with high Murray River flows to maximise attraction/immigration of upstream migrating juvenile golden perch and silver perch in late summer. The probability of silver perch moving into and then staying in other more upstream tributaries of the Murray River (Goulburn and Campaspe rivers) is elevated in March-May (Koster et al. 2020), so delivering attraction flows in the Edward/Kolety-Wakool river system at this time or before (e.g. January-March) may be optimal for this more downstream tributary.

Recommendation: In watering years where risk of hypoxic blackwater events is probable, consider how CEW watering actions could be used to mitigate effects on fish populations. One option to explore could be use of flows to encourage movement out of high risk reaches.

CEWO Adaptive Management Response:

- A number of the recommendations above are linked to recommendations in Chapter 7. The CEWO will seek to implement these recommendations via multi-objective watering actions, as it has done so in the past.

9 PHYSICAL HABITAT

Authors: Neil Suton and Geoff Vietz

9.1 Key Findings

Table 9.1. Key Findings from physical habitat research in the Edward/Kolety-Wakool system

Flow period	Bank/Veg condition response
What were the features of flow regime that drove erosion and deposition?	Operational flows that produce prolonged invariable periods of inundation to riverbanks within a defined bank zone appear to be the main driver of notching on riverbanks in the Edward/Kolety River downstream of Stevens Weir. The position of the notch relative to the water level of the flow delivery was a critical variable which implicated the scale and pattern of the erosion response. Commonwealth environmental water (CEW), operational flows and unregulated flows can result in periods of inundation of the bank zone above the notch, and upon draw-down can result in large quantities of unstable sediment. It is the combination of the following processes in sequence that are the driving force behind extensive areas of channel widening resulting from mass-failure events: a) *Summer operational flows:* create a deep notch and drying of the upper bank b) *Environmental or unregulated flows:* saturation of upper bank and drawdown of the water level. This highlights that the influence of CEW actions or unregulated flows on bank condition cannot be measured in isolation. Preparation of the bank during summer operational flows must be considered, as they play a critical role in driving erosion events throughout the entire year and in years following. The erosion volumes at the Edward/Kolety River site downstream of Stevens Weir are linked to the historic pattern of operational flows. If the management of operational flows does not change, then the potential benefits to bank condition as a result of CEW actions-will not materialise. Flows which resulted in the most deposition relative to erosion were unregulated flows during the winter months. This was due to the following combination of factors, a) the source of the water delivered during this period (high tributary %), b) the range of these flows (between 500 - 3,000 ML/day), and c) the lack of erosion evident in response to these events. CEW actions that are delivered with a gradual draw-down of the receding limb are likely to result in less erosion due to mass-failure events, and higher levels of deposition as a result of mud-draping. However, this will have a limited impact if summer operational flows are not re-designed.
What were the features of flow regime that affect riverbank and aquatic vegetation?	Prolonged inundation (+30 days) during spring appeared to result in reduced riparian vegetation cover above the bank zone relating to 3,000 ML/day in the Edward/Kolety River downstream of Stevens Weir. This was evident in Colligen Creek and the Edward/Kolety River, however some of this vegetation grew back over the summer and autumn months, highlighting the ability of vegetation to recolonise under favourable conditions. Data for vegetation cover in the lower half of the bank (<3,000 ML/day) was less consistent, however drone photography supports the hypothesis that vegetation that is negatively impacted by summer operational flows can struggle to re-establish in the seasons that follow (autumn, winter and spring).

9.2 Background

Bank condition is explicitly linked to flows. The risk to biota from changes in bank morphology and sediment liberated by erosion make bank condition an important and explanatory variable for assessing the value of environmental water actions for achieving ecosystem objectives.

Riverbanks influence the velocity of flow, depth of water, and provide the sediment conditions for biota including flora and fauna. Riverbank condition can alter conditions for biota, and this is often related to the extent of bank activity and river flow.

Riverbank vegetation richness and diversity are also impacted by flows, due to flow characteristics such as prolonged inundation, high velocities, and smothering by sediment. Changes in vegetation can be independent of bank condition, or inextricably linked. There are considerable advantages to monitoring bank condition concurrently with riverbank vegetation condition.

Quantifying the relationship between flows and bank condition can assist with understanding flows that enhance ecological objectives and reduce any potential unintended consequences. The Cause and Effect Diagrams (CEDs) developed as part of the original Long-Term Intervention Monitoring (LTIM) (Figure 9.1) illustrate some of the linkages between bank condition and a range of ecological and ecosystem values.

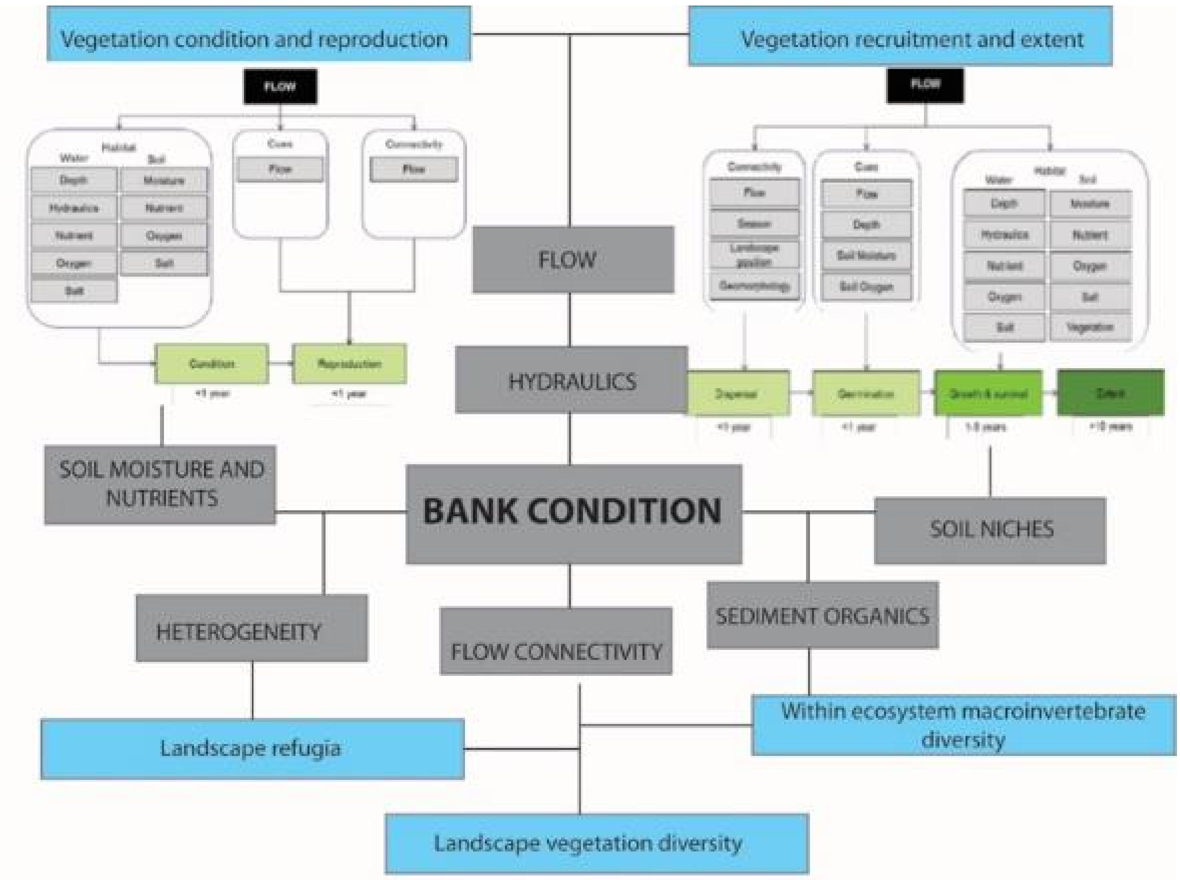


Figure 9.1. Contribution of bank condition monitoring to example CEDs developed for the CEW monitoring program.

For this project Streamology focused on using Unmanned Aerial Vehicle (UAV) technology and photogrammetry methods to generate spatial and temporal data to create data sets to investigate the impacts of flow events on physical habitat in reaches of the Edward/Kolety River and Colligen Creek. UAVs were used throughout this project to capture high resolution aerial imagery to process with photogrammetry methods to produce:

1) Detailed digital elevation models (DEMs), DEMs of Difference (DEMODs) and quantifying bank condition changes
2) Riparian vegetation maps displaying spatial and temporal differences associated with flow events, quantification of the percentage loss of riparian vegetation, and locating areas of most/least impact.

The methodology used, insights gained, and conclusions made as a result of these process will be documented in this report.

9.3 Research questions

- *What are the features of the flow regime and river operations that drive erosion and deposition?*
- *What are the features of flow regime and river operations that affect riverbank vegetation and aquatic vegetation cover?*

9.4 Methodology

The infographic (Figure 9.2) provides an overview of the methodology used to monitor bank and vegetation condition in response to hydrological characteristics within the Edward/Kolety-Wakool system.

Figure 9.2. Infographic providing an over-view of the methodology applied to bank and vegetation condition analysis using UAV technology

The drone method

The drone method is a 3-step process (Figure 9.3):

- the site must be prepared, this involves the distribution of ground control points (GCPs), which enable the accurate comparison of multiple drone surveys over time.
- the drone must be flown in a particular way to capture the entire face of the chosen area of interest on the bank. This involves several flights using an oblique image capture protocol.
- the enormous amount of data must be processed. This processing focusses on a) stitching images together using a combination of geospatial data and visual bank features b) processing these into points to enable the creation of digital elevation models (DEMs) or point clouds
- steps 2 and 3 are repeated so that datasets from two different time periods can be compared, enabling the creation of DEMs of difference (DEMODs), which highlight areas of erosion and deposition across a bank face.

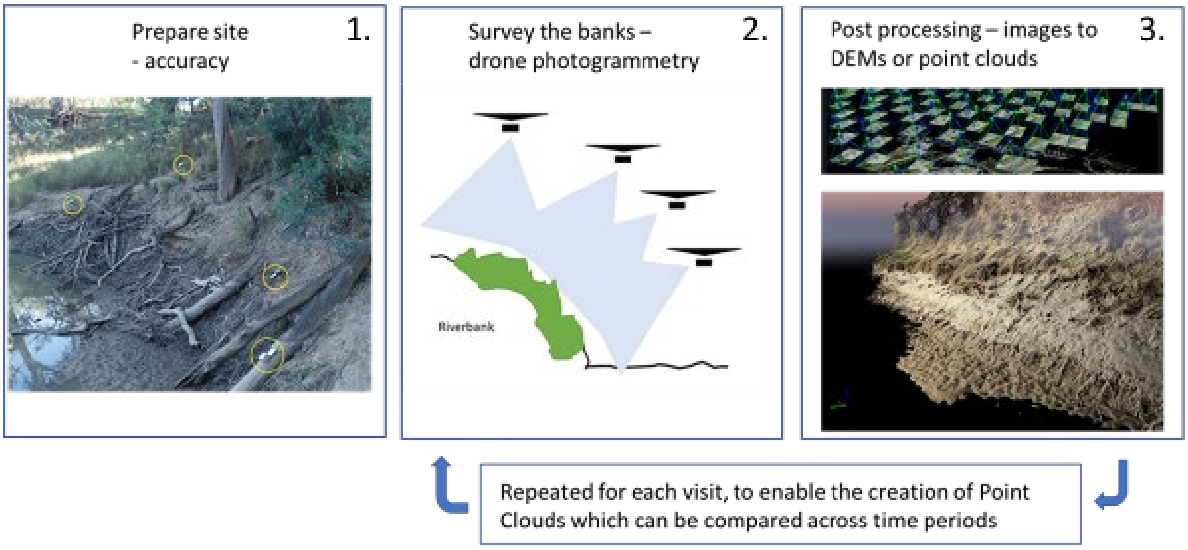


Figure 9.3 Infographic illustrating the three steps to collect field data using drones and to process this data into 3D models.

Monitoring sites

This project included three monitoring sites; two on the Edward/Kolety River and one on Colligen Creek (Figure 9.4).

Figure 9.4. Location of study sites in the Edward/Kolety River and Colligen Creek.

Visit timing related to daily discharge

The varying discharge made determining ideal trip visits challenging as flows had to be considered prior to each visit in addition to weather conditions. Where possible, visit dates were aligned with troughs in flow, to ensure that the largest amount of bank face was visible during the drone monitoring flights. As illustrated in the hydrograph (Figure 9.5) the largest flow event at the Edward/Kolety River site downstream of Stevens Weir peaked in October 2019 when discharge reached over 3,500 ML/day. This event was recorded between visit 1 and visit 2 and the response regarding bank condition is discussed later in this report (Figure 9.5).

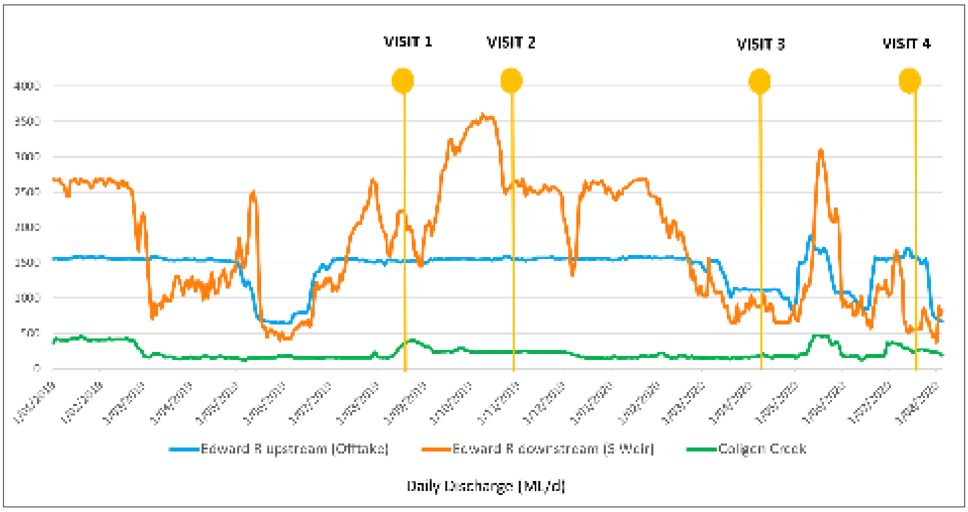


Figure 9.5 Hydrograph illustrating daily mean discharge for each site and the timing each of each visit for drone monitoring. Data sources from WaterNSW Realtime website; site downstream of Stevens Weir is gauge 409023, the Edward River offtake is gauge 409008, Colligen Creek is gauge 409024.

9.5 Drone visits relative to hydrologic events

Edward/Kolety River upstream site

The hydrograph in Figure 9.6 shows the discharge throughout the monitoring period was fairly uniform (near 1,600 ML/day) until March 2020 where a large reduction in flow was evident prior to visit 3. Between visit 3 and 4 there was an unregulated flow event, resulting in a pulse from the River Murray through the offtake into the Edward/Kolety River.

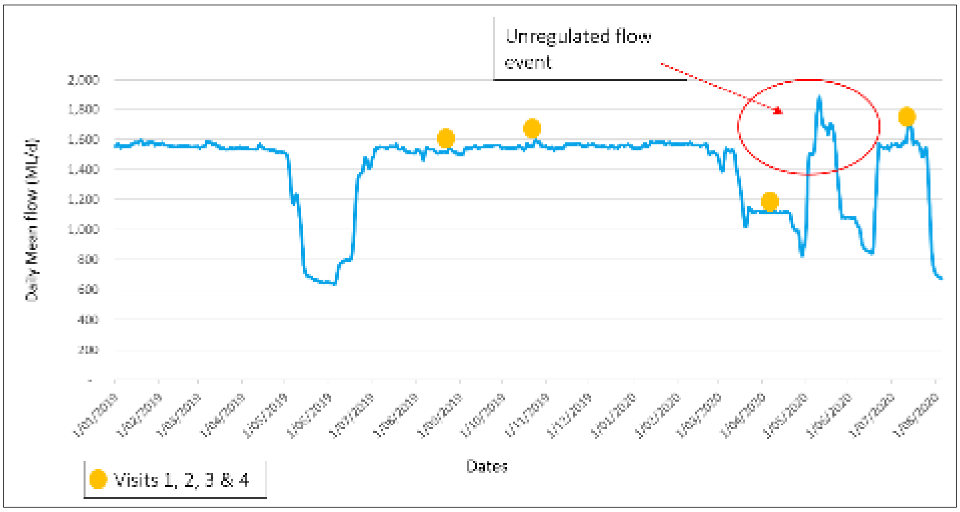


Figure 9.6 Hydrograph illustrating the flow regime at upstream Edward/Kolety River site and visit timings. Daily discharge data for the Edward River offtake are from gauge 409008 (https://realtimedata.waternsw.com.au/).

The two flow pulses that occurred between visit 3 and visit 4 were 1,750 – 2,000 ML/day which represents the unregulated flow event and between 1,000 – 1,250 ML/day which represents regulated flow. The unregulated event will be the major focus for deposition/erosion bank condition analysis and vegetation analysis at this site.

Edward/Kolety River downstream of Stevens Weir

Due to the diversity in volume and characteristics of flow events and the physical qualities of this reach, this site will be the primary focus of this report.

This reach received flows as part of the Southern Connected Flow in the Murray system. This is referred to in the hydrograph in Figure 9.7 as Southern Connected Flow (SCC) flow event.

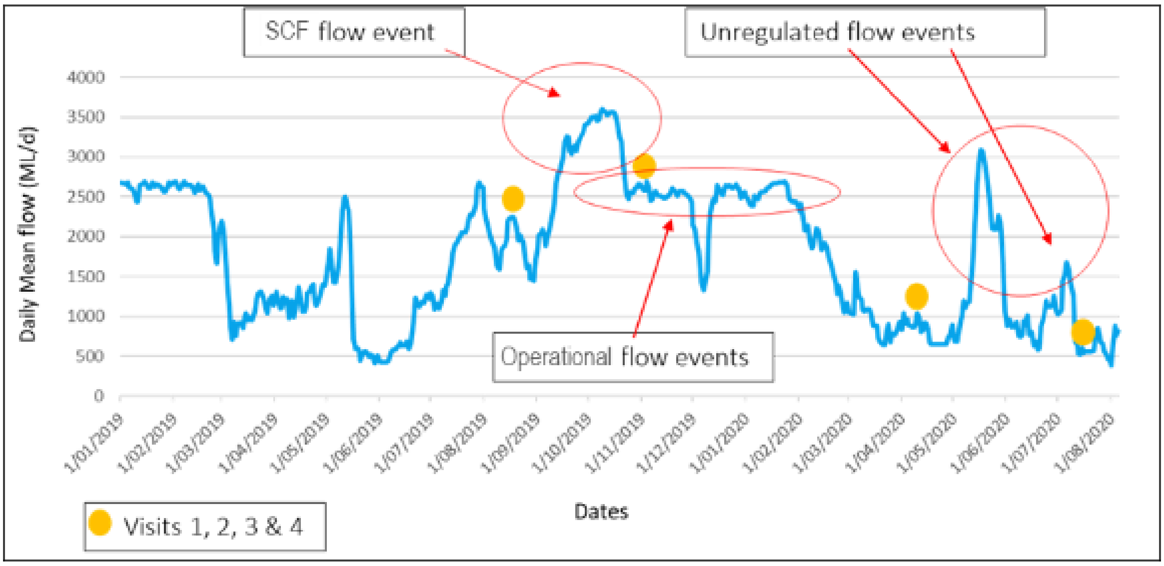


Figure 9.7 Hydrograph illustrating flow at Edward/Kolety River downstream Stevens Weir site with visit dates and flow events plotted

Visits 1 – 2: Southern Connected flow

The Southern Connected Flow during August and September 2019 resulted in an extended period of discharge of up to 3,500 ML/day (Figure 9.7). This represents the maximum flow event monitored across all sites throughout the 12 month study. The major flow pulse of consideration will be 3,000 – 3,500 ML/day which was 28% of total days (23 days). As such, the bank zone corresponding to this flow will be the focus of the analysis for this event.

Visits 2 – 3: Operational flow and Visits 1 – 3 (SCF + Operational Flow)

On more than 50% of days the daily mean discharge was between 2,100 – 3,100 ML/day representing the invariable nature of this operational flow period. The operational flows occurred over almost 3.5 months (October 2019 to February 2020) during the hottest days of the year. The bank zone corresponding to the upper half of this flow will be the key focus during this period. Due to the high water level during the 2nd field visit, analysis can only be done on the bank face relating to flows >2,650 ML/day. To help understand the impact of the prolonged operational flows during this period, DEMOD V1 – V3 was analysed, which covers both the SCF and the operational flows where the flow between 2,300-2,850 (ML/day) is represented greater than 40% of the time period as illustrated in Figure 9.8.

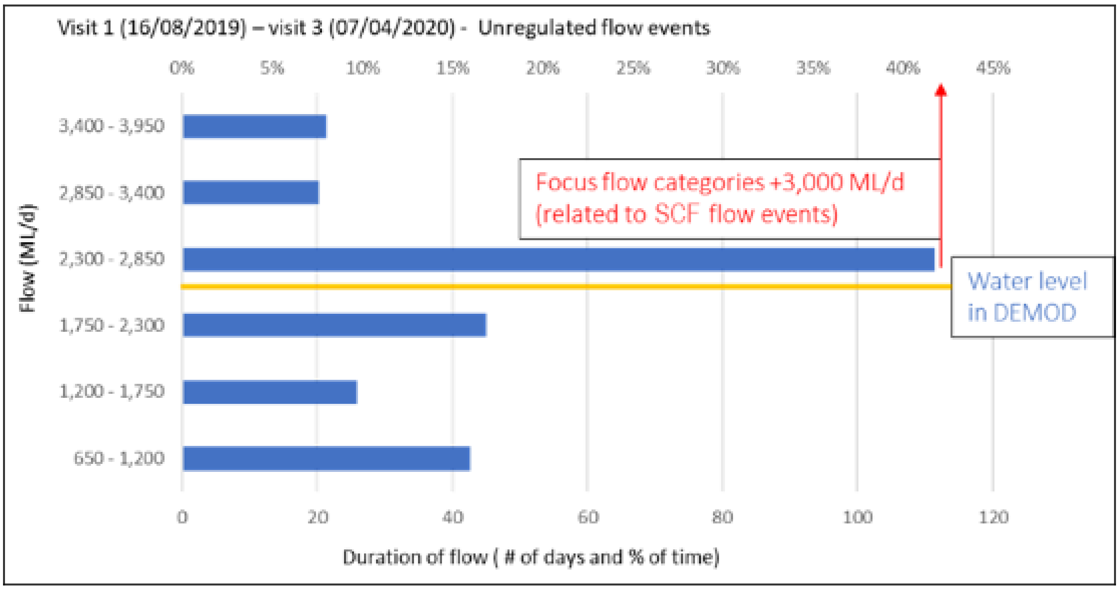


Figure 9.8 Histogram illustrating mean daily discharge by flow range and the number of days and % of time the flow was received between visit 1 and 3 at the Edward/Kolety River downstream of Stevens Weir.

Visits 3 -4: Unregulated flow:

The largest range of mean daily flow (500 – 3,500 ML/day) was experienced during this period and due to low water levels during each visit the DEMODs shows the largest range of bank. The two large peaks in discharge between visits 3 and 4 (Figure 9.7) are a result of unregulated flow events. These unregulated flows are managed quickly and, as such, result in the rapid recession of water level in the reach. These spikes in discharge are being analysed as they have historically resulted in extensive erosion along the Edward/Kolety system, and as such these are the focus of analysis during this time period (bank zone correlating to >1,200 ML/day flows).

Colligen Creek

The Colligen Creek reach received a CEW delivery which influenced the hydrology at this site from early August 2019 to December 2019. The resulting increase to the base flow at Colligen Creek influenced flow across Visits 1 and 2 (Figure 9.9).

The SCF during August and September resulted in the peaks in flow (Figure 9.9) between visit 1 and visit 2 with discharge peaks of 400 ML/day and 300 ML/day respectively. However, due to the height of the water level on visit 1, only the first event will be analysed for impact to vegetation and bank condition changes as a response to flow. Figure 9.10 illustrates that this flow represents <20% of days during this time period, so we considered that significant change to bank condition was unlikely.

As illustrated in the hydrograph in Figure 9.9, unregulated flows in Colligen Creek between visits 3 and 4 were the largest experienced during the study period with the first reaching +450 ML/day and the second reaching +350 ML/day. Additionally, these events were sustained for longer periods than the SCF between visits 1 and 2 (Figure 9.10). These flow events will be reviewed for bank and vegetation condition changes.

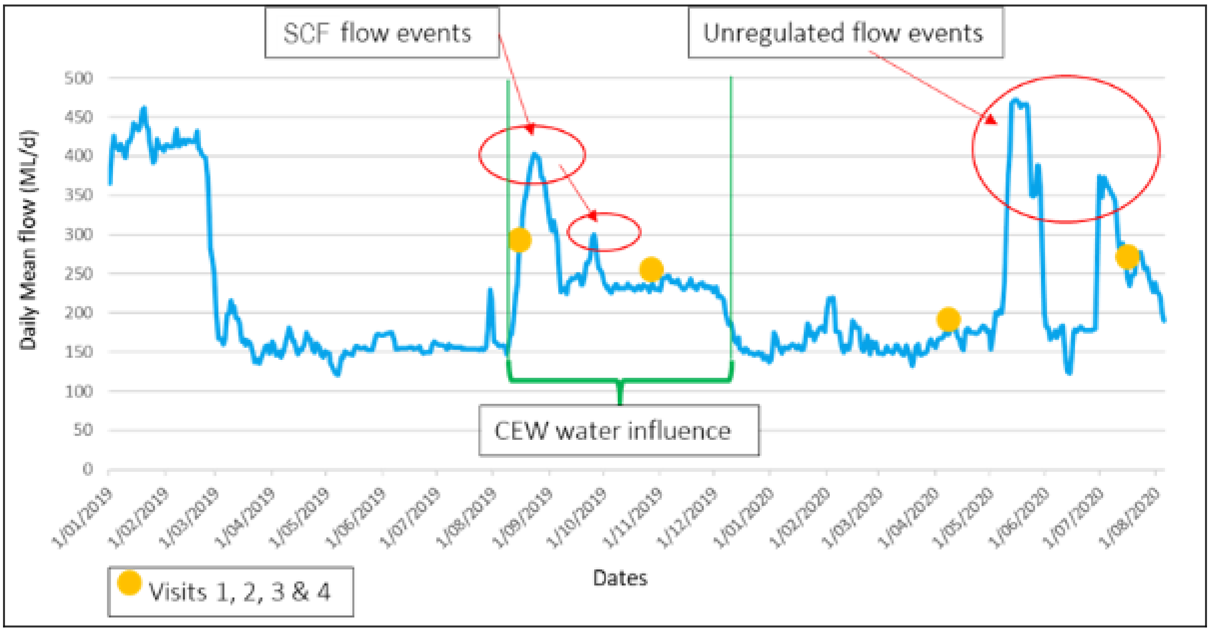


Figure 9.9 Hydrograph illustrating flow at Colligen Creek with visit dates and flow events plotted

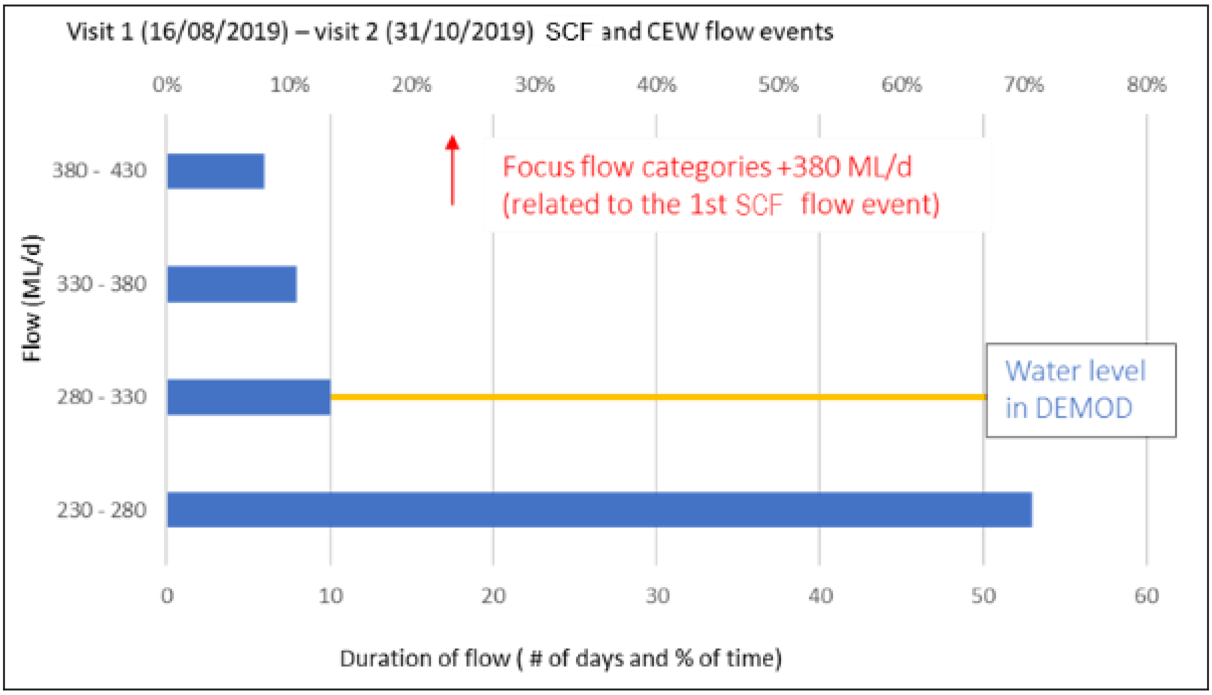


Figure 9.10 Histogram illustrating mean daily discharge (ML/day) by flow range and the number of days and % of time the flow was received between visit 1 and 2 at the Colligen Creek site.

9.6 Results

Bank Condition

Edward/Kolety River (upstream site)

The upstream Edward/Kolety River monitoring site is based in the Murray Valley National Park downstream of the confluence with the River Murray and upstream of Deniliquin. One bank of interest was chosen for analysis and one vegetation zone (Figure 9.11).

Figure 9.11. Location of banks of Interest within the Edward/Kolety River (Upstream) monitoring Site.

Visit 1 – Visit 2

As detailed in the hydrograph (Figure 9.6) there was a uniform discharge between visits 1 and 2. The flow was uniform at 1,500 ML/day and accordingly resulted in no significant change that could be seen on the DEMOD, or registered through volume metric outputs. The DEMODs for this period are in the appendix.

Visit 3 – Visit 4 (Unregulated flow events)

The monitoring period between visit 3 and 4 is of most interest due to the variation in flow during this period. However, due to increases in flow above the constant water level threshold being relatively minor it appears that erosion/deposition has been relatively minimal in response to these unregulated flow events with only scattered areas of erosion in areas above the notch visible (Figure 9.12).

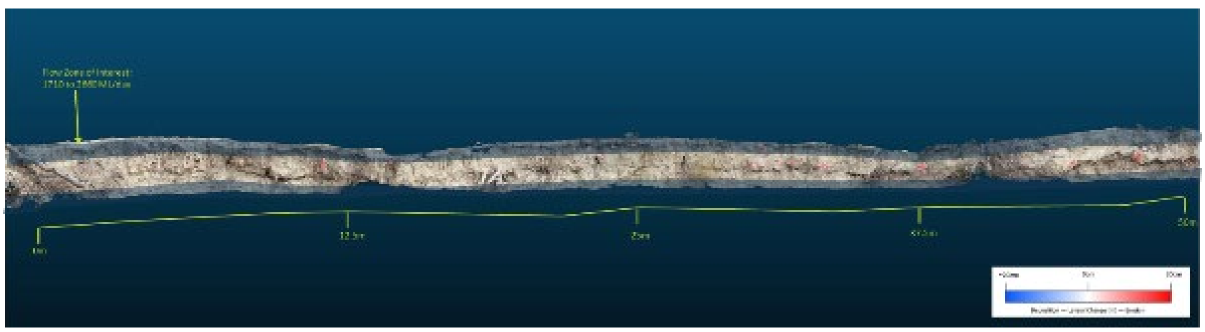


Figure 9.12 DEMOD comparing visit 3 to 4 at Edward/Kolety River upstream site.

The volume metric change output from this model could not be resolved as a result of a) very little change, and b) some distortion in aligning visits. Accordingly, volume metric change for this period is not presented. The data can be found in the Appendix.

Edward/Kolety River downstream of Stevens Weir

The downstream Edward/Kolety River site is located approximately 15 kilometers downstream of Stevens Weir, north-west of Deniliquin. One bank of interest was monitored at this site (Figure 9.13).

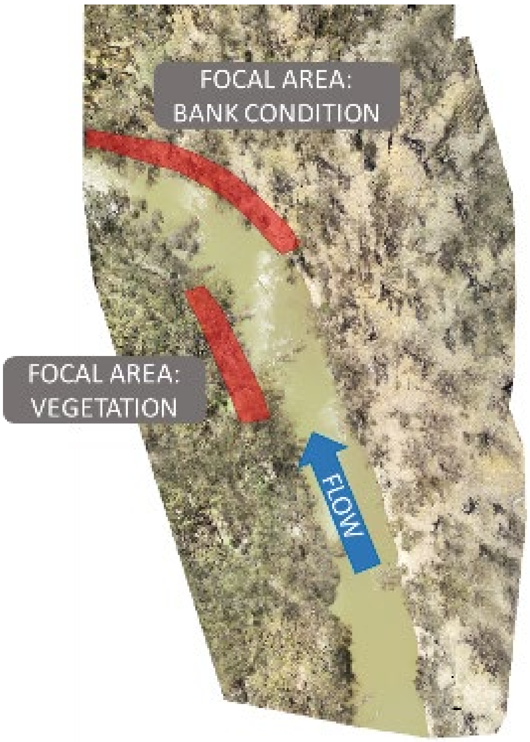


Figure 9.13 Location of banks of interest within the Edward/Kolety River downstream of Stevens Weir.

Visit 1 – Visit 2 (SCF events)

We predicted that the SCF with an extended period of max flow (17 days above 3,500 ML/day) and a medium paced falling limb (<14 days from >3,500 to 2,500 ML/day) (Figure 9.14) would lead to erosion high on the bank, in the zone relating to 3,000-3,500 ML/day, and some deposition in areas below points major erosion. We also predicted that the event would result in significantly less erosion than the operational flow, and more erosion than the unregulated flow event (v3-v4).

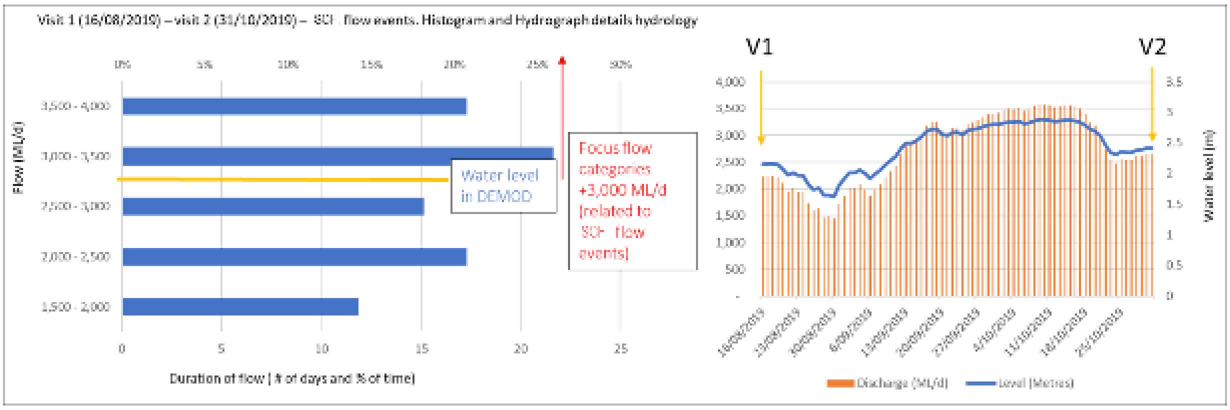


Figure 9.14 Hydrology detailed between visit 1 and 2 (SCF events). Histogram and hydrograph.

The highest volume of erosion (1.82cm^3/m^2) and deposition (1.2cm^3/m^2) occurred between v1 and v2 compared to operational flows (v2-v3) and unregulated flows (v3-v4) equating to net erosion -0.62cm^3/m^2. Erosion is expressed primarily on the right bank half and above the existing notch in small and larger patches which correlate directly with the SCF peak flow bank zone (3,000-3,500 ML/day). Larger patches of deep (+20cm) erosion appear to be a result of mass-failure (slumping). Beneath these areas of mass-failure can be found the major areas of deposition (Figure 9.15 and 9.16). It is likely that the notch would have reset/reduced in areas beneath the mass-failure events and the bank re-profiled as a result. Accordingly, there is an opportunity in these areas for the bank to gain more stability with an intact toe, however, this dependant on subsequent flow events.

Table 9.2 Volume change output from Edward/Kolety River DS Site v1 to v2 DEMOD

DEMOD comparison and bank side	Bank area (m2)	Total Erosion (m3)	Total Deposition (m3)	Tot erosion (m3) per m2 of bank	Tot depos (m3) per m2 of bank	Erosion as a % of depos
V1 – V2: Left bank	83.40	1.58	0.82	0.02	0.01	+94%
V1 –V2: Right bank	84.00	1.46	1.19	0.02	0.01	+23%
V1 – V2: Total	167.40	3.04	2.01	0.02	0.01	+52%

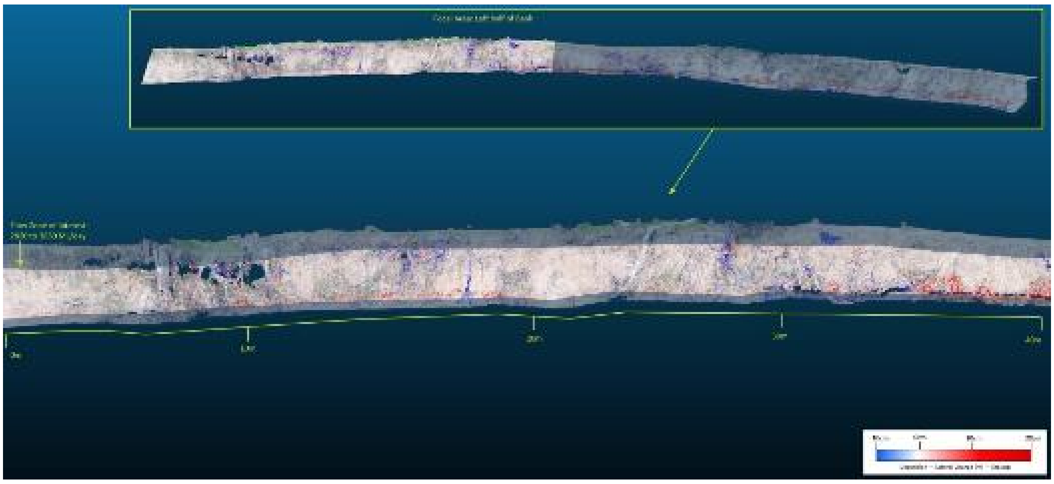


Figure 9.15 DEMOD comparing Edward/Kolety Rver DS Site v1 to v2 (left side of bank).

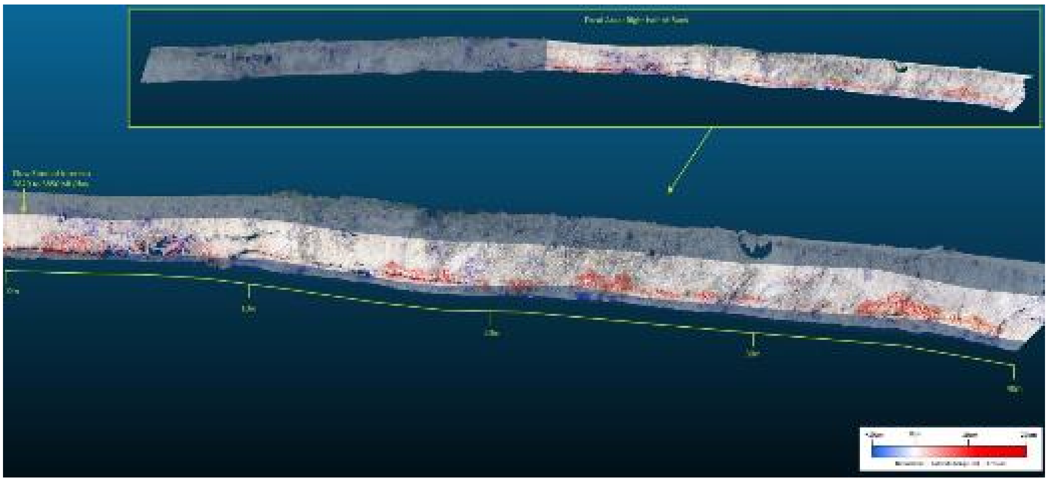


Figure 9.16 DEMOD comparing Edward/Kolety River DS Site v1 to v2 right side of bank).

Visit 2 – Visit 3 (operational flow)

We predicted that due to the extended period of inundation between 2,300 – 2,800 ML/day over more than 90 days and the uniform profile of the hydrology during the majority of this monitoring period (Figure 9.17), that unregulated flows would result in the largest volume of erosion and the least volume of deposition due to defined erosion at the related bank zone to this flow regime. The cyclical nature of flow is likely to remove the majority of deposition.

The average erosion of 0.82 (cm^3/m^2 of bank) was relatively low compared to volumes recorded against the SCF event, and deposition was the lowest across all comparison windows. Considering the limitations of analysis due to the water level this is not surprising. Erosion was considerably higher than the deposition received (>42%) resulting in -0.26 net erosion (Table 9.). Erosion is primarily expressed below the notch, consistently (along right half of bank and some of left half), and deeply (between 15-20cms) relating to the flow zone relative to 2,300-3,800 ML/day. Deposition cannot be seen within the model (Figures 9.18 and 9.19).

As illustrated in Figure 9.17, the water level during v2 was >2,600 ML/day, this has limited the range of available bank for monitoring, and thus data from this DEMOD will not show the full extent of the change resulting from the flow received. Only change related to flow above 2,600 ML/day can be fully analysed.

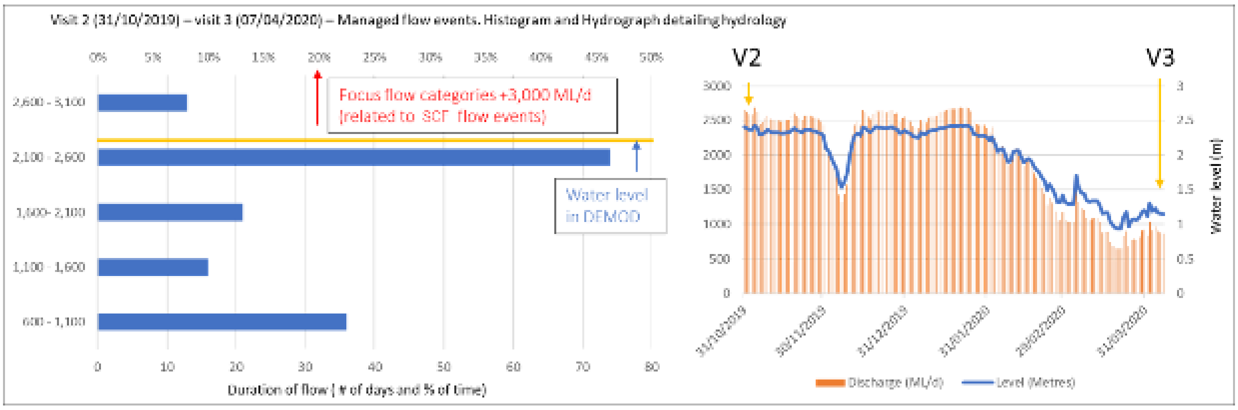


Figure 9.17 Hydrology detailed between v2-v3 (operational flow events). Histogram and hydrograph.

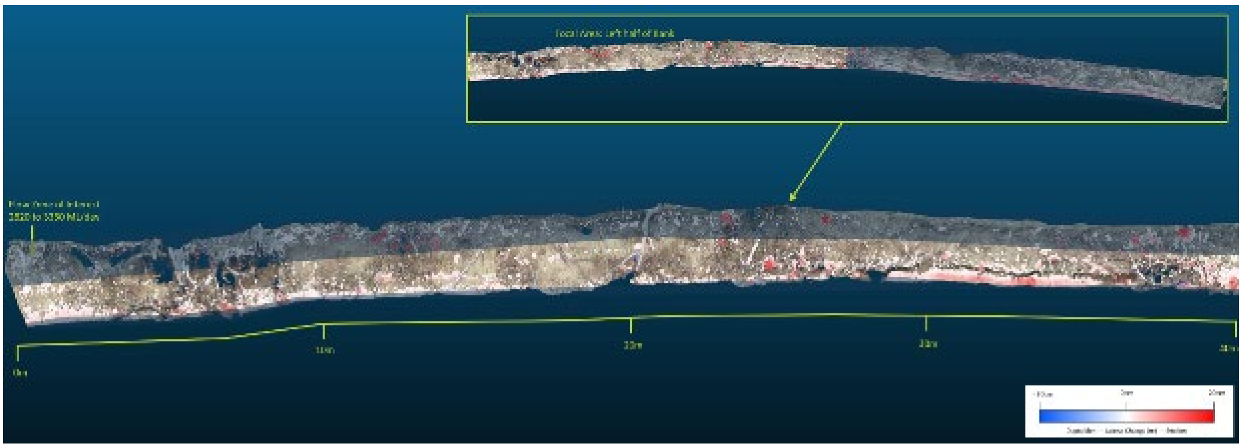


Figure 9.18 DEMOD comparing Edward/Kolety River DS Site v2 to v3 (Left bank).

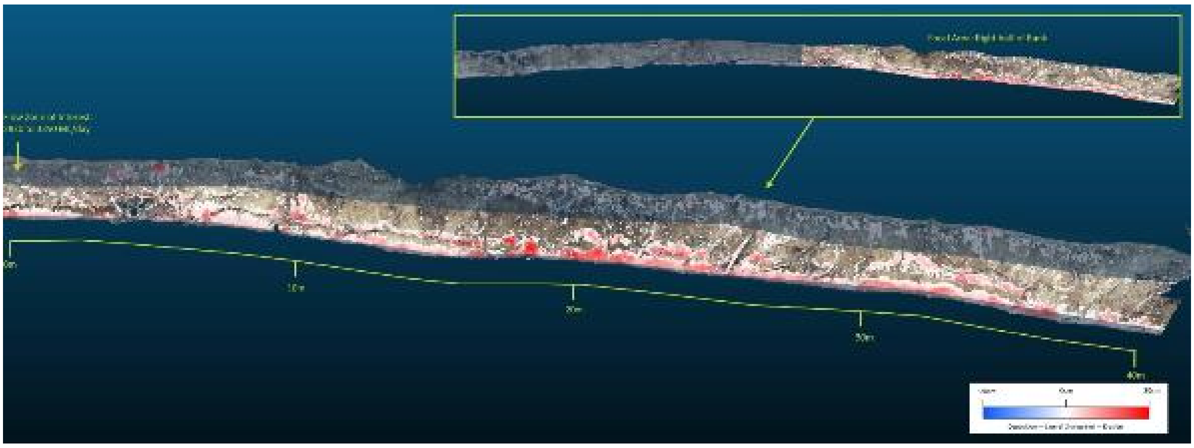


Figure 9.19 DEMOD comparing Edward/Kolety River DS Site v2 to V3 (Right bank).

Table 9.3 Volume change output from Edward/Kolety River DS Site v2 to v3 DEMOD

DEMOD comparison and bank side	Bank area (m2)	Total Erosion (m3)	Total Deposition (m3)	Tot erosion (cm3) per m2 of bank	Tot depos (cm3) per m2 of bank	Erosion as a % of depos
V2 – V3: Left bank	105.76	0.98	1.05	0.93	0.99	-6%
V2 –V3: Right bank	146.61	1.20	0.50	0.82	0.34	+142%
V2 – V3: Total	252.37	2.18	1.54	0.87	0.61	+42%

Visit 3 – Visit 4 (unregulated flow)

We predicted that the unregulated flows during period v3-v4 (Figure 9.20) would lead to areas of mass-failure, due the rapid receding limb of the first event (which reached >3,000 ML/day). However, this hypothesis largely depends on a) whether bank sediment is submerged long enough to reach saturation, and b) the stability of banks in these zones of inundation following the summer operational flows. It was expected that deposition would be high in response to erosion in areas above.

There were relatively low levels of erosion and deposition in comparison to other monitored periods (Figure 9.21), however, deposition relative to erosion was higher than any other period (Table.4). Erosion (<20cms) was expressed primarily, beneath the notch (flow zone between 1,500 – 2,000 ML/day: Figure 9.22) in addition to above and below. Deposition was present primarily beneath the notch, but also with high diversity both laterally and vertically across the bank face. The patterns of change evident within the DEMODs (Figure 9.21 and 9.22) are relative to the large fluctuations of discharge experienced during the unregulated flow events.

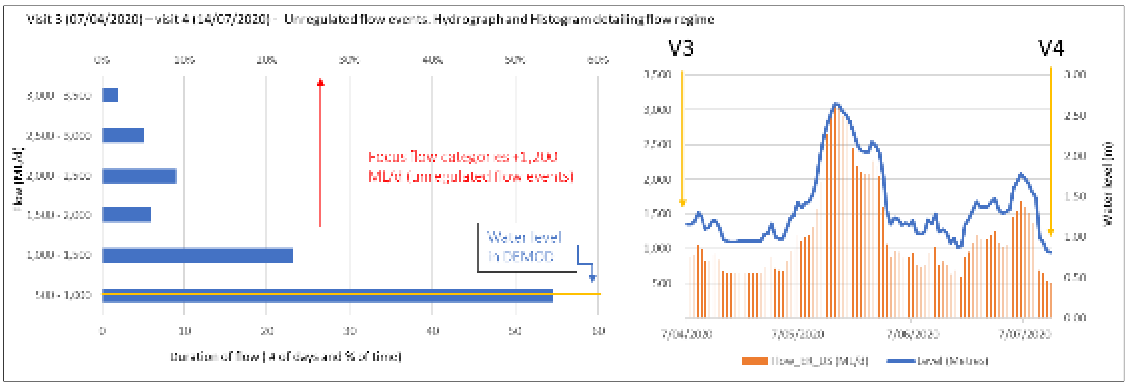


Figure 9.20 Hydrology detailed between v3-v4 (unregulated flow events). Histogram and hydrograph.

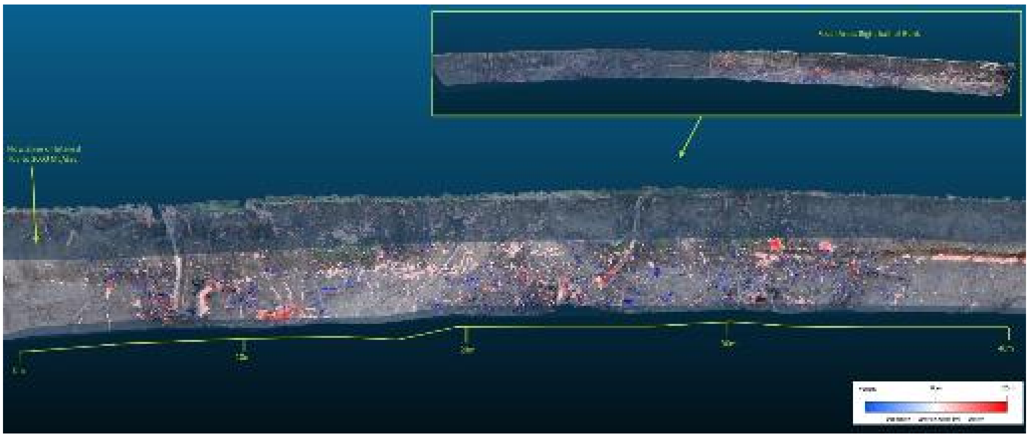


Figure 9.21 DEMOD comparing Edward/Kolety River DS Site v3 to v4 (Left bank).

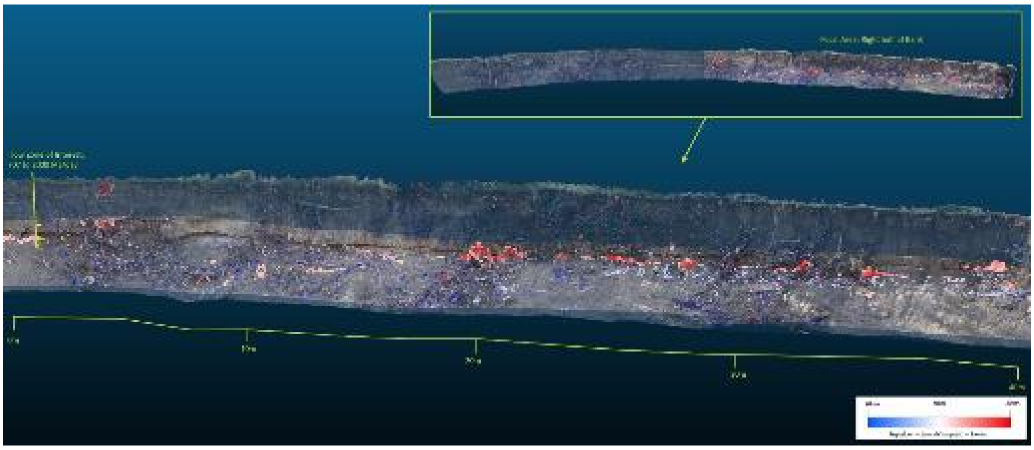


Figure 9.22 DEMOD comparing Edward/Kolety River DS Site v3 to v4 (Right bank).

Table 9.4 Volume change output from Edward/Kolety River DS Site v3 to v4 DEMOD

DEMOD comparison and bank side	Bank area (m^2)	Total Erosion (m^3)	Total Deposition (m^3)	Tot erosion (cm^3) per m^2 of bank	Tot depos (cm^3) per m^2 of bank	Erosion as % of depos
V3 – V4: Left bank	144.48	1.25	2.92	0.87	2.06	-26%*
V3 –V4: Right bank	181.46	0.94	1.74	0.52	1.02	-55%*
V3 – V4: Total	325.94	2.19	2.19*	0.67	0.67*	0%*

**Deposition results were distorted as a result of vegetation and slight model miss-alignment in this DEMOD. Deposition numbers were cautiously reduced to be in-line with erosion; therefore this site is net neutral (erosion = deposition).*

Visit 1 – Visit 3 (SCF > operational flow events)

It was expected that due to the long period of inundation between 2,300-2,850 ML/day (relating to the operational flow event (Figure 9.23) that erosion would be higher than all other comparisons, and evident in a specific area below and just above the notch due to the destabilisation of bank in response to a deepening notch.

These DEMODs (Figure 9.24 and 9.25) compare visits 1-3 and should give more clarity on the true impact of the operational flow period (v2 - v3) due to the available bank face that can be analysed because water levels were considerably lower for these visits. With water level on visit 1 at 2,200 ML/day the entire impact of the prolonged operational flow events can be recorded, which adds up to 111 days as illustrated in the histogram (Figure 9.23). Erosion is expressed extensively and deeply (>40cms) below and above the notch resulting in significant loss of 2.31 (cm^3/m^2) which was >50% vs deposition, resulting in net erosion of -1.49 which is >140% higher than that observed during the SCF alone (9.5).

Large areas of mass-failure are evident along the bank and shaving beneath the notch is clear and consistent. The erosion zone relates to both the SCF peak flows (>3,000 ML/day) and the prolonged (>90 days) operational flow period (2,300-2,800 ML/day). Deposition was relatively low and difficult to visualise on the DEMODs (Figure .24 and 9.25).

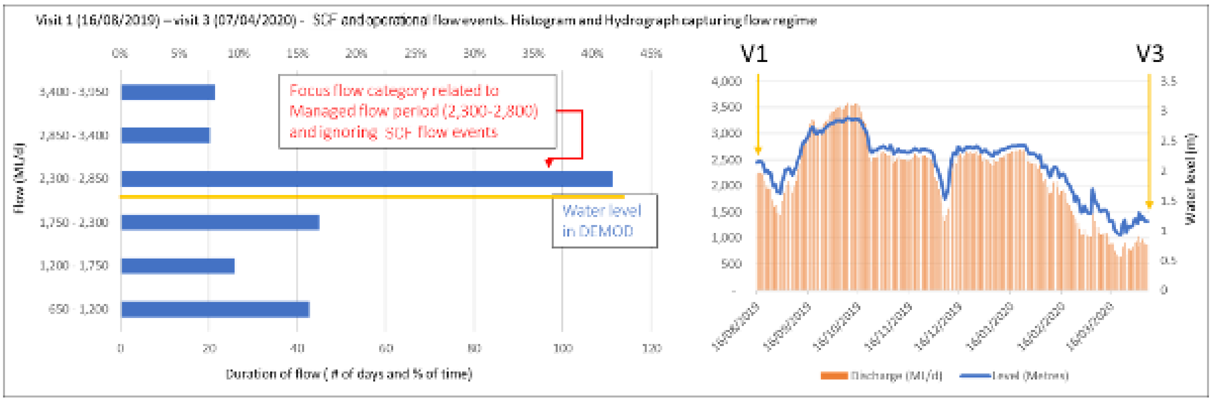


Figure 9.23 Hydrology detailed between v1-v3 (SCF and operational flow events). Histogram and hydrograph.

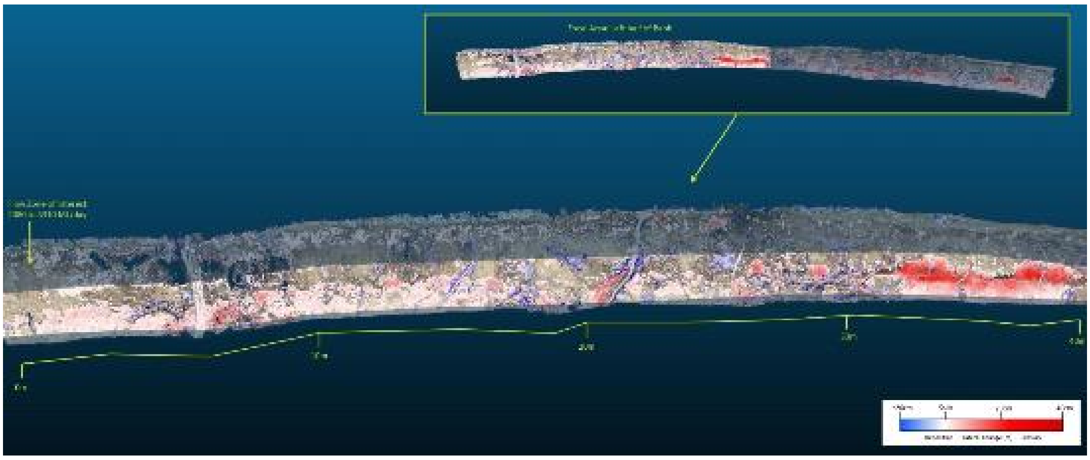


Figure 9.24 DEMOD comparing Edward/Kolety River DS Site v1 to v3 (Left bank).

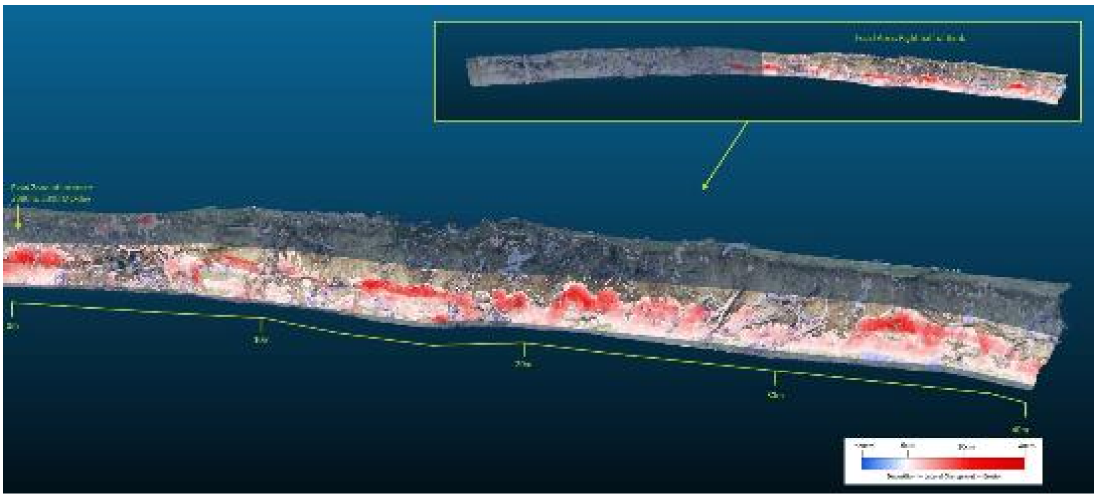


Figure 9.25 DEMOD comparing Edward/Kolety River DS Site v1 to v3 (Right bank).

Table 9.5 Volume change output from Edward/Kolety River DS Site v1 to v3 DEMOD

DEMOD comparison and bank side	Bank area (m2)	Total Erosion (m3)	Total Deposition (m3)	Tot erosion (cm3) per m2 of bank	Tot depos (cm3) per m2 of bank	Erosion as a % of depos
V1 – V3: Left bank	125.99	2.28	1.23	1.81	0.98	+85%
V1 –V3: Right bank	126.51	3.55	`0.84	2.81	0.67	+321%
V1 – V3: Total	252.50	5.83	2.08	2.31	0.82	+181%

Colligen Creek

The Colligen Creek monitoring site is located in Calimo, NSW, northwest of the township Deniliquin. One bank of interest and one primary vegetation zone was monitored for this project as detailed in Figure 9.26. Colligen Creek was the only site to have received CEW water through return flows resulting from an environmental water action delivered to Millewa Forest.

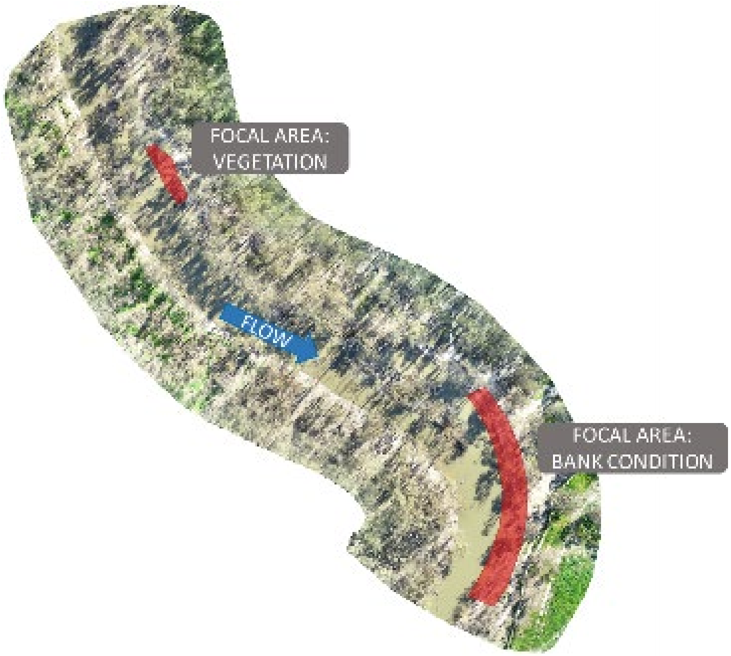


Figure 9.26 Image illustrating the location of the bank and vegetation monitored at Colligen Creek Visit 1 – Visit 2 (SCF and CEW flow events)

Change detected across each of the monitoring periods was minimal. Insights cannot be gathered from either visual expressions of change apparent on the DEMODs (Figure 9.9.27, Figure 9., Figure 9.29 and Figure 9.) or volume metric change numbers (Tables 9.6 and 9.7) from the calculated outputs. With this considered, any conclusions made from this data around geomorphic processes in response to flow events could be misinterpreted. Very low margins of change (-0.5cm/m^2 of bank) are unreliable as distortion in response to vegetation or slight miss-alignment can impact the data.

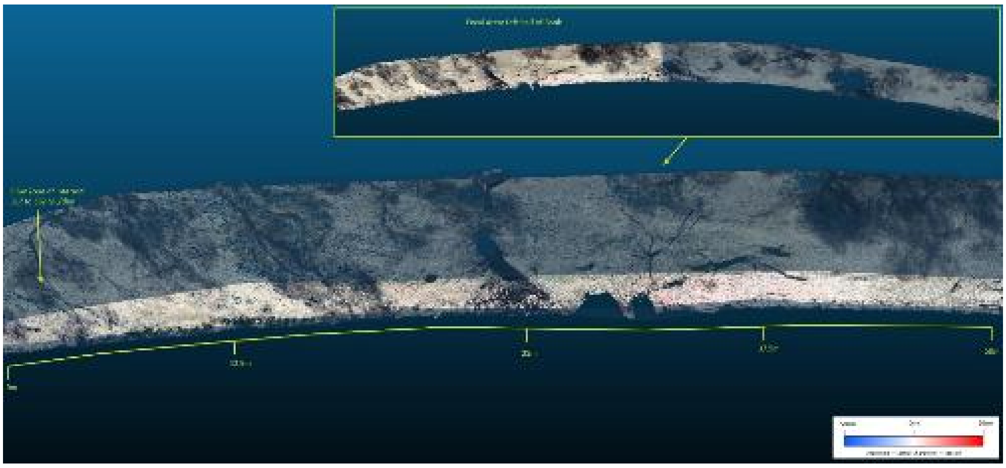


Figure 9.27 DEMOD comparing Colligen Creek Site v1 to v2 (Left bank).

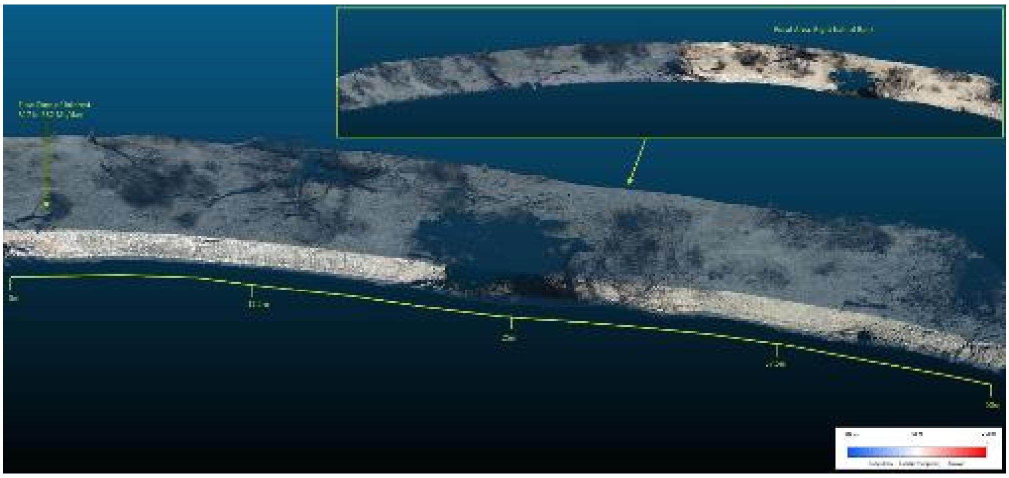


Figure 9.28 DEMOD comparing Colligen Creek Site v1 to v2 (Right bank).

Table 9.6 Volume change output from Colligen Creek v1-v2 DEMOD.

DEMOD comparison and bank side	Bank area (m2)	Total Erosion (m3)	Total Deposition (m3)	Tot erosion (cm3) per m2 of bank	Tot depos (cm3) per m2 of bank	Erosion as a % of depos
V1 – V2: Left bank	192.84	0.15	0.31	0.08	0.16	-52%
V1 –V2: Right bank	160.86	0.04	0.05	0.03	0.03	-23%
V1 – V2: Total	353.72	0.19	0.36	0.05	0.10	-47%

Visit 3 – Visit 4 (Unregulated flow events)

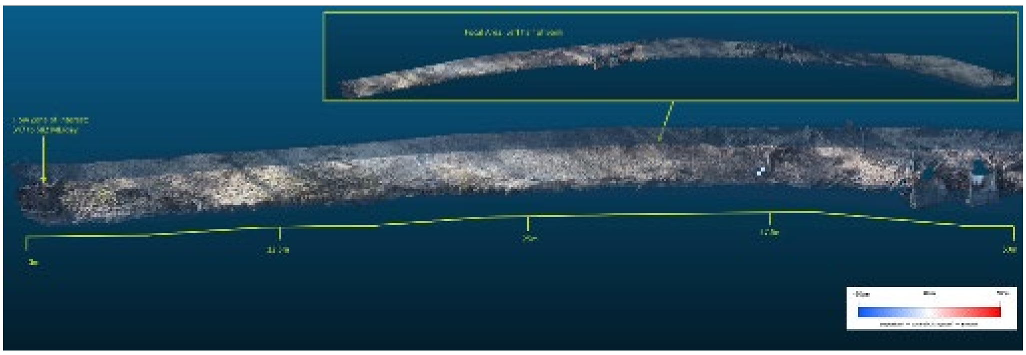


Figure 9.29 DEMOD comparing Colligen Creek Site v3 to v4 (Left bank).

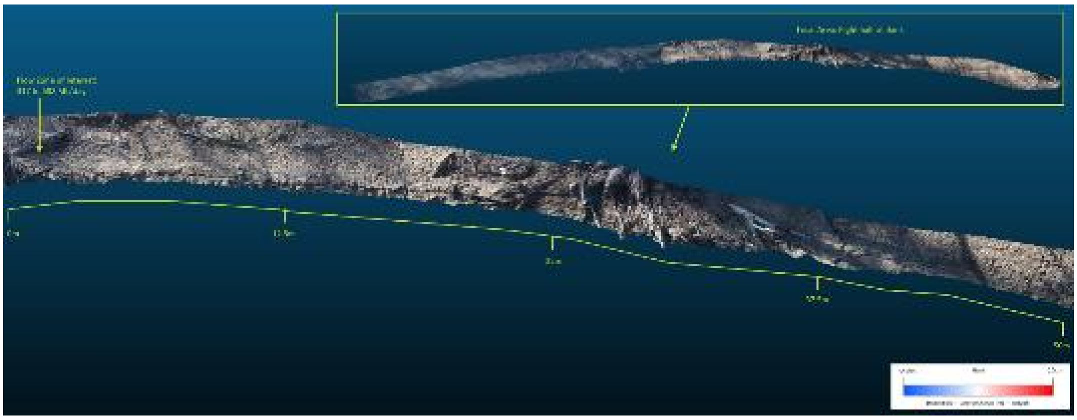


Figure 9.30 DEMOD comparing Colligen Creek Site v3 to v4 (Right bank).

Table 9.7 Volume change output from Colligen Creek v3-v4 DEMOD.

DEMOD comparison and bank side	Bank area (m2)	Total Erosion (m3)	Total Deposition (m3)	Tot erosion (cm3) per m2 of bank	Tot depos (cm3) per m2 of bank	Erosion as a % of depos
V3 – V4: Left bank	230.74	0.12	0.05	0.05	0.02	>143%
V3 –V4: Right bank	168.06	0.04	0.22	0.03	0.13	-81%
V3 – V4: Total	398.79	0.67	0.27	0.04	0.07	-39%

Summary of change linked to hydrology

Due to the accuracy and volume of data gathered from the Edward/Kolety River downstream of Stevens Weir this is being used for analysis of bank condition in response to flow events.
relates the pattern of erosion and deposition to flow and discusses the geomorphic processes driving the recorded change. Table 9.8 describes how the raw numbers (cm^3/m^2) relate to different flow packets. It is clear to see in Figure 9.31 the huge impact that the SCF and operational flows combined have on total erosion and net change, this highlights the importance of considering the combined (rather than isolated) impact of flow events.

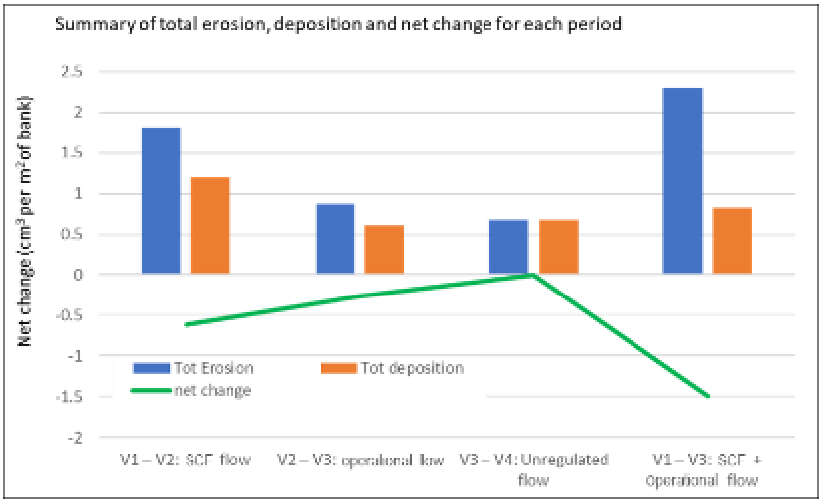


Figure 9.31 Bar chart summarising total deposition, erosion and net sediment change (deposition minus erosion)

Table 9.8 Summary of observations for each monitoring window

Visit and Flow type	Observations summarised with reference to flow and processes
Visit 1 – 2 (SCF Period) Aug, Sep, Oct 2019	*Change was recorded on the bank zone corresponding to >2,500 ML/day* **Erosion:** largest volume recorded across all periods. Primarily located (vertically) above the existing notch in bank zones relating to flow packet 3,000 – 4,000 ML/day (Table 9.9) and in patches laterally across the entire bank face up to depths of 20cms. **Deposition:** largest volume recorded across all periods. Primarily located in areas directly beneath areas of erosion. Deposits from erosion and up to 20 cms in depth. **Process:** Evidence of extensive mass-failure events in response to the rising (above) and falling (below) the existing notch, leading to critical stress reached on the drawdown and subsequent destabilisation of sediment in these bank zones.
Visit 2 – 3 (operational flow Period) Nov 2019- Feb 2020	*Change was recorded above 2,650 ML/day due to the water height on survey, and change beneath this bank zone was not recorded, so to fully understand the response v1-v3 must be analysed.* **Erosion:** Lowest volume of erosion across all periods. Located primarily vertically below the existing notch in bank zones relating to 2,600 – 2,800 ML/day (Table 9.9) and across the entire face of the bank (laterally) in depths of up to 20cms. Also located in some areas above the notch. **Deposition:** Lowest volume of deposition across all periods **Process:** Cyclical rising and falling of water level resulting in corresponding wetting and drying of sediment with a defined vertical zone of the bank beneath the notch. Resulting in the deepening of the notch in areas and the resetting of the notch in areas where mass-failure events in response to the prior SCF event were apparent. In areas the deepening notch resulted in further mass-failure events to sediment above the notch.
Visit 3 – 4 (Unregulated flow period) Apr – Jul 2020	*Change was recorded above 1,000 ML/day due to low water levels.* **Erosion:** second largest volume of erosion recorded primarily in patches vertically beneath the notch (mostly <10cms in depth), in addition to minor erosion above and below the notch. The diversity of these locations reflects the diversity in flow during this period and the area of bank monitored (Table 9.9). Less erosion than hypothesised could be due to a combination of short max flow period (7days) meaning less saturation, and prior mass-failure events in response to SCF flows removing unstable upper bank. **Deposition:** was the highest relative to erosion, resulting in net neutral change during this period. Deposition located primarily in patches above and below the notch and laterally across the bank face responding to areas of roughness and shallower bank profile (up to 20cms in depth), but not corresponding to areas of erosion in most cases. Initial results presenting a net positive deposition outcome were scaled back due to distortion from vegetation in the lower section of the DEMOD). **Processes:** Minor erosion due to wetting and drying of the recently aggravated notch, in addition to some impact of woody debris colliding with the lower bank during high flow periods. Minor deposition primarily due to the consolidation of sediment on areas of roughness on the drawdown of the receding limb.
Visit 1 – 3 (SCF and operational flow events)	*Change was recorded above 2,300 ML/day and as a result this DEMOD is being used to understand the full extent of operational flow events (v2-v3)* The combined impact of SCF + operational flow periods gives a clear view of the combined impact of a max flow event rising over the notch and an operational flow rising and falling below the notch. **Erosion:** expressed consistently above and below the notch at up to 40cm depths relating primarily to 99 day period in which the operational flows delivered discharge between 2,300-2,850 ML/day, but also to flow categories +3,000 (SCF events) (Table 9.9). **Deposition:** is relatively minor compared to erosion and is barely visible in the DEMOD **Processes:** as discussed in sections for v1-v2 (SCF) and v2-v3 (operational flow). The lack of deposition recorded across this window is likely to be a result of the cyclical nature of operational flows after the SCF flows. The sequential positioning of these flow events, combined with the vertical location of the notch, has resulted in significant erosion and channel widening within the system.

Table 9.9 Summary of volume change relative to flows and related drone survey

Visits compared, flow event & flow range (ML/day)	Duration of focus flow event (days)	Vol change (D* E* and net outcome* (cm^3/m^2)
V1- V2: RMC flows 2,800 – 3,800 (1,000)	Total = 43 2,800 – 3,000 = 7 3,000 – 3,500 = 20 3,500 – 4,000 = 16	Erosion = 1.82 Deposition = 1.20 -0.62 (net erosion)
V2 – V3: Managed 2,600 – 2,800 (300)	Total = 13 2,600 – 2,800 = 13	Erosion = 0.87 Deposition = 0.61 -0.26 (net erosion)
V3 - V4: Unregulated* 1,000 – 3,500 (2,500)	Total = 45 1,000-1,500 = 23 1,500 - 2,200 = 6 2,000 - 2,500 = 9 2,500 - 3,000 = 5 3,000 - 3,500 = 2	Erosion = 0.67 Deposition = 0.67 Neutral
V1 - V3: RMC and Managed 2,300 – 3,800 (1,500)	Total = 127 2,300 – 2,850 = 99 +3,000 = 28	Erosion =2.31 Deposition = 0.82 -1.49 (net erosion)

Key Insights

1. The position of an existing notch relative to the receiving bank zone of prolonged periods of flow, is a critical factor in regard to the expression and volume of change (erosion/deposition).
    a. If the existing notch is beneath (e.g. 50cms) a zone of prolonged (>20 days) inundation, bank saturation in addition to a subsequent rapid drawdown of water level, is likely to lead to mass-failure as demonstrated in the response to the SCF.
    b. If the existing notch is positioned just above (e.g. >50cms) a zone of prolonged inundation with a very small flow range, the cyclical process of wetting and drying due to the minor rises and falls in water level is likely to result in significant increases to the existing notch. This is demonstrated in the bank response to operational flows (v2-v3)

When these two events are combined, in sequence, the result is large scale erosion across and above the existing notch as demonstrated in Figure 9.32.

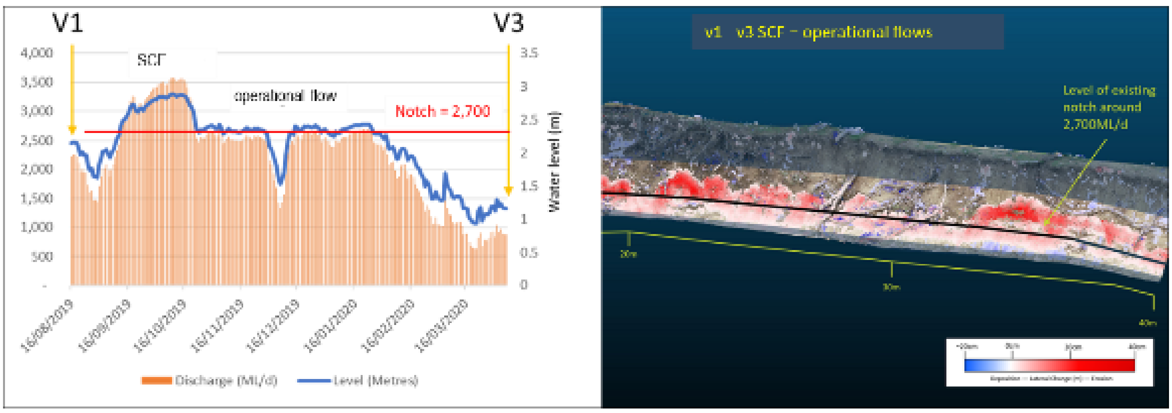


Figure 9.32 Level of the notch in relation to daily mean flow (left) and responding erosion shown in the DEMOD (both related to the period between v1 – v3 which covers SCF + operational flow events.

2. The length of inundation and the speed of drawdown are both critical in determining the extent of erosion due to the fact that saturation plays a key role in determining the volume of erosion. Figure 9.33 compares the difference in erosion between SCF (v1-v2) and unregulated (v3-v4) flow events which received >20 days and <7 days of discharge above the notch respectively.

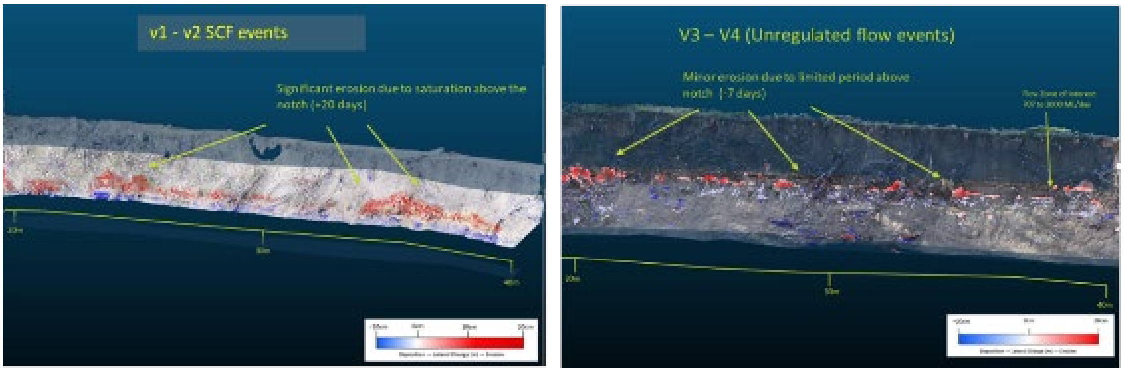


Figure 9.33 Difference in erosion in response to SCF (v1-v2) flow versus unregulated (v3-v4) flow events arguably due to the duration of inundation above to bank zone above the notch.

3. Operational flows that result in periods of invariable flow, result in deep and defined erosion creating a notch and under-cutting. Figure 9.34 illustrates that between Aug-2015 – Aug 2020 there has been four operational flow periods all within the very defined discharge range of 2,300 – 2,800 (ML/day). This equates to 22% of the total time period (388/1,800 days) during the hottest months of the year, where the process of cyclical wetting and drying of banks has the most impact on clay rich soils.

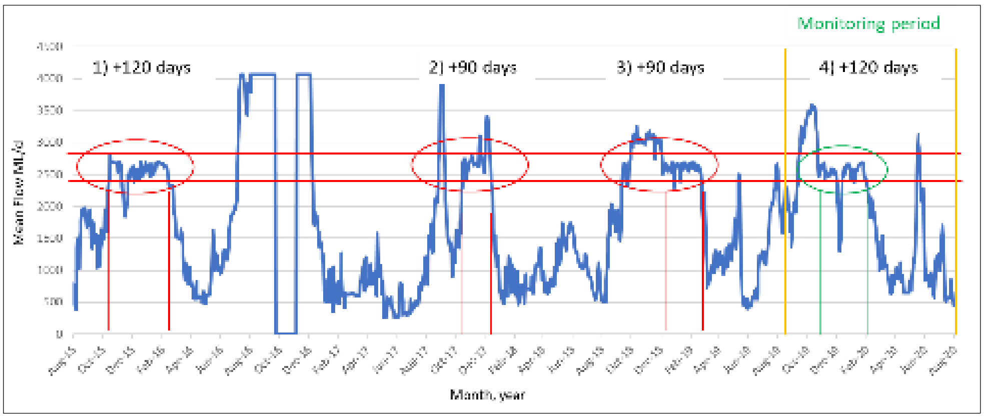


Figure 9.34 Hydrograph indicating daily mean flow at DS Stevens Weir gauge from Aug 2015-Aug 2020 and highlighting areas of operational flow which occurred in the *notch zone* corresponding to discharge between 2,300 – 2,800 ML/day. The maximum discharge that occurred during the flood in 2016 have been truncated in this figure.

4. Figure 9.35 highlights that there is a distinct pattern in flow regime from year to year which can be summarised into a 3 step process, and also highlights how a simple sequence of events can result in large scale erosion, and arguably extensive channel widening, within a system:
   1) Prolonged (+30 days) cyclical wetting and drying of a defined bank zone. ***Bank Response:*** Notch Created
   2) Prolonged (+7 days) Inundation of bank zone above the notch. ***Bank Response:*** Saturation
   3) Rapid draw-down of water level over the notch. ***Bank Response:*** Mass-failure events above notch

Areas of bank prior to and post mass-failure (Figures 9.36 and 9.37, respectively) illustrate the significant impact this 3 step process had on areas of the the Edward/Kolety River downstream of Stevens Weir.

5. Deposition at this site is intricately linked to the receding limb of diverse and large flow events:
   a. Where bank saturation above the notch is reached, deposition recorded on the lower bank appeared to be primarily a result of erosion above (Figure 9.38 left).
   b. When the bank above the notch was not saturated, deposition appeared to be linked to sediment being input to the system and residing on the lower bank (Figure 9.38 right).

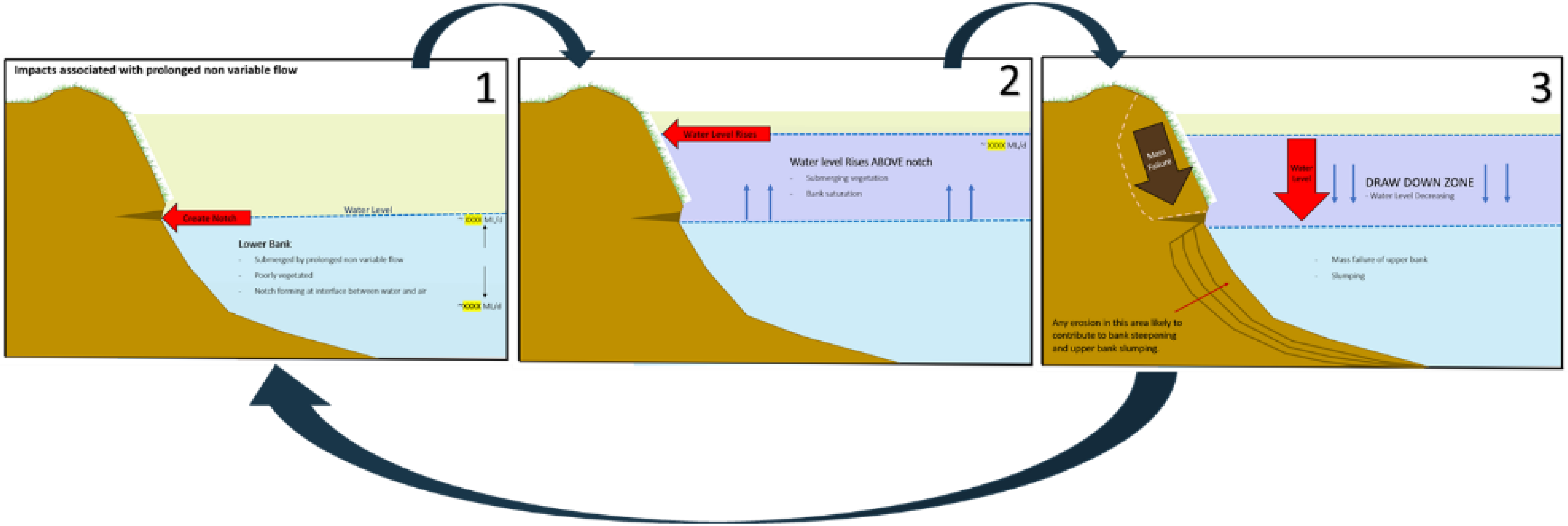


Figure 9.35 Conceptual diagram detailing the 3 stages of channel widening linked to the sequence of events occurring at the Stevens Weir site. This are repeatedly annually in sequence.

Figure 9.36 Drone photograph taken on the 3rd visit (07/04/2020) highlighting the notch at the site in the Edward/Kolety River downstream of Stevens Weir

Figure 9.37 Drone photograph taken on the final visit (visit 4: 14/07/2020) highlighting areas of the bank that appear to have experienced mass-failure events within the monitoring period. Edward/Kolety River downstream of Stevens Weir.

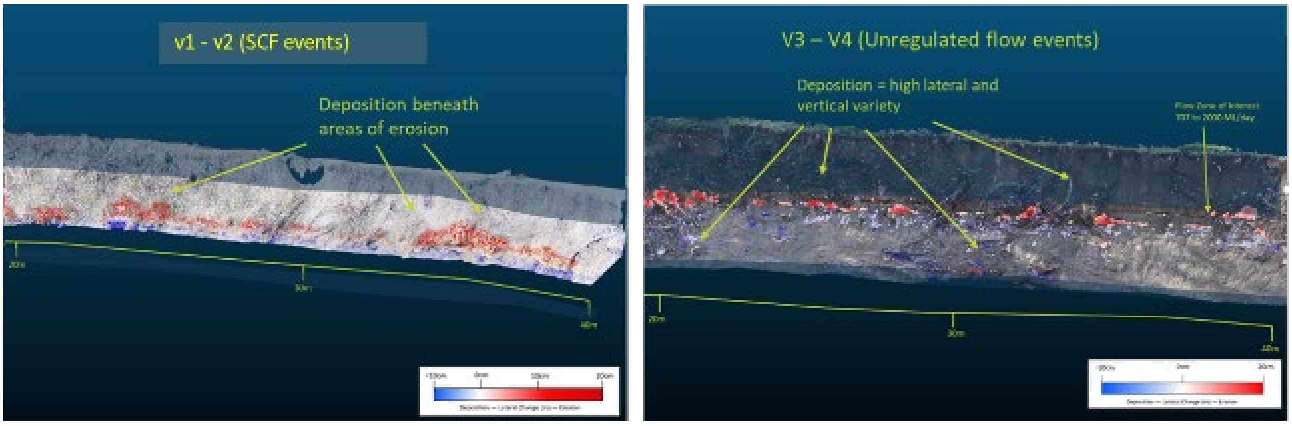


Figure 9.38 Figure comparing the difference between locations of deposition after the receding limb of flow events expressions of change between v3-v4 unregulated flow event (left) and v1-v2 SCF flow event (right). Expressing the areas at which deposition resided on respective banks.

Vegetation Condition Analysis

Colligen Creek site

Three zones of interest were selected within the site at Colligen Creek. These zones were monitored over three visits occurring in September 2019, November, 2019 and April 2020. Figure 9.39 highlights the locations of these three zones of interest within Colligen Creek.

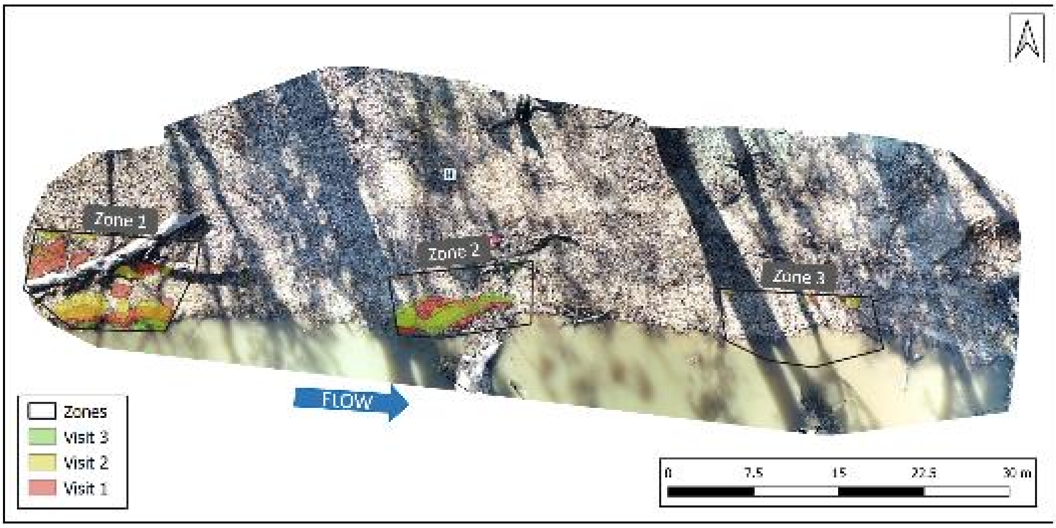


Figure 9.39 Site map of vegetation zones of interest at Colligen Creek site.

Figure 9.40 represents the spatial extents of vegetation from visits 1 – 3 within zone 1. Evident in this figure is the consistent decrease in vegetation from visit 1 to 3. A net loss of 1.4m^2 of vegetation was experienced across the monitoring period (September 2019-April 2020).

In this zone, a recession of vegetation from the water's edge was experienced between visit 1 and visit 2, and over the summer months (between visit 2 and 3), this vegetation died off, with new growth occurring in smaller clumps directly at the bank-water interface.

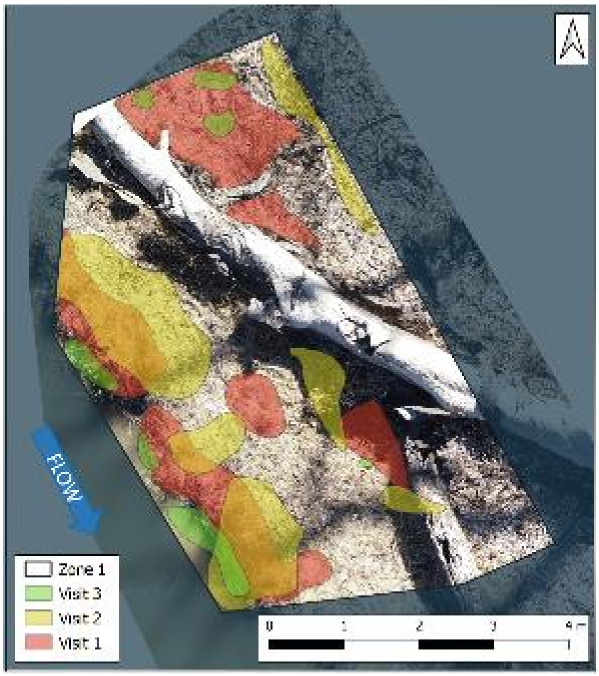


Figure 9.40 Spatial extent of vegetation across 3 sequential site visits within zone 1 at Colligen Creek.

Figure 9.41 presents the spatial extent of vegetation from visits 1 – 3 within zone 2. Evident in this figure is the consistent decrease in vegetation from visit 1 to 2 (loss of 26% vegetation) with an increase in vegetation occurring between visit 2 and 3 (increase of 12% vegetation cover). The loss of vegetation between visit 1 and 2 was observed with vegetation not only reducing in size but also receding up the bank. This was reflected in zone 1 with vegetation receding up the bank

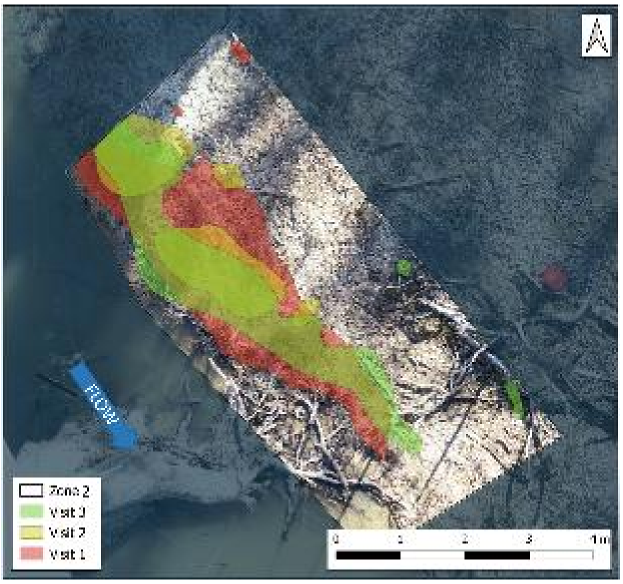


Figure 9.41 Spatial extent of vegetation across 3 sequential site visits within zone 2 at Colligen Creek.

Figure 9.42 presents the spatial extent of vegetation from visits 1 – 3 within zone 3. This zone exhibits a different composition to the other zones at Colligen Creek, exhibiting a vastly smaller amount of initial vegetation cover and larger proportion of bare bank to the other zones. Across the monitoring period, this zone exhibited a 42% increase in vegetation between visits 1 and 2 followed by a 64%*reduction of vegetation between visits 2 and 3. It is worth noting that due to the initial vegetation cover being so low, these percentage changes between visits appear large, but in reality reflect very minor changes in vegetation cover, a net change of approximately 12cm^2 equating to the loss of approximately one small shrub.

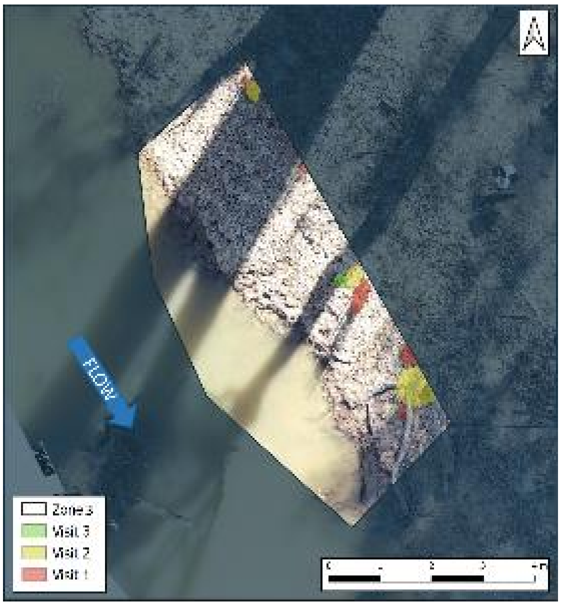


Figure 9.42 Spatial extent of vegetation across 3 sequential site visits within zone 3 at Colligen Creek.

Table 9.10 provides a tabulated summary of the changing vegetation extents across the 3 site visits for zones 1-3 at Colligen Creek. This table highlights the percentage change in vegetation cover experienced between visits 1 and 2 and between visits 2 and 3.

Table 9.10 Tabulated summary of changing vegetation extent across 3 site visits at 3 zones of interest at Colligen Creek.

Zone	Visit Number	Sum Vegetation (m^2)	Percentage Change Between Visits
1	1	6.1	N/A
	2	5.1	8% reduction
	3	0.9	34% reduction
2	1	4.5	N/A
	2	1.9	26% reduction
	3	3.1	12% increase
3	1	0.2	N/A
	2	0.5	42% increase
	3	0.03	63% decrease

<u>Edward/Kolety River downstream of Stevens Weir</u>

Two zones of interest were selected within the Edward/Kolety River downstream site. These zones were monitored over 2 visits occurring in September 2019 and April 2020. Figure 9.43 highlights the locations of these two zones of interest within Edward/Kolety River downstream site.

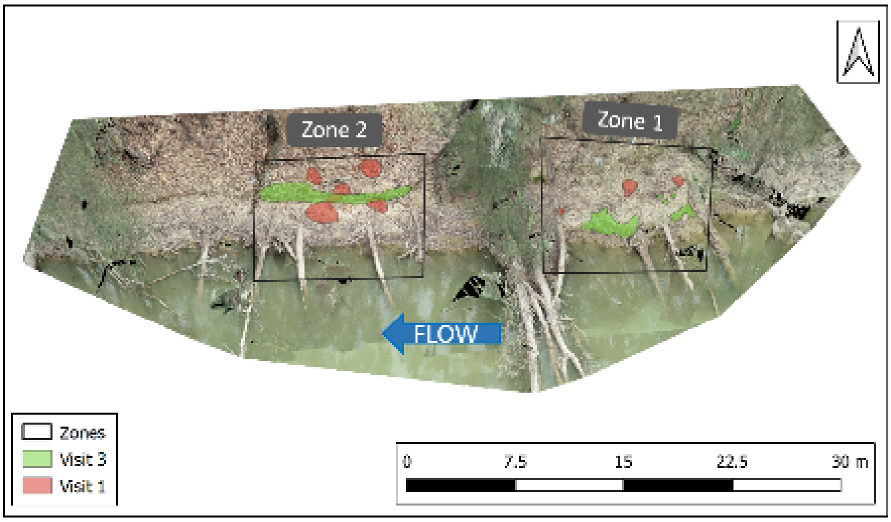


Figure 9.43 Site map of vegetation zones of interest at Edward/Kolety River downstream site.

Zone 1

Figure 9.44 presents the spatial extents of vegetation from visits 1 and 3 within zone 1. This zone exhibits a significant change in vegetation cover and extent between visits 1 and 3. Overall, a 60% increase in vegetation occurred between these two visits, and the location of the vegetation was drastically changed with the existing mid-bank vegetation completely lost and replaced with larger clusters of vegetation lower on the bank towards the water-bank interface. Figure 9.45 provides photographic evidence of this, with new clusters of vegetation evident on the previously bare lower bank. With no visit 2 data available, it is not clear whether there was a decrease in vegetation during the periods of sustained higher flows, or whether there was a consistent increase in vegetation throughout the whole period between September 2019 and April 2020.

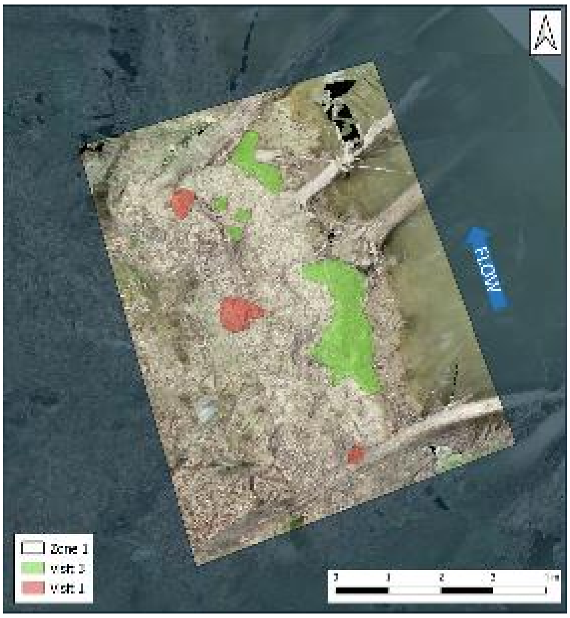


Figure 9.44 Spatial extent of vegetation across 3 sequential site visits within zone 1 at Edward/Kolety River downstream site.

Figure 9.45 Sequential photographs of Zone 1 at Edward/Kolety River downstream site between visit 1 and 3 highlighting new riparian vegetation recruitment on lower bank.

Zone 2

Figure 9.46 presents the spatial extents of vegetation from Visits 1 and 3 within zone 2. This zone experienced a significant change in the location of the vegetation, but only an 8% change in the total vegetation cover. The location of vegetation during visit 1 was clumped in 4 central locations along the mid bank. By visit 3, all of this existing vegetation cover was lost with new vegetation located on the mid bank in a long strip, indicative of vegetation that has grown at the water-bank interface during a period of higher flow. Figure 9.47 provides photographic evidence of this, with a long strip of new vegetation evident on the mid-exposed bank. These photographs highlight a clear line where flows have remained consistent for an extended period of time. Similar to zone 1, it is hypothesized that between visit 1 and visit 2 that a vegetation die off event occurred due to elevated flows sitting at 3,500 ML/day, with a recruitment event following between visits 2 and 3 along the water-bank interface.

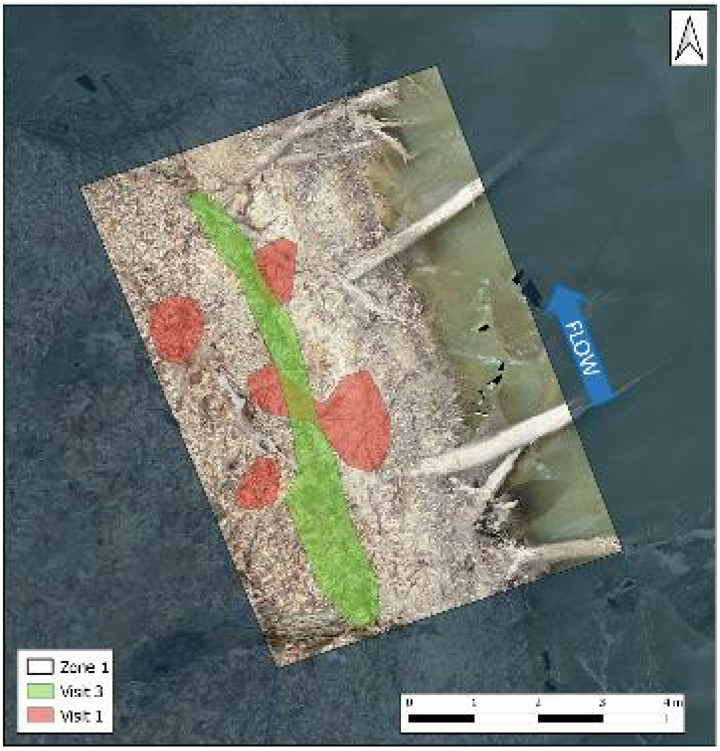


Figure 9.46 Spatial extent of vegetation across 3 sequential site visits within zone 2 at Edward/Kolety River downstream site.

Figure 9.47 Sequential photographs of Zone 2 at Edward/Kolety River downstream site between visit 1 and 3 highlighting new riparian vegetation recruitment on lower bank.

Table 9.11 provides a tabulated summary of the changing vegetation extents across the 3 site visits for zones 1 and 2 at Edward/Kolety River downstream site. This table highlights the percentage change in vegetation cover experienced between visits 1 and 2 and between visits 2 and 3.

Table 9.11 Tabulated summary of changing vegetation extent across 3 site visits at 3 zones of interest at Edward/Kolety River downstream site.

Zone	Visit Number	Sum Vegetation (m^2)	Percentage Change Between Visits
1	1	1.1	N/A
	3	4.4	60% increase
2	1	5.9	N/A
	3	6.9	8% increase

Figure 9.48 Drone image capture of Area 4. Across from the main carpark on site. Recorded on Visit 4 14/07/2

<u>Summary of findings</u>

- A trend in vegetation composition was observed with a consistent loss of vegetation experienced between visits 1 and 2 (the SCF event period). During this period elevated flows inundated vegetated areas for a period of +20 days.
- Between visit 2 and visit 3 (the operational flow period) there was a consistent increase in vegetation cover present, with this occurring primarily across the lower bank.
- There appears to be very little vegetation cover changes between visit 3 and visit 4 (the unregulated flow period), however, Figure 9.48 illustrates that there were signs of plants germinating on lower bank features across the site. This time period was relatively short and the two unregulated flow events during his period resulted in inundation for a duration of less than 7 days.

9.7 Discussion

This report identifies insights that can be gained by using drone technology to analyse bank and vegetation response to different characteristics of hydrological regime. The Digital Elevation Models of Difference (DEMODs) compare two drone surveys at different points in time – before and after influential hydrological events. The DEMODs provide a high resolution mosaic of change (deposition/erosion), over time, in response to the hydrology in the monitoring periods. The results from this research provide sub-centimetre data regarding the morphology of riverbanks in response to variable flow events which cannot be gathered through other means such as LIDAR. The results give insights into the factors that should be considered in flow delivery strategies, within systems that have experienced extensive notching as a result of prolonged operational flows.

What characteristics of flow regime drive erosion and deposition?

It is clear based on the findings from this report that characteristics of flow regime cannot be analysed without consideration of the existing notch and the role this can play in responses of erosion/deposition to different patterns of flow. On the Edward/Kolety River downstream of Stevens Weir, the existing deep notch at the bank zone relating to 2,700 ML/day, was a major contributor to the extensive erosion and deposition that occurred in response to the environmental flow event during September 2019. Prolonged inundation above the notch led to sediment saturation and the corresponding sediment instability and slumping upon the draw-down of the receding limb. If the notch is considered in the evaluation, then it enables the attribution of erosion not to the isolated flow event, but rather to the historical sequence of flow events prior to it (the preparation). On the Edward/Kolety River downstream of Stevens Weir, 20% of days over the last 5 years relate to operational flows and within these periods the mean daily discharge has been between 2,300 – 2,800 ML/day. The result is a deep and defined notch at the bank zone corresponding to 2,700 ML/day.

If the mass-failure events are attributed to the historical pattern of operational flows rather than the isolated environmental flow action, it highlights the need to address how operational flows can be designed to have less impact on the wider system. If operational flows are design with more variability during the summer months then environmental flows (e.g. spring freshes) can deliver positive results within rivers like the Edward/Kolety River. If a holistic approach is not taken to water delivery within the Edward/Kolety Wakool system then it is unlikely that environmental flows will lead to positives within the system (Vietz et al 2016, Vietz et al 2017).

This study highlighted the importance of the sequence of flow events and the role that inundation periods play on sediment saturation. The former is important as the order of flow events determines the preparation that prior events have on future events, the latter is important to understand as flow deliveries could be managed to minimise the impact of wetting and drying sediment that resides above an existing notch. We saw in this study that the unregulated event (which rose above and fell below the notch) resulted in minor erosion. It could be that during this event the bank above the notch did not become fully saturated, and thus mass-failure events were minimised. Establishing a mean period which results in bank saturation could be critical in guiding flow regimes in systems like the Edward/Kolety-Wakool where existing notches need to be considered when dictating flow regimes.

Deposition appeared after all the event types, however in most cases it was intricately linked to erosion events to the bank area directly above the deposition. It appears that unregulated events with shorter duration above the notch (less saturation) and more extended periods of recession result in reduced net erosion. For example, although the unregulated flow event (between visits 3-4) occurred after events which lead to extensive mass-failure, it resulted in minor levels of change and was net-neutral regarding sediment change.

To conclude, this study highlights the important role that historic flow patterns play on influencing future erosion events. In systems like the Edward/Kolety-Wakool, where historic flow patterns have led to excessive notching within channels, bank responses to flow events cannot be looked at in isolation. Thus, to be able to correctly assess the outcome of CEW on riverbanks, the impacts of operational flow strategies must be considered and addressed. If this is not possible then environmental flow deliveries need to be designed with the position of the existing notch considered and with close attention to the rate of fall (regarding the receding limb of flow deliveries) as to minimise responding mass-failure events.

What were the features of flow regime that affect riverbank and aquatic vegetation?

A trend emerged with vegetation recession consistently occurring between visit 1 and visit 2 during periods of elevated SCF flows. It is likely that vegetation die-back occurred along the lower bank due to persistent inundation during this period. Between visits 2 and 3 (during the operational flow period) there was an observed increase in vegetation along the bank-water interface, with vegetation recolonizing the areas where the bank had been inundated during SCF flows.

Between visits 3 and 4 (unregulated flow period) new growth began to mature, but no new germination occurred on the lower banks. This highlights vegetation resilience with the prompt recolonization of bare banks after the SCF. However, the operational flows appear to inhibit vegetation growth on the lower bank. It is likely that prolonged operational flows will result in the loss of vegetation from the lower banks of these waterways, reducing the ability of these banks to withstand future peaked or prolonged flow events. Without vegetation on these lower banks, it is likely that further steepening and slumping will occur, facilitating a positive feedback loop exacerbating this cycle (reduced vegetation = increased erosion).

A knowledge gap exists with respect to the amount of time the riparian vegetation may be inundated before it dies. It is possible that an altered flow management approach (i.e. cyclic pulsing) may improve vegetation retention across the operational flow period and may provide subsequent erosion control to banks where scour, notching and steepening may otherwise become an issue.

Knowledge Gaps

Adaptive flow management is critical to improve the management of flows through a regulated river system, whilst seeking to maintain ecological, social and physical elements that support a healthy river. Further understanding of the relationships between flow and riverbank morphologic condition and vegetation is required. Research questions may include:

Geomorphology

- How long does an upper bank zone need to be inundated to reach a point of saturation?
- How much has the Edward/Kolety River reach downstream of Stevens Weir widened over recent years?
- When analysing change in response to unregulated flow events that follow a period of mass-failure, is the extent of saturation more critical or the sequence of events (with unstable bank removed prior) more important to understanding bank condition response to discharge regime?
- Can environmental flows realistically be analysed when there is major notching within a system due to past prolonged and repeated operational flows within the same defined bank zone over many years?
- How long does it take for a prolonged flow level to produce a notch?
- Does sun and temperature play a role in drying riverbanks and preparing them for erosion, if so to what extent, and what role can vegetation play here?

- How much of a role does the flow play in preparing riverbanks for failure during subsequent flows?
- What is the interaction between vegetation density and erosion/deposition?
- Does vegetation present in the early phases of the flow (~two months) reduce the severity of erosion?
- To what extent does vegetation protect riverbanks through shading, increased structure, increased roughness, or increased organic matter?
- What proportion of sediments is provided by tributary inflows?
- What rate of drawdown is most conducive to reducing bank mass failure (slumping) and creating mud drapes?
- How effective are mud drapes at 'patching' riverbanks?

Vegetation

- How does the depth and duration of inundation influence survival of key native vegetation taxa?
- Does providing short intervals of low flow during flow delivery improve vegetation survival?
- Do fine scale variations in inundation depth improve plant establishment and growth?
- What is the time frame for key taxa to germinate, mature and set seed in the field?
- What is the abundance and composition of the soil seed bank at different geomorphic features?
- Does prolonged summer submergence deplete the soil seed bank?
- Does the availability of seeds limit plant establishment?
- How does the availability of suitable hydraulic habitat for vegetation change with river discharge?
- How can an area of vertical bank with notching be revegetated?

9.9 Appendix

Volume change numbers

Table 9.12 Volume change metrics converted to rate of change m^3/m^{-2} (m a $^{-2}$)

Visit Comparison	Bank area (m2)	Total Erosion (m3)	Total Deposition (m3)	Net change (m3)	Tot erosion (cm3) per m2 of bank	Tot deposition (cm3) per m2 of bank	net change cm3	net change M3	Rate of Change (m^3 m^{-2}) (m m^{-2})
V1 – V2: SCF	167.4	3.04	2.01	-1.03	1.82	1.2	-0.62	-0.006	-0.0062
V2 – V3: Operational flow	252.37	2.18	1.54	-0.64	0.86	0.61	-0.26	-0.003	-0.0025
V3 – V4: Unregulated flow	325.94	0.00	0.00	0	0.00	0.00	0	0.000	0.0000
V1 – V3: SCF + Operational flow	252.5	5.83	2.08	-3.75	2.31	0.82	-1.49	-0.015	-0.0149

DEMODs and volume change for Edward/Kolety River (upstream site)

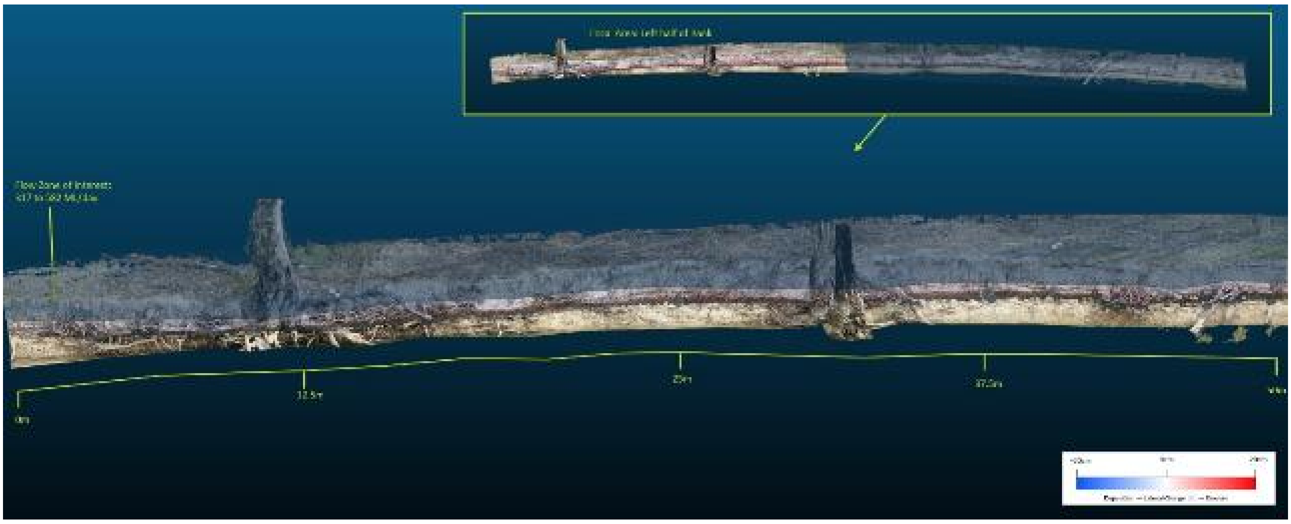


Figure 9.49 DEMOD comparing Edward/Kolety River Upstream Bank v1 to v3 (Left side of bank).

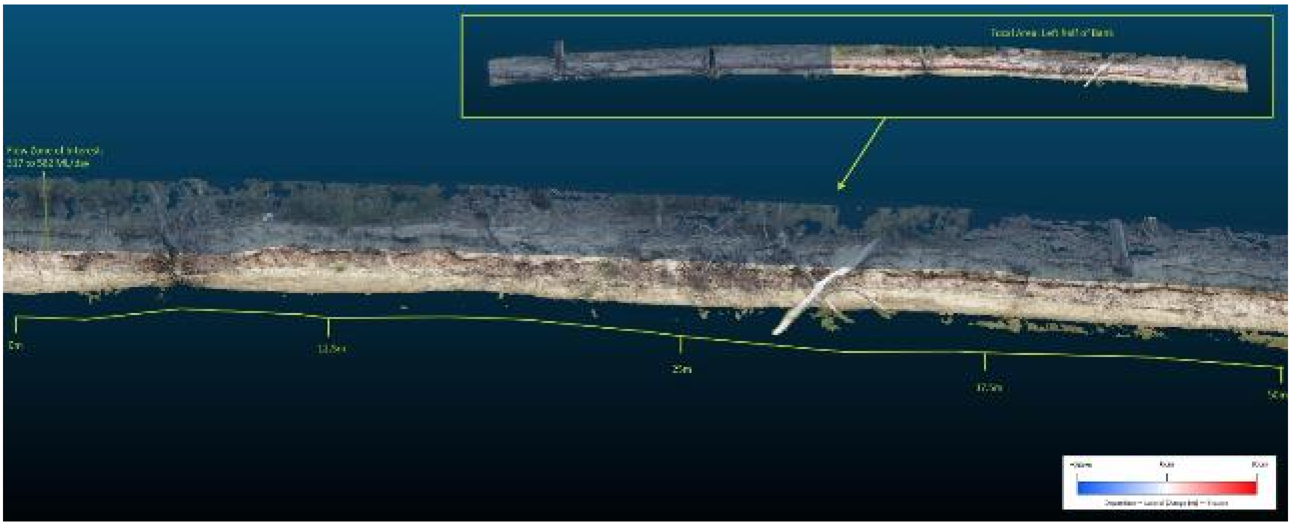


Figure 9.50 DEMOD comparing Edward/Kolety River Upstream Bank v1 to v3 (Right side of bank).

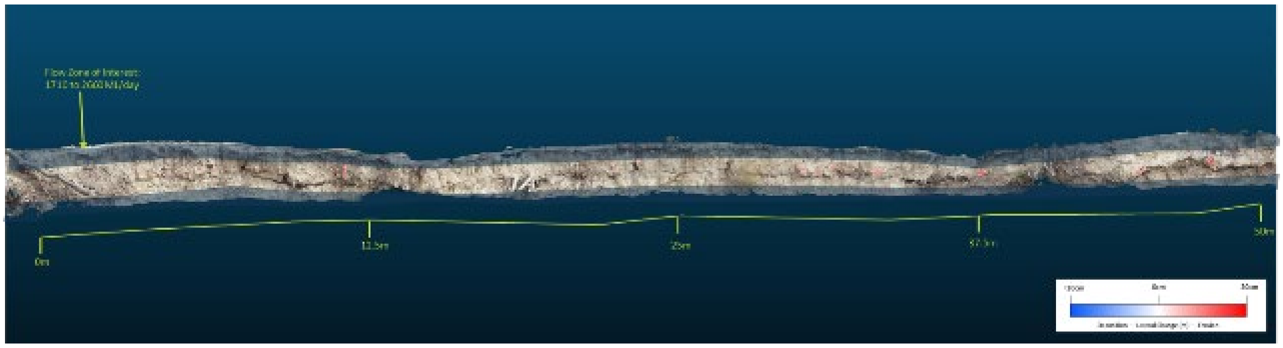


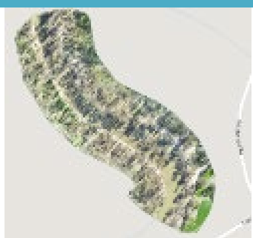
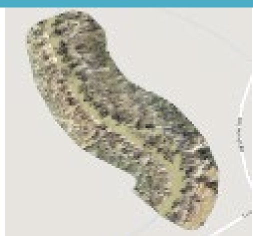
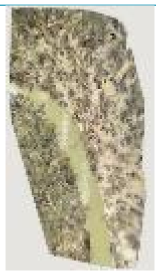
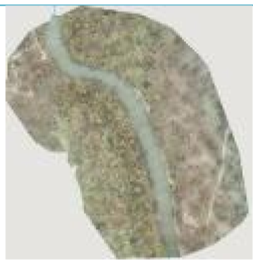
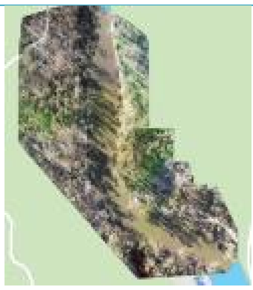
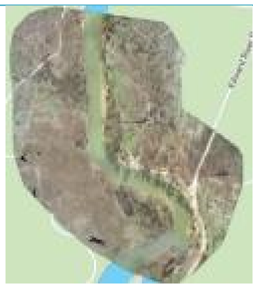
Figure 9.50 DEMOD comparing visit 3 to 4 at Edward/Kolety River upstream site.

Table 9.13 Table showing volume metric change resulting from unregulated flow events at Edward/Kolety River upstream

DEMOD comparison and bank side	Bank area (m2)	Total Erosion (m3)	Total Deposition (m3)	Tot erosion (cm3) per m2 of bank	Tot deposition (cm3) per m2 of bank
V3-V4 left	93.21	0.13	0.06	0.14	0.07
V3-V4 right	47.17	0.17	0.19	0.36	0.40
V3-V4 total	**140.40**	**0.30**	**0.25**	**0.22**	**0.18**

Drone orthomosaics (by site and visit)

Table 9.14 Hyperlinked images linking each site and visit to the cloud-based location where the interactive orthomosaic is stored.

Visit	V1	V2	V3	V4
Colligen Creek				
Edward-Kolety river DS				
Edward-Kolety river US		N/A		N/A

10 EDWARD/KOLETY RIVER PRIMARY PRODUCTIVITY RESEARCH

Authors: Nick Bond, Andre Siebers and Nicole McCasker

10.1 Background

Stream metabolism evaluates the processes of Gross Primary Production (GPP) and Ecosystem Respiration (ER) that support and sustain aquatic food webs and are directly related to ecosystem health. Flow variability is a key factor influencing rates of GPP and ER in river systems, and one of the aims of the MER project is to improve understanding of how Commonwealth environmental water influences GPP and ER within river channels under different flow conditions. Due to the nature of delivery constraints in the Edward/Kolety-Wakool system, the LTIM/MER projects to date have focussed on the influence of in-channel flow variability on GPP and ER (Watts et al. 2019). The influence of higher flows that inundate low lying forested areas and thus connect anabranches, benches, and low-lying floodplains on GPP and ER has therefore been under-represented in the LTIM/MER results to date, with the exception of the evaluation of upstream flows returning from Millewa Forest flow into the Edward/Kolety-Wakool system.

Shallow, wide, and slow-flowing inundated benches and low-lying floodplains provide an ideal environment for the growth of algae and aquatic plants, subject to other rate-limiting factors (e.g., temperature, light, and nutrient availability). In addition, the inundation of previously dry floodplains can release large quantities of terrestrial carbon and nutrients to support both respiration and primary production. In large floodplain rivers, infrequent overbank flood events can account for a large proportion, or even the majority of annual ecosystem metabolism. Further, high levels of primary production can support large increases in populations of zooplankton and macroinvertebrates that provide the basal food sources for higher order consumers such as fish. Flows that inundate low lying forested areas can also improve retention of carbon and nutrients within floodplain systems, which may make the metabolic regime of downstream reaches more stable (i.e., less prone to high-rate events such as algal blooms or anoxia). Increased connectivity might therefore both enhance the productivity of the Edward/Kolety-Wakool system, as well as benefiting the productivity of forested areas through enhanced nutrient retention or plant recruitment.

The Edward/Kolety River downstream of Steven's Weir experiences higher discharge than many of the existing MER sites (Section 4). Flow events downstream of Stevens Weir have the potential to inundate parts of Werai Forest, connecting low-lying floodplains and floodplain wetlands and runners that sometimes return discharge back into the Edward/Kolety River. Consequently, the Edward/Kolety River downstream of Stevens Weir provides an opportunity to investigate the influence of lateral connectivity on GPP and ER. The aim of this research component is to advance understanding of how GPP and ER in the Edward/Kolety River may differ from the existing MER sites in other parts of the system due to potential for flows within the Edward/Kolety River to increase connectivity between Werai Forest floodplain wetlands and the Edward/Kolety River. This research is focussed on the Werai Forest, and the lessons learned from this project may be transferrable to other low lying forested areas within the Edward/Kolety-Wakool system, such as Koondrook Perricoota Forest.

10.2 Research question

The relationship between higher-flow events that connect low-lying floodplains and floodplain wetlands and stream metabolism is complex and has not been assessed in the Edward/Kolety-Wakool system. The unregulated overbank floods in this system during 2016 could not be analysed for GPP and ER, as wide-spread anoxia from blackwater events precluded the use of metabolic models (Watts et al. 2017). Small freshes (c. 800 ML/day flows) in the Edward/Kolety-Wakool system have been demonstrated to substantially increase production (Watts et al. 2019), but these effects could potentially be much greater if the wetted area was increased by connecting anabranches and low-lying floodplains. The effects of flow events on in-channel GPP and ER can be influenced by direct lateral connectivity with floodplains as well as the contributions of upstream floodplain connections. Consequently, this initial research in 2019-20 was focused on understanding baseline GPP and ER at sites that have the potential to be used to evaluate the influence of future flows downstream of Stevens Weir that connect low-lying floodplains and floodplain wetlands.

In 2019-20, the question relating to the stream metabolism research component was:

- How does variation in the flow regime downstream of Steven's Weir relate to patterns of GPP, ER, and NEP (net ecosystem production; GPP – ER) in the Edward/Kolety River?

The key focus of this research is a comparison of GPP and ER at sites upstream and downstream of Werai Forest with respect to variation in hydrology. In the future these sites have the potential to experience higher flows that will connect with anabranches and low lying floodplains along the Edward/Kolety River and through Werai Forest.

10.3 Methods

Sites

Two sites with newly installed DO loggers were included in this initial phase of the research project. The first site was located along the Edward/Kolety River, approximately 10 km downstream of Steven's Weir and downstream of where Colligen Creek splits from the Edward/Kolety River (Figure 10.1). The second site was located downstream of Werai Forest, nearby to Balpool Road Bridge (Figure 10.1). The two sites are connected via Edward/Kolety River flows. During high-flow events, flows can also connect through the centre of Werai Forest and outflow into the Edward/Kolety River upstream of the junction of the Edward/Kolety River and Colligen/Niemur Creek systems (Figure 10.2). The second site therefore integrates a substantial stretch of the Edward/Kolety River bordering Werai Forest as well as outflows from Werai Forest.

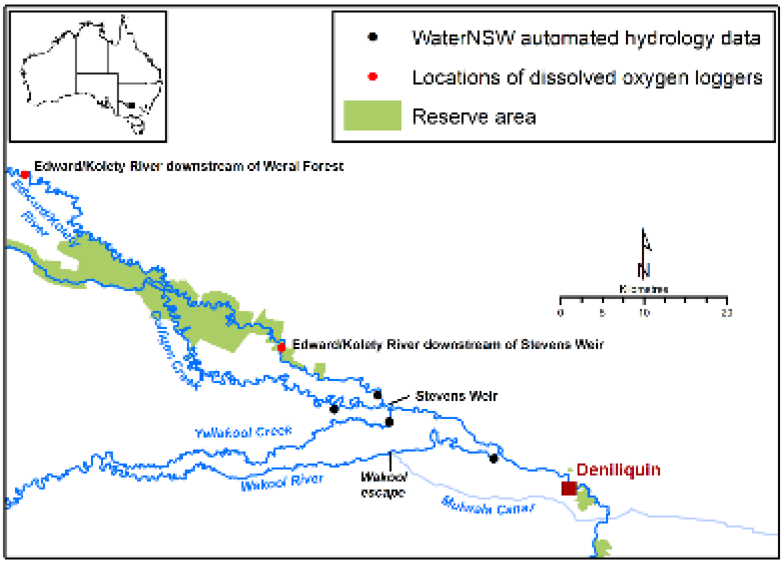


Figure 10.1 Map of the Edward/Kolety-Wakool Selected Area showing initial sampling sites for the stream metabolism research component (red circles). Black circles indicate location of hydrological gauges. Red square shows the location of Deniliquin.

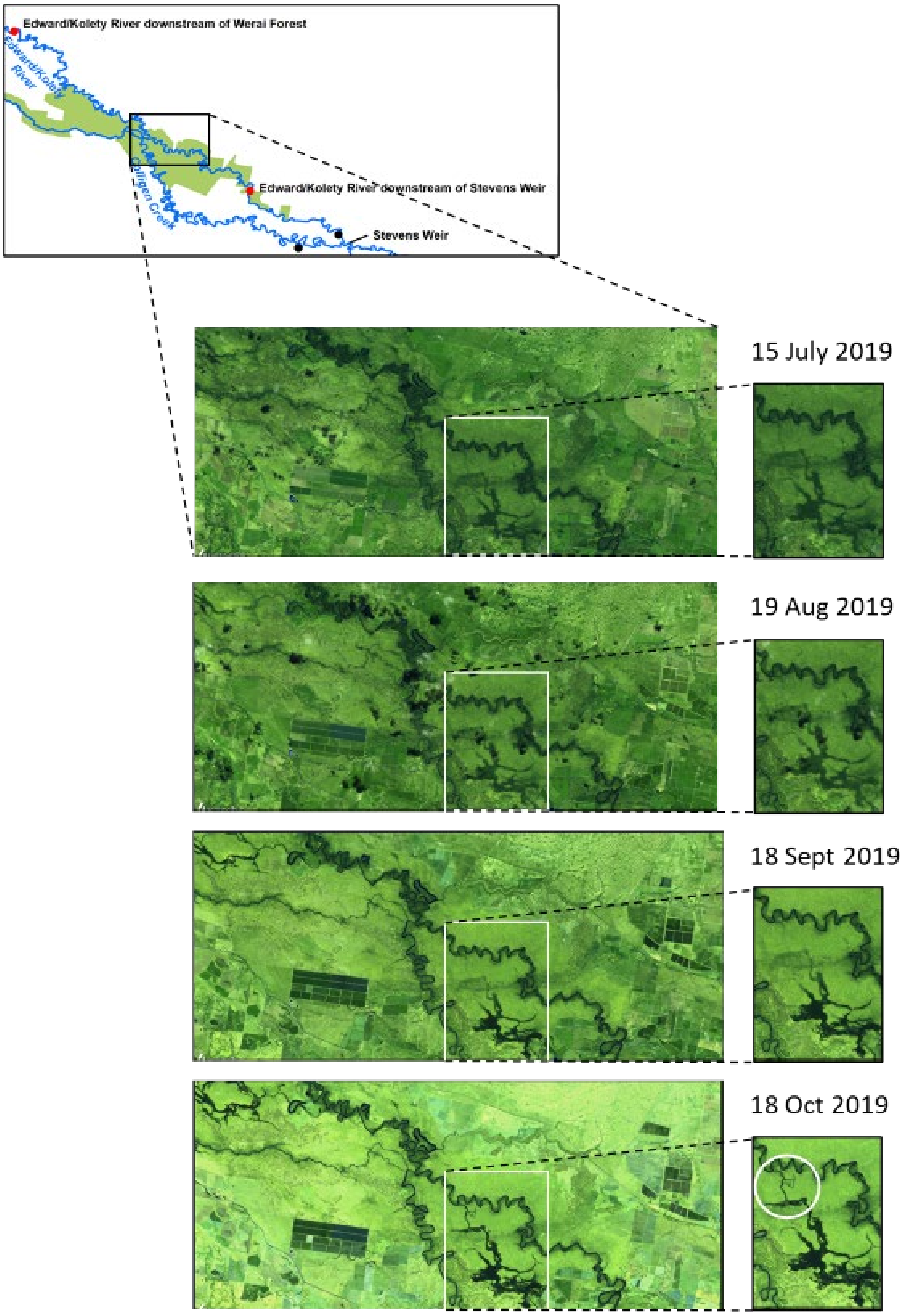


Figure 10.2 False-colour Sentinel imagery of the Edward/Kolety River adjacent to Werai Forest (large map shows location of imaged area within the Edward/Kolety-Wakool Selected Area). Inset images show expanded view of inundated areas within Werai Forest in relation to the Edward/Kolety River. The progression of water through the forest from August 2019 to October 2019 is evident in the images. The image from 18 October 2019 shows connection of Werai Forest outflows into the Edward/Kolety River (white circle).

Data collection

Stream metabolism measurements were performed in accordance with the LTIM Standard Operating Procedure (Hale et al. 2014). Water temperature and dissolved oxygen (DO) were logged every ten minutes with one logger placed at each of the two study sites. Data were downloaded and loggers calibrated approximately once per month if sites were accessible, and more frequently (often fortnightly) during summer to avoid problems with probe biofouling. Light loggers were deployed alongside oxygen loggers and data were downloaded monthly (approximately).

Data analysis

Estimation of stream metabolism rates followed Section 6: first, we used BASEv2 to model daily rates of GPP and ER (mg O_2/L/Day) from oxygen, temperature and light measurements. Acceptance criteria for inclusion of daily results were that the fitted model for a day must have (i) an R^2 value of at least 0.90 and a coefficient of variation for the GPP, ER, and K parameters of < 50%, (ii) a reaeration coefficient (K) within the range 0.1 to 15, and (iii) model fit parameter PPfit within the range 0.1 to 0.9.

Rates of carbon produced and consumed each day were calculated by converting GPP or ER in mg O_2/L/Day by a factor of 12/32 (ratio of atomic mass of C to molecular mass of O_2). Total production for each day (kg C/day) was estimated by multiplying the rate of production derived for that day (in kg C/L/day) by the observed discharge on that day (L). As the site downstream of Werai Forest is ungauged, we used discharge measurements from the WaterNSW Moulamein gauge (Section 4) to perform these calculations. This gauge site can be influenced by inflows from Billabong Creek, but it is currently the best hydrological data that is available for this site. Except during times of large unregulated flows, the inflows from Billabong Creek are small relative to discharge in the Edward/Kolety River.

There are no directly measured, continuous data on the extent and timing of overbank flow events within Werai Forest. We therefore estimated periods of potential floodplain or wetland inundation within Werai Forest with three metrics. First, we estimated the potential for inundation via overbank flow as flow events where discharge below Steven's Weir exceeded 2700 ML/day. We refer to these events here as "high-flow events", i.e. events which are likely large enough to have the potential to cause flow events that connect with Werai Forest. We also estimated the possibility of floodplain or wetland inundation across the entirety of Werai Forest by (i) the proportion of Werai Forest covered with open water and wet vegetation derived from Digital Earth Australia (DEA) satellite imagery (Ramsar Wetlands Insight Tool v3.0) for 2019, and (ii) daily cumulative rainfall measured across 2019-20 at the nearby Wakool (Calimo) weather station (Australian Bureau of Meteorology).

10.4 Results

Rates of stream metabolism

Using the acceptance criteria for each day's diel DO curve, the acceptance rate for downstream of Steven's Weir was 15% and downstream of Werai Forest was 20% (Table 10.1). Large flows in the Yallakool and Wakool systems have previously precluded collection of data meeting acceptance criteria (Watts et al. 2018), and may account for the low data acceptance rates here given that flows downstream of Steven's Weir (mean 1807 ML/day) were substantially higher than those in Yallakool Creek (mean 280 ML/day; Section 4). Examination of the underlying dissolved oxygen data suggests that the low acceptance rate was not due to anoxic events (i.e., blackwater) during high flows (e.g., Watts et al. 2016). Instead, the higher flows here as opposed to the Yallakool and Wakool systems likely dilute any change in dissolved oxygen concentrations driven by GPP or ER: this likely accounts for the low rates of data acceptance, as metabolic models have intrinsic difficulties with producing good fits to data when daily oxygen concentration ranges are small (Appling et al. 2019).

The median GPP value for the site downstream of Steven's Weir (2.79 mg O_2/L/day) was roughly twice that of GPP downstream of Werai Forest (1.35 mg O_2/L/day). In addition, maximum GPP and ER rates were much higher downstream of Steven's Weir than downstream of Werai Forest, despite similar minima and GPP/ER ratios (Table 10.2). As above, major events such as large flows and anoxia can often preclude data meeting acceptance criteria. These comparisons are therefore made using metabolic rates obtained primarily during stable flow conditions.

Table 10.1 Summary of data availability for the two data logger sites, July 2019 – June 2020.

Site	Total days	Days with acceptable data	% Acceptable data days
Downstream of Steven's Weir	291	43	15
Downstream of Werai Forest	291	59	20

Table 10.2 Summary of gross primary production (GPP) and ecosystem respiration (ER) rates and GPP/ER ratios for the two sites, July 2019 – June 2020. Each metabolic parameter is expressed as a median and mean with minimum and maximum values also included. 'n' is the number of days for which successful estimates of metabolic parameters were obtained.

	Downstream of Steven's Weir (n = 43)			
	Median	Mean	Min	Max
GPP (mg O_2/L/day)	2.79	1.50	0.30	18.35
ER (mg O_2/L/day)	5.85	4.60	0.95	23.01
GPP / ER	0.55	0.49	0.12	1.46

	Downstream of Werai Forest (n = 59)			
	Median	Mean	Min	Max
GPP (mg O_2/L/day)	1.35	1.37	0.24	2.55
ER (mg O_2/L/day)	2.51	2.27	0.93	6.62
GPP / ER	0.59	0.63	0.09	1.15

High-flow events (>2700 ML/day) occurred downstream of Steven's Weir in October 2019 and June 2020. However, acceptable BASE results at both sites were scarce during these times. Acceptable data was therefore largely only available for early-mid 2020, and there was insufficient GPP and ER data to evaluate the potential effects of overbank flow events within Werai Forest on metabolism at either site (Figure 10.3). The most notable trends in the available data were (i) a greater occurrence of high GPP and ER events downstream of Steven's Weir when compared with downstream of Werai Forest, and (ii) a seasonal progression from higher to lower GPP/ER ratios from summer to winter 2020 (Figure 10.3). As with the Wakool/Yallakool sites (Section 6), or for that matter most other flowing waters (Bernhardt et al. 2018), warmer, longer days with more intense sunlight likely drive this trend by causing higher rates of GPP.

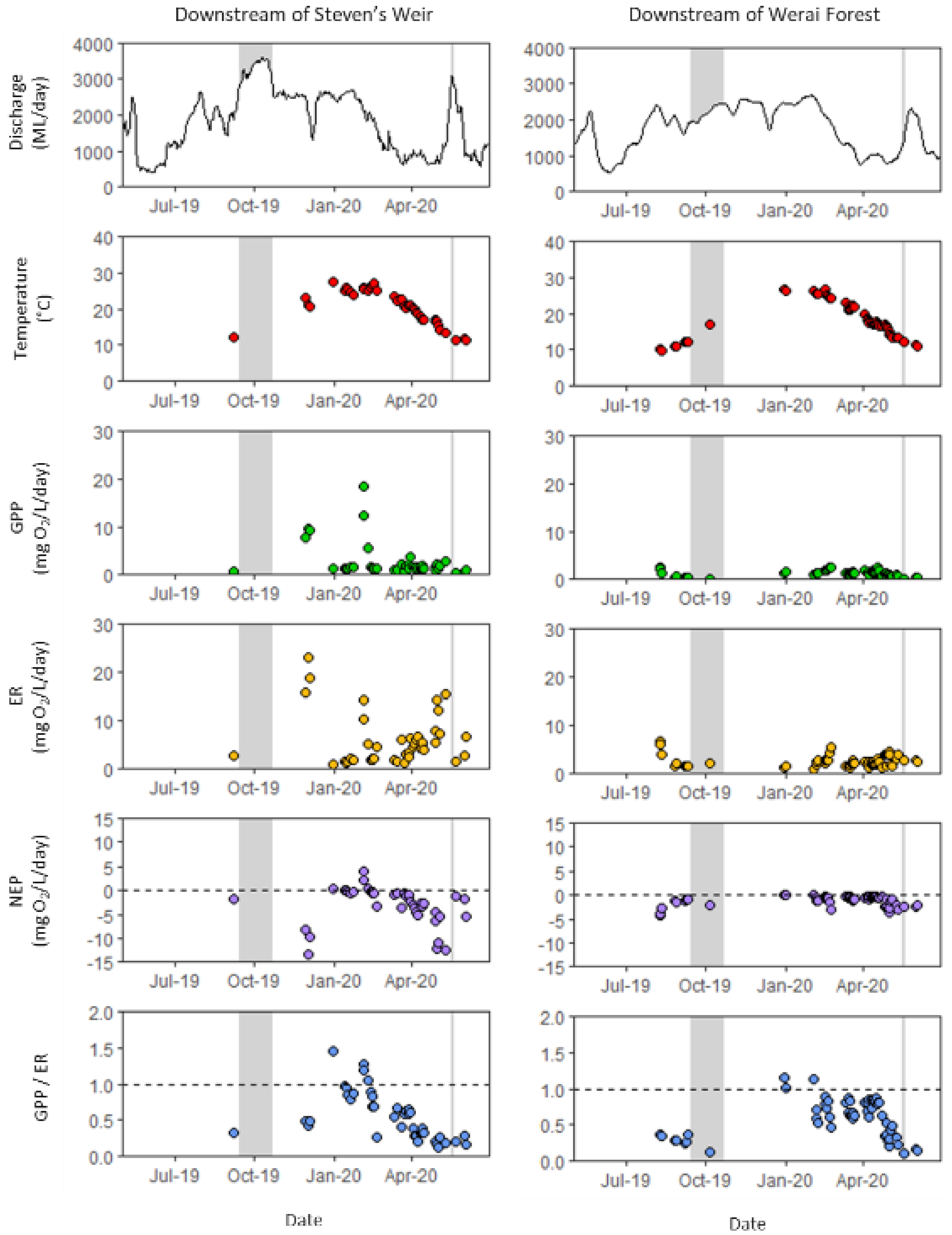


Figure 10.3 Plots of discharge, average daily water temperature, oxygen production (GPP), consumption (ER), net production (NPP) and production: consumption ratio (GPP / ER) over both sites in 2019-20. Potential for high flow events that may connect Werai Forest, flows >2700 ML/day downstream of Steven's Weir (13/9/19 - 22/10/19 and 16/5/20 - 20/5/19), are indicated by shaded bars. Shaded bars for site downstream of Werai Forest are indicated from downstream Steven's Weir gauge data. Discharge for the site downstream of Werai Forest is estimated from Moulamein gauge data.

When converted to units of C produced and consumed per day, the main patterns observable were again a seasonal decrease in GPP from summer to winter and the greater number of high GPP and ER events downstream of Steven's Weir (Figure 10.4). Again, however, the lack of useable data constrains the conclusions that are able to be made around responses in GPP and ER to potential overbank flow events.

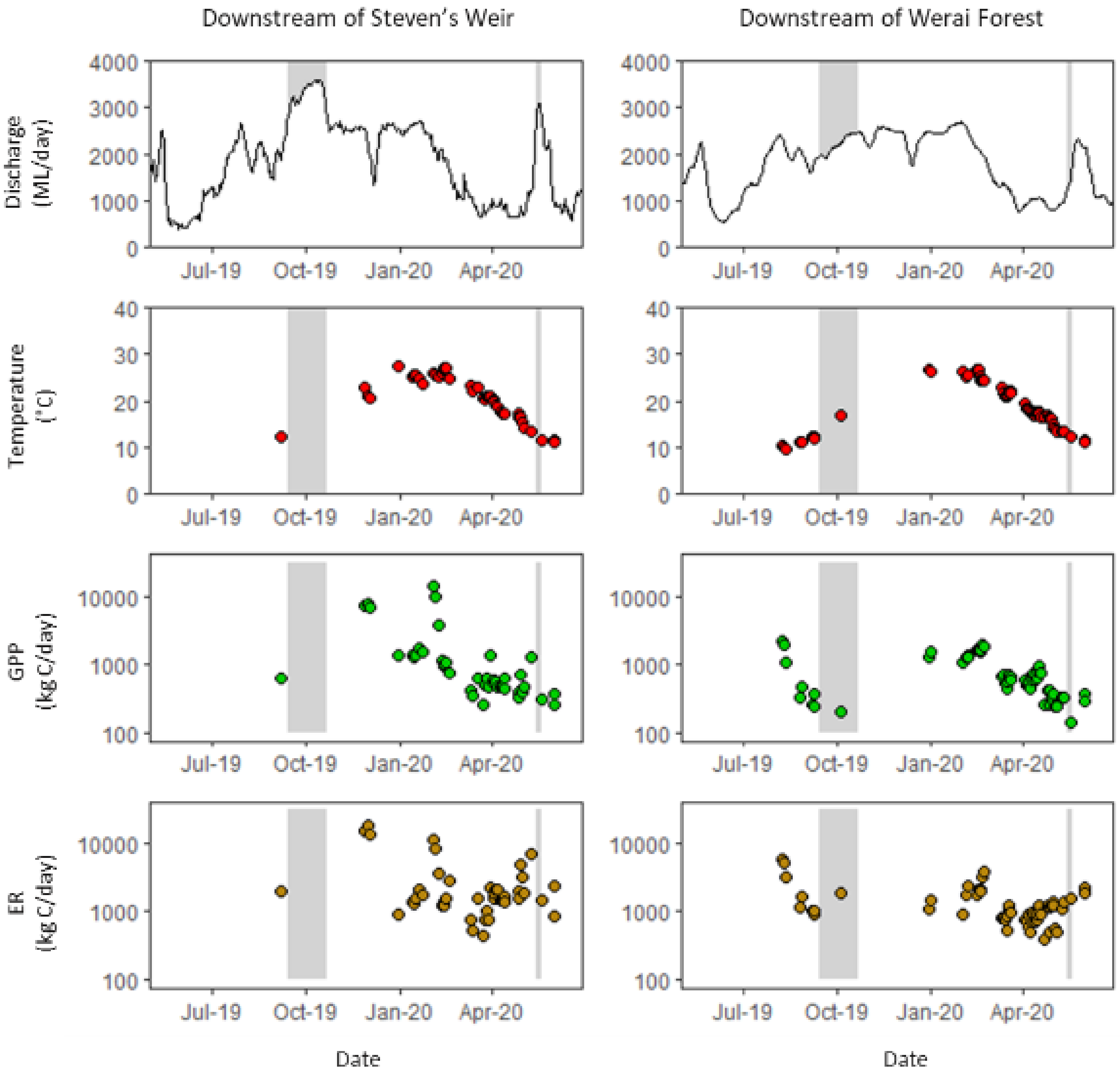


Figure 10.4 Plots of discharge, average daily water temperature, total daily production of C (GPP), and total daily consumption of C (ER) over both sites in 2019-20. Note that both GPP and ET are plotted on logarithmic scales so that variation around the flow pulse in June/July 2020 can be visualised. Potential high flow events for Werai Forest, as indicated by flows >2700 ML/day downstream of Steven's Weir (13/9/19 - 22/10/19 and 16/5/20 - 20/5/19), are indicated by shaded bars. Shaded bars for site downstream of Werai Forest are indicated from downstream Steven's Weir gauge data. Discharge for the site downstream of Werai Forest is estimated from Moulamein gauge data.

There was also little correlation between other potential indicators of inundation within Werai Forest (% inundation, daily rainfall) and any visible changes in GPP or ER rates (Figure 10.5). An approximately 10% drop in the estimated inundation percentage of Werai Forest between July and August 2019 may have led to a reduction in both total daily production and consumption of C (Figure 10.5). However, there was no corresponding change in GPP/ER ratios, and changes in GPP and ER rates may thus largely correlate with variation in in-channel discharge.

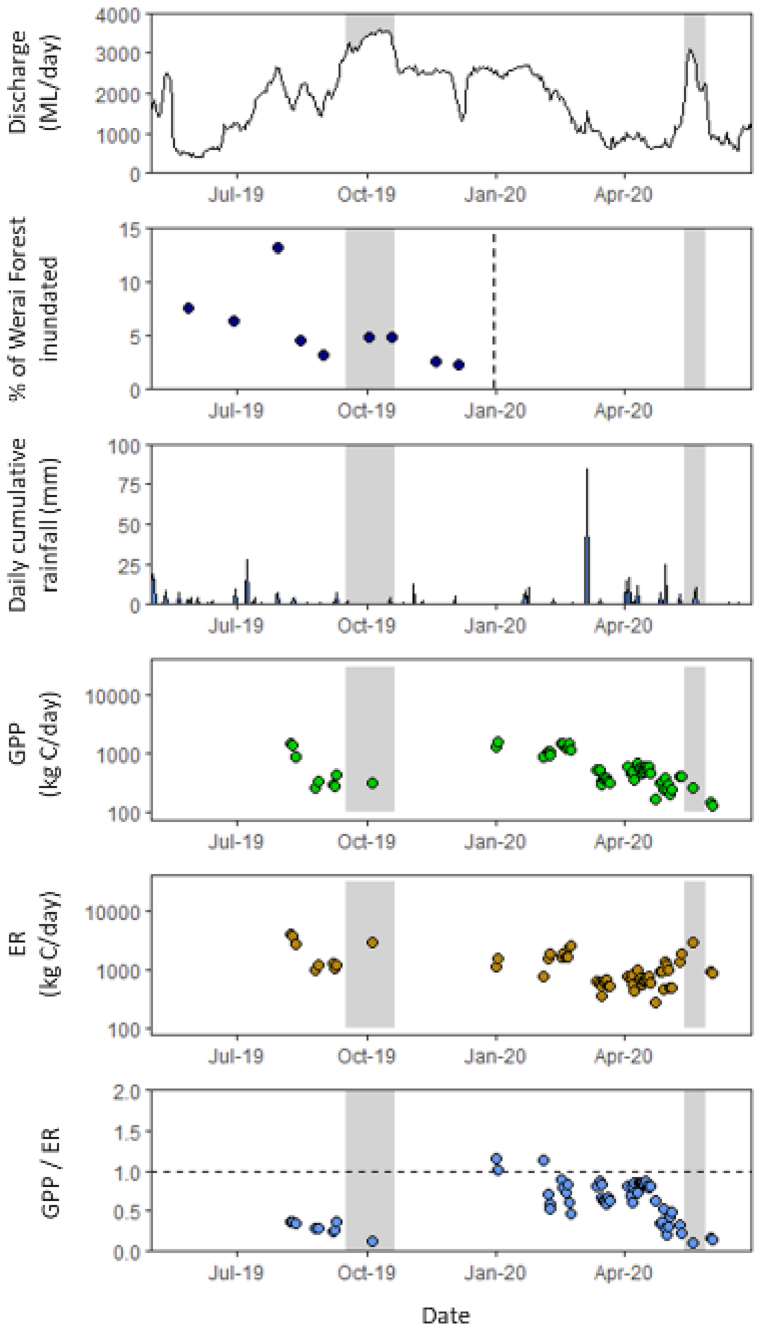


Figure 10.5 Plots of discharge downstream of Steven's Weir for 2019-20, percentage of Werai Forest estimated to be inundated (open water or wet vegetation) at various points throughout 2019, daily cumulative rainfall recorded by the Wakool (Calimo) weather station for 2019-20, total daily production of C (GPP), total daily consumption of C (ER), and GPP/ER ratios for the site downstream of Werai Forest in 2019-20. Dashed vertical line on the % inundation figure indicates limits of Werai Forest inundation data. Potential overbank flow events for Werai Forest, as indicated by high flows downstream of Steven's Weir (13/9/19 - 22/10/19 and 16/5/20 - 20/5/19), are indicated by shaded bars.

10.5 Discussion

Ultimately, there was not enough useable data in 2019-20 to answer the research question – how does variation in the flow regime downstream of Steven's Weir drive changes in rates of GPP, ER and NEP (net ecosystem production; GPP – ER) in the Edward/Kolety River. As with the regularly monitored sites (Section 6), the clearest pattern in rates of GPP and ER was a seasonal trend. The reduction in rates of GPP from summer into winter 2020, and greater reduction in GPP relative to ER, indicates that the higher discharge in the Edward/Kolety River relative to the regularly monitored Edward/Kolety-Wakool sites does not alter (i) the primary drivers of GPP and ER (i.e., light and temperature) or (ii) where these processes occur (i.e., biofilms on the riverbed and hard substrates within the river). However, much of this trend occurred during a period of declining or lower overall discharge and can therefore not be assessed in the context of variation driven by high-flow events.

Examination of Sentinel satellite imagery (Fig. 10.2) suggests that low-lying areas prone to inundation are more likely to occur in the centre of Werai Forest than immediately adjacent to the Edward/Kolety River channel. Rather than increasing the area of inundated benches and floodplains along the Edward/Kolety River, high-flow events that inundate the forest and result in return flows to the Edward/Kolety River may therefore have had the greatest impact on the relative source proportions and quality of water contributing to discharge downstream of Werai Forest. Inundated floodplain wetlands within Werai Forest are likely to act as a strong "sink" for nutrients (i.e., they are retained within the system and either incorporated into organism biomass or sedimented, rather than exported downstream). In particular, reactive phosphorus is the nutrient most likely to limit GPP in the Edward/Kolety-Wakool systems (Watts et al. 2019) and is likely to be strongly retained in floodplain ecosystems. Slow-flowing floodplain wetlands may also retain large quantities of both terrestrial and aquatic carbon which might otherwise support in-channel respiration. The main effect of high-flow events on the productivity regime of the Edward/Kolety River may thus be higher proportions of discharge derived from nutrient and carbon-depleted Werai Forest outflows, which then contributes to lower rates of GPP and ER. Retention of nutrients and carbon within Werai Forest may therefore also lead to a lower frequency of pulsed events (i.e., days with very high GPP and/or ER) within the Edward/Kolety River downstream of the forest.

Much of the carbon cycled during inundation events can be both produced and consumed within shallow, slow-flowing anabranches and inundated floodplains. This carbon cycling might therefore not be reflected in oxygen cycles within main channels, particularly where the loggers (as here) are not located within reaches of the channel which are directly connected or adjacent to floodplains. Effects of floodplain inundation on main-channel GPP and ER are also likely to be highly pulsed, particularly with transport of terrestrial carbon, sediment and nutrients into river channels during the initial flooding front. Quantifying rates of GPP and ER within inundated floodplains presents challenges due to the unpredictable and pulsed nature of overbank flow events, difficulties with site selection and access, and methodological issues of monitoring metabolism in shallow, slow-flowing aquatic habitats. Yet a more comprehensive understanding of how inundation events in Werai Forest influences whole-river metabolism will likely necessitate monitoring of several floodplain sites within the forest itself. In turn, related productivity outcomes of high flows (e.g., increased food supply for higher-order consumers) might also be concentrated within the floodplain wetlands of Werai Forest. Expansion of the metabolism research project into these sites could greatly increase our understanding of the benefits to productivity from anabranch and connection flows.

Unlike for sites on Yallakool Creek, the Wakool River, and Colligen-Niemur River (Section 6), we did not convert volumetric rates of GPP and ER (mg O_2/L/day) to areal rates (g O_2/m^2/day) by multiplication with average depth. Discharge was similar between the two sites monitored here and areal rates are thus likely to follow the same patterns as the volumetric rates presented. However, hydrological modelling of both the Edward/Kolety River channel and the extent of inundation within Werai Forest is likely to prove valuable in future comparisons of GPP and ER between these two hydrologically-disparate environments; characterising areal rates may allow estimates of total production to be modelled over the total area of inundation if floodplain metabolism proves difficult to measure directly (see above).

We recommend that a campaign/intervention monitoring type of study be undertaken during a flow event >2700 ML/day that inundates low lying parts of Werai forest and is likely to return flows to either Colligen Creek or the Edward/Kolety River. The evaluation of primary productivity associated with the event would be enhanced by the installation of temporary gauges to collect data on the inflows to the forest. Analysis of Sentinel images would also quantify extent of inundation within Werai Forest.

11 EDWARD/KOLETY RIVER FISH SPAWNING RESEARCH

Authors: Nicole McCasker, Robyn Watts, Dan Hutton, Troy Bright, Anthony Jones, John Trethewie

11.1 Background

Fish reproduction has been a core component of the ongoing monitoring of Commonwealth Environmental Water in the Edward/Kolety-Wakool Selected Area. Throughout the LTIM project (2014-2019) one noticeable observation was the lack of golden perch spawning in the upper and Mid- Wakool River and Yallakool Creek. A small number of silver perch larvae were collected in Yallakool Creek in late spring/early summer 2017 (Watts et al. 2018) and again at several sites in Yallakool Creek and the Wakool River in late spring early summer 2018 (Watts et al. 2019) suggesting spawning in Yallakool Creek and the Wakool River is limited. For silver perch, spawning is likely to be dependent on the delivery of pulses in early summer once water temperatures reach around 23-25 degrees C. For golden perch, spawning is likely to be dependent on a large and sharp rise in flows once water temperatures have reached about 18 degrees C.

There has been a lack of information as to whether golden and silver perch could be spawning in unmonitored parts of the Edward/Kolety-Wakool system, such as the Edward/Kolety River where there are possibly higher flow velocities in the main channel as well as the connection to wetlands that may have the potential to serve as nursery areas. Local fishers have observed golden perch congregating downstream of Stevens Weir during late spring, further prompting the importance of evaluating whether the hydrological conditions of Edward/Kolety River may be suitable for the spawning of golden perch and silver perch.

Golden perch and silver perch are valued by the local community as iconic species, a target for recreational fishing, and are used by the community as an informal indicator of river health. Community Fish Forum's organised by Murray Local Land Services are well attended by members of the community. Recreational fishing is an important activity for the community in the Edward/Kolety-Wakool area and the Edward-Wakool Angling Association (EWAA, https://www.facebook.com/EdwardWakoolAnglingAssociation/) represents fishers and community groups throughout the Edward/Kolety and Wakool River systems. EWAA is involved in rehabilitation of fish communities in the Deniliquin Lagoons, release of fish larvae to assist recovery of native fish populations, riverbank vegetation revegetation, a weekly wetland workshop with the Deniliquin High School, and organisation of events such as the Wakool Fishing Classic and Deniliquin Fishing Classic. These activities are well attended by the community and involve local high school students. EWAA plays an important role in engaging the community in river and wetland related projects.

Charles Sturt University and EWAA discussed the opportunity to collaborate to assess the spawning of golden and silver perch in the Edward/Kolety River to address both the knowledge gap about the spawning of these species and as an opportunity to engage with community. This aim of this research is to investigate if golden and silver perch spawning occurs in the Edward/Kolety River, and if they are, to understand what aspects of the flow regime are associated with spawning.

11.2 Research Question

Did golden perch and silver perch spawn in the Edward/Kolety River downstream of Steven's Weir in 2019-20?

11.3 Methods

EWAA/CSU collaboration

The collaboration was initiated through telephone conversations and followed by a workshop held in Deniliquin in October 2019 where CSU staff and EWAA members discussed the research project, methods, field safety and logistics for the collaborative project. Dan Hutton was EWAA coordinator for the collaborative project, providing a key link between CSU and EWAA and central coordination of logistics and EWAA member involvement. John Trethewie from CSU provided logistical support and field training of EWAA members. Kris Gibbs (CSU) provided administrative support for EWAA members. The MER program funded the costs of the field work, employment of EWAA members on the project, and travel and boat fuel costs. Members of EWAA from Deniliquin undertook the weekly larval fish sampling in the Edward/Kolety River (Figure 11.1) over a period of twenty-two weeks in 2019-20.

Figure 11.1 Members of the Edward/Kolety River fish spawning monitoring team setting drift nets and retrieving and preserving samples. Left, John Trethewie (CSU) and Anthony Jones (EWAA). Right: Dan Hutton and Anthony Jones (EWAA).

Field monitoring

Occurrence of golden and silver perch spawning was monitored at three sites in the Edward/Kolety River. All three sites were located between Stevens Weir, Deniliquin and Werai State Forest (refer to section 3; site 1 - 35.38765 S 144.65224 E; site 2 - 35.38599 S 144.65079 E; site 3 - 35.37516 S 144.65654 E).

Three drift nets were deployed at each of 3 sites weekly, from 11 Oct 2019 – 22 Feb 2020 (22 sampling nights). Drift nets were constructed from 500 µm mesh and had an opening diameter of 50 cm which tapered over 1.5 m to an end of 9 cm, to which a reducing jar was fitted. Drift nets were set by boat and attached to snags in good flowing sections of the Edward/Kolety River channel. Nets were set late afternoon and retrieved the following morning. Upon retrieval, drift nets were rinsed down, and entire samples preserved in 70% ethanol, and returned to the CSU laboratory for processing. All eggs and fish larvae were picked from the drift samples, identified to species and enumerated.

Any eggs or poorly preserved larvae collected were sent to Australian Genome Research Facility (AGRF) for species determination. Since both Murray cod and trout cod are known to the Edward/Kolety River, a subsample of cod larvae was also sent to AGRF for species confirmation. Nucleic acid extraction and subsequent verification of species assignment was based on dual direction sequencing following PCR amplification. Results of the PCR amplification revealed all cod larvae to be Murry cod, and thus, from here on, we report all cod larvae collected in the study zone to be Murry cod. The developmental stage of each individual was recorded as egg, larvae, or juvenile/adult, according to classifications of Serafini and Humphries (2004). Only the trends in abundances of eggs and larvae are reported.

11.4 Results

Hydrological and temperature conditions

River Murray Channel (RMC) flows in the Edward/Kolety River resulted in an increase in discharge throughout the study reach from 1500 ML/day 1 Sep 2019 up to 3500 ML/day in mid-October, when weekly drift netting commenced. This rise in flow corresponded with a 1 to 1.5 m increase in river height (Figure 11.2). The elevated flows resulting from the RMC spring fresh was evident throughout the Edward/Kolety River (refer to Hydrology section), highlighting the longitudinal extent to which the RMC influenced flow conditions throughout the Selected Area at this time. Discharge remained relatively stable 3500 ML/day peak for two weeks in October before dropping to 2500 ML/day by the start of November. Discharge from Nov 2019 through to mid-January remained stable around 2500 ML/day, with the exception of a short period of lower discharge in early December. There was a steady recession from February into March after the sampling had ceased. Mean daily water temperature ranged from 16.9 °C (11 Oct 2019) to 27.7 °C (2 Feb 2020) during the sampling period.

Detection of fish eggs and larvae

A total of 353 Murray cod larvae, 2 Murray cod eggs and 1 Australian smelt egg were collected from the three study sites during the October to March sampling period (Table 11.1). There was no indication of golden or silver perch spawning in the study reach during the sampling period, as evidenced by the lack of eggs or larvae recorded for these two species (Table 11.1). Murray cod larvae appeared in drift nets from 1 Nov 2019 through to 6 Dec 2019.

Table 11.1 Combined catch of fish larvae and eggs at the three Edward/Kolety River drift net sites located between Steven's Weir and Werai State Forest. Three nets were deployed at each site weekly from October 2019 to March 2020.

	golden perch		silver perch		Australian smelt		Murray cod	
site	eggs	larvae	eggs	larvae	eggs	larvae	eggs	larvae
site 1	-	-	-	-	-	-	-	267
site 2	-	-	-	-	1	-	-	43
site 3	-	-	-	-	-	-	2	43
total	-	-	-	-	1	-	2	353

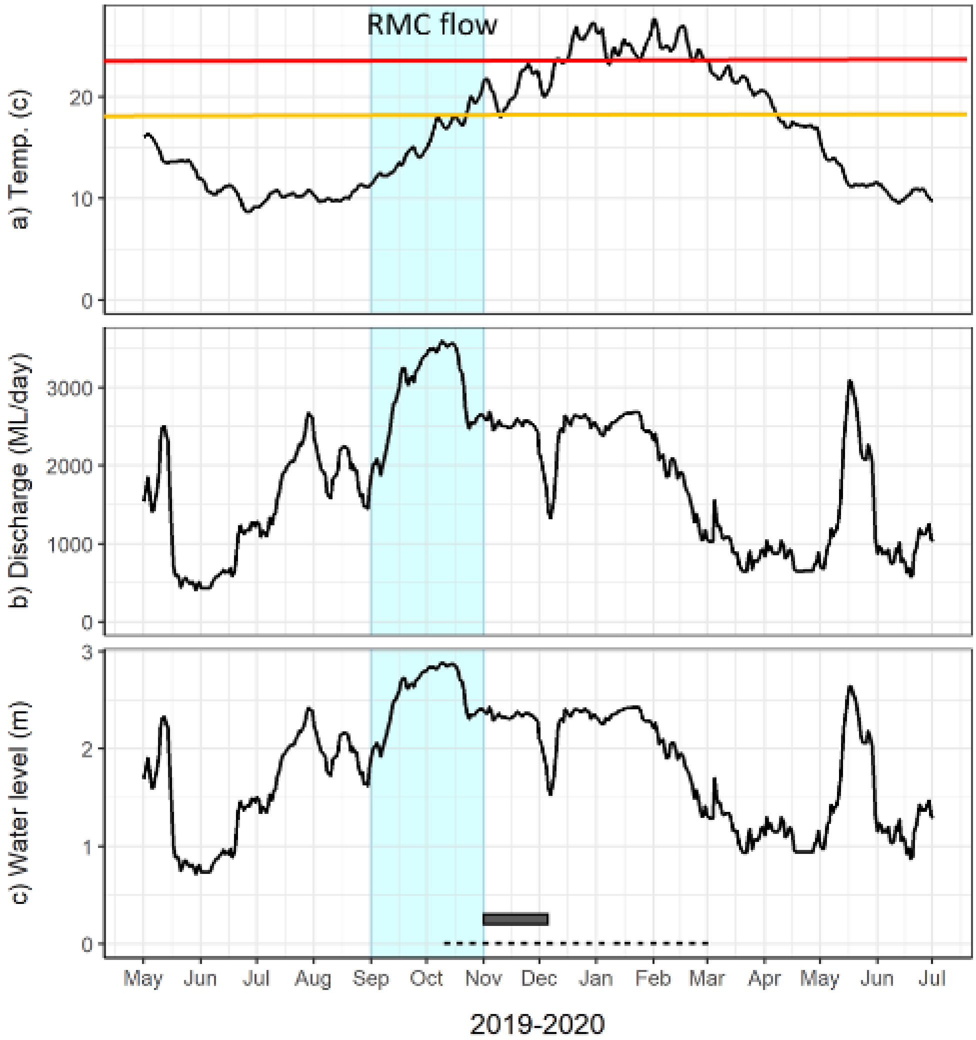


Figure 11.2 Occurrence of Murray cod larvae in the Edward/Kolety River (between Stevens Weir and Werai State Forest) during the 2019-20 watering year in relation to a) Water temperature b) Discharge, and c) Water level. Drift nets were deployed weekly at 3 sites (n=9), over the time period denoted by the dashed horizontal line. The black horizontal bar denotes the dates when Murray cod larvae were detected (1 Nov – 6 Dec 2019). The blue vertical bar denotes the River Murray Channel flow. The red horizontal line indicates the temperature threshold at which silver perch are known to spawn. The orange horizontal line indicates the temperature threshold at which golden perch are known to spawn.

11.5 Discussion

Golden perch and silver perch are long-lived, large bodied native fish species which are dependent on the presence of suitable hydrological conditions to initiate spawning. Understanding the flow related conditions required for successful spawning and recruitment in golden and silver perch has been a priority for LTIM/MER environmental watering monitoring programs in the Goulburn River, mid Murray, Murrumbidgee, Lachlan and Edward/Kolety-Wakool River Selected Areas. Over the past 5 years of LTIM/MER monitoring silver perch spawning has been recorded in all of these Selected Areas, however only small number of silver perch eggs have been recorded in Yallakool Creek in the Edward/Kolety-Wakool system. Over the past years LTIM/MER monitoring golden perch spawning has been recorded in the Goulburn River, mid Murray and Murrumbidgee systems, but golden perch eggs or larvae have not been recorded in the Edward/Kolety-Wakool and Lachlan River systems.

In the Edward/Kolety-Wakool MER fish monitoring program (section 8), we describe that the monitoring of fish spawning monitoring has previously focussed on the Wakool River and Yallakool Creek, as these systems have been a focus for the delivery of Commonwealth water for the environment. The research on fish spawning undertaken by the EWAA and CSU in this current study in the Edward/Kolety River will provide insight as to whether there are suitable hydrological conditions for the spawning of these two flow-dependent species in the Edward/Kolety River and this will inform future delivery of Commonwealth environmental water.

The temperature thresholds at which golden perch and silver perch spawn are well documented. Silver perch have been observed to to spawn when water temperature is above 23 degrees celcius, and golden perch spawns at temperatures over 18 dgrees celcius. The monitoring in the Edward/Kolety River commenced just before the water temperature was 18 degrees and well before the critical spawning temperature for silver perch. So water temperature during the monitoring was in the range suitable for these species.

Although there were no golden or silver perch eggs or larvae detected in the Edward/Kolety River in 2019-20, further monitoring over a longer period of time is warranted. Obligate riverine species, particularly golden perch, are likely complete their lifecycles over a broader geographic scale, that is, the scale of 100’s of kilometres (Zampatti et al. 2015, Mallen-Cooper and Zampatti 2018). For example, immigration of juveniles from the nearby mid-Murray River, and sometimes as far afield as the Darling River, appear to play a major role in structuring the sub-adult and adult populations observed in the Edward/Kolety-Wakool River System (Mallen-Cooper and Zampatti et al. 2018, Watts et al. 2019).

The hydraulic conditions (e.g. minimum or threshold velocity paths) required for successful spawning and recruitment of golden and silver perch are not yet clear. Recent insights from other Selected Area LTIM/MER monitoring projects suggests that traditional ideas proposing that these species required river level rises or changes in discharge may not be the relevant hydrological measure to which these species respond to (King et al. 2005, Wassens et al. 2020). Instead, for species with drifting eggs and larval phases, the provision of suitable water velocities over large distances may be the relevant ecological requirement – ensuring that eggs and larvae maintain their position in the water column while maximising their opportunity to disperse downstream to suitable, productive and safe nursery environments (Stuart and Sharpe 2020). The growing appreciation of large spatial scales at which these species operate, highlights the need for continued monitoring of spawning and recruitment indicators across key main channel and off-channel environments in both the southern and northern Murray-Darling Basin. Ongoing monitoring and analysis of the pattern of flow delivery and water velocities across multiple years will be able to better inform a discussion about spawning of silver perch and golden perch in the Edward/Kolety River.

Although no golden or silver perch eggs or larvae were detected during this study, the project demonstrated that collaboration between researchers and community groups is an effective way to undertake research and engage the local community. Local community fish experts from the Edward-Wakool Angling Association are involved in several projects related to the rehabilitation of fish communities in the region, and this project provides them an opportunity to collaborate with scientists from Charles Sturt University. Involving local community in this project ensures multiple successful outcomes by tapping into local expert knowledge, providing local employment and training, program cost savings and reduced carbon emissions due to reduced travel, and ensuring the results and findings are quickly communicated to the local community (Section 11.8). The collaboration between CSU and EWAA was successful due to the expertise, local knowledge, dedication and commitment of EWAA members to undertake weekly monitoring, the appointment of a local coordinator being a key link between EWAA and CSU staff, and availability of funding from CEWO.

11.6 Acknowledgements

Dan Hutton coordinated the Edward-Wakool Angling Association participation in the project. We thank Anthony Jones, Warren Parsons, Zac McCullock, Alec Buckley and Dan Hutton for their valuable contribution to this project through undertaking the field work to set and retrieve drift nets during the 2019-20 sampling season. John Trethewie (CSU) provided support to the Edward-Wakool Angling Association. Dale Campbell (CSU) undertook the laboratory work to sort and identify eggs and fish larvae from the drift samples. Thank you to Ian Fisher from the Edward-Wakool Angling Association for use of his boat for the field work.

11.7 Appendices

Working together on fish project

Article published in Deniliquin Pastoral Times, 5 March 2020

Working with local fish experts has been the key to extending the region’s native fish breeding conservation efforts.

A study to monitor species of fish larvae in the Edward-Wakool River system started in October 2019 and thanks to promising initial results, the program has been extended.

The project has been a part of a multi-year Charles Sturt University study, initially to gauge environmental responses within the river system.

After research showed species of fish were breeding specifically further down the Edward River, the university paired up with local experts to monitor fish larvae trends.

Local environmental consultant Dan Hutton says the initial results from CSU yielded promising signs for the Murray Cod but further study was needed downstream.

‘‘For a number of years CSU has been monitoring environmental responses within the Edward-Wakool River system, this monitoring is ongoing and includes native fish breeding,’’ he said.

‘‘The collected data has been utilised to manage flows within the system to successfully promote and support native fish breeding.

‘‘Murray cod have responded well, however thus far there has been limited evidence of silver perch breeding.

‘‘This raises the question of whether flow-dependent spawning species (eg. golden and silver perch) may be spawning in other parts of the E-W system, such as the Edward River. Local fishers have observed fish, including golden and silver perch, congregating downstream of Stevens Weir during late spring, which suggests the Edward River may be a spawning area for this species.

‘’This spring (in 2019) CSU extended their fish breeding monitoring to the Edward River downstream of Stevens Weir and approached Edward Wakool Angling Association (with whom they had a long relationship) to conduct the monitoring of those sites.

‘’The monitoring consists of setting three drift nets at each of three locations in late afternoon, then retrieving the nets and collecting and processing the sample the following morning. The sample are then viewed under a microscope to identify the species of any fish larvae.’’

After seeing positive results from the first round of the program, CSU has extended it and intend to continue entrusting the project to locals.

‘’The monitoring program commenced in October and has been conducted by Anthony Jones, Warren Parsons, Alec Buckley and Zak McCullock. They detected the first Murray cod larvae in the samples before Christmas,’’ Mr Hutton said.

‘’By utilising locals with appropriate skill and knowledge, CSU is able to expand the monitoring program at relatively low cost by avoiding travel and accommodation expenses.

‘’Having local field technicians conduct the monitoring includes the local community in the program, providing an important platform for valuable local input into management of the local waterways.’’

The project includes contributions from Commonwealth Environmental Water Office (funding), Charles Sturt University (contracted to conduct the monitoring program) and Edward Wakool Angling Association (conducting some of the fish breeding monitoring for CSU).

Mr Hutton believes the use of local experts not only ensures positive results but also helps contribute back to the Deniliquin community, hoping it will be continued into the future.

‘’Utilising existing, local field technicians ensures multiple successful outcomes by tapping into local expert knowledge, providing local employment and training, program cost savings and ensures monitoring results and findings are quickly communicated to the local community.

‘’It is proposed EWAA repeat the monitoring next summer and we hope it will be expanded from three to six sites.’’

CSU has also engaged other local field technicians to conduct some turtle and riverbank vegetation surveys.

Focus on Edward/Kolety River fish spawning project

From: Watts, R.J., Liu X., Healy S., Trethewie J., Vietz G., Sutton N. and Hutton D. (2020)

Edward/Kolety-Wakool System Environmental Flows Newsletter, Issue 3. Charles Sturt University

Fish larvae have been monitored in Yallakool Creek and the Wakool River for over 5 years as part of the LTIM program and the data have been used to inform water management and promote and support native fish breeding. Between 2014 and 2019 there was limited evidence of silver perch spawning and no evidence of golden perch spawning in the Wakool River and Yallakool Creek. Local fishers have observed fish, including golden and silver perch, congregating downstream of Stevens Weir during late spring, suggesting the Edward/Kolety River may be a spawning area for this species. A collaborative research project on fish spawning in the Edward/Kolety River involving Charles Sturt University and the Edward-Wakool Angling Association (EWAA) was initiated as a result of these observations.

Each week from October 2019 through to the end of February 2020 members of EWAA set drift nets at three locations in late afternoon and then retrieved the nets and collected the samples the following morning. The monitoring program commenced in October and has been conducted by Anthony Jones, Warren Parsons, Alec Buckley and Zak McCullock. They detected the first Murray cod larvae in the samples before Christmas. The samples are being processed through microscopy at Charles Sturt University to identify the very small eggs or fish larvae in the samples.

Local EWAA member Dan Hutton, who coordinated the EWAA field work, said that “employing locals with appropriate skills and knowledge to undertake the field work ensures multiple successful outcomes. The collaboration has provided local employment and training, program cost savings and ensures monitoring results and findings are quickly communicated to the local community. This research provides an important platform for valuable local input into management of the local waterways’’.

Fish spawning research in the Edward/Kolety River near Werai Forest. Left: Anthony Jones and Dan Hutton from the Edward-Wakool Angling Association. Right: Anthony Jones (EWAA) and John Trethewie (CSU) setting larval fish nets.

12 Environmental DNA biodiversity research

Authors: Meaghan Duncan, Jackson Wilkes Walburn, Elka Blackman, Jason Thiem and Robyn Watts

12.1 Introduction

Monitoring aquatic species using traditional methods can ineffectively sample rare or cryptic species. It is critical that the distribution of these species be accurately understood given that their continued survival will depend on appropriate water management. Environmental DNA (eDNA) provides an indirect approach to detecting the presence or absence of a species (Taberlet *et al.*, 2012). eDNA includes any DNA found in a wide range of substrates, including water, soil, ice or air. In the aquatic environment, eDNA is continuously shed by organisms when they defecate and shed cells, including gametes. This eDNA can be captured by filtering the water, extracting the eDNA and using targeted PCR to identify if the species is present or absent.

eDNA has some benefits over traditional techniques given that it does not require the species to be physically sampled, it is less labour intensive, economical and it can potentially detect species that are not targeted or not efficiently sampled as part of the Cat 1 and 3 components due to their low abundance and/or cryptic nature. The added benefit of collecting eDNA is that the extracted DNA sample contains a snapshot of the species present at that location and time. Therefore, these samples can be stored and used to identify other species of interest in the future, which can allow for range expansions/contractions to be tracked.

The aim of this research was to use eDNA to identify the presence and spatial distribution of threatened, uncommon and iconic species of crustacean, turtles, fish and aquatic mammals in the Edward/Kolety-Wakool system. This question is a priority given the presence of any of the species listed under the research proposal may result in changes to the way environmental water is managed. If the outcome of this project indicated eDNA is a suitable method for documenting the distribution of the target species, it could potentially be used in the future to identify population expansion as a result of targeted environmental watering.

There are two approaches for identifying species from eDNA: a targeted approach (to detect a single species) or multispecies (or metabarcoding) approach. The choice of approach depends on the study's aims and the resources available (McColl-Gausden *et al.*, 2019). For example, a targeted approach can be carried out with relatively standard genetic equipment located in a dedicated eDNA laboratory. Primer design allows flexibility in the choice of the target gene to maximise the chance that the target species can be detected and differentiated from congeneric species. The metabarcoding approach requires an accurate reference library containing sequences from the target gene region from all known species in the target group. In some cases, metabarcoding may not be able to identify species beyond family or genus. Furthermore, metabarcoding requires complex bioinformatic tools that can be labour intensive to develop and require greater expertise to develop in comparison to the targeted approach (McColl-Gausden *et al.*, 2019). While metabarcoding is valuable for identifying whole communities, primer specificity biases may result in a failure to detect some species, while the eDNA of extremely abundant species could reduce the probability of detecting rare species (Bylemans *et al.*, 2018; Kelly *et al.*, 2014). While few studies have directly compared the sensitivity of the targeted approach and metabarcoding at detecting the presence of a single species, it is likely that detectability may be higher with a targeted approach (Bylemans *et al.*, 2019; Harper *et al.*, 2018).

Given the information above, here we apply a targeted eDNA approach to develop assays to detect up to 13 aquatic species in the Edward/Kolety-Wakool system; Murray cod (*Maccullochella peelii*), trout cod (*M. macquariensis*), silver perch (*Bidyanus bidyanus*), freshwater catfish (*Tandanus tandanus*), river blackfish (*Gadopsis marmoratus*), dwarf flathead gudgeon (*Philypnodon macrostomus*), unspecked hardyhead (*Craterocephalus fulvus*), obscure galaxias (*Galaxias oliros*), Murray crayfish (*Euastacus armatus*), river mussel (*Alathyria jacksoni*), eastern long-necked turtle (Chelodina longicollis), short-necked turtle (*Emydura macquarii*) and platypus (*Ornithorhynchus anatinus*).

12.2 Methods

Targeted assay development

Assay design

Note, assay design was attempted for all 13 species (except for platypus as the assay was available in the literature). However, for various reasons assay design did not progress further for six species (see results). Here we describe assay development for six species.

Targeted quantitative PCR (qPCR) assays were developed to amplify a short region of the mitochondrial 12S rRNA gene in Murray cod, trout cod, silver perch, dwarf flathead gudgeon, freshwater catfish and Murray crayfish. Fin clips from all target fish species were sourced from at least five individuals from the Murray-Darling Basin in New South Wales (NSW), Australia in order to generate sequences from the target region. Note that sequences were not generated for Murray crayfish and we relied on GenBank sequences for this species. Fish genomic DNA was extracted using the PureLink Genomic DNA Mini Kit (Invitrogen) according to the manufacturer's instructions. A 390 bp region of the 12S rRNA gene was amplified using universal vertebrate primers MT1091L (50-CAAACTGGGATTAGATACCCCACTAT-30) and MT1478H (50-TGACTGCAGAGGGTGACGGGCGGTGTGT-30) (Fuller *et al.*, 1998). Quantitative PCR was performed using a QuantStudio3 PCR System (Applied Biosystems) with 50 µL reactions containing 25 µL of TaqMan Environmental Master Mix 2.0 (2X), 2.5 µL forward primer, 2.5 µL reverse primer, 15 µL DNase/RNase free water and 5 µL of template DNA. PCR cycling conditions consisted of an initial denaturation step at 94 °C (2 min) followed by 35 cycles of 94 °C (30 sec), primer annealing at 55 °C (1 min) and extension at 72 °C (30 sec), with a final extension at 72 °C (10 min). PCR products were purified using the PureLink PCR Purification Kit (Invitrogen) and then sent to the Australian Genome Research Facility (AGRF) (Sydney, Australia) for forward and reverse Sanger sequencing on a 3730xl DNA Analyzer using the above primers.

Sequences were imported into Geneious Prime (2020.0.5) and aligned to all other 12S rRNA sequences recorded for the target species and all closely-related species (at the family level) that co-occur in the Murray-Darling Basin (Appendix 12.1; Hardy *et al.*, 2011). Primers and probe were designed manually to amplify a short fragment of the 12S rRNA gene in the target species (Table 12.1), maximising the number of bp mismatches with all other species that potentially co-occur with the target species. Secondary structure was checked using the in-build Primer3 software in Geneious Prime. The primer/probe assays were then tested for specificity in silico against the National Centre for Biotechnology Information (NCBI) GenBank database using Primer-BLAST (Ye *et al.*, 2012). Any matches to non-freshwater species not found in Australia were disregarded.

Assay specificity and sensitivity assessments

The assays were tested for specificity by performing qPCR on genomic DNA from the target species and non-target closely related species (Appendix 12.1). Quantitative PCRs were carried out in triplicate 20 µL reactions, consisting of 10 µL Environmental Master Mix 2.0, 1 µL TaqMan Gene Expression assay 20X, 7 µL DNase/RNase free water and 2 µL of genomic DNA from at least three individuals of each species. PCR cycling was conducted on a QuantStudio 3 PCR System with thermal cycling conditions set at 50 °C (2 min), 95 °C (1 min), followed by 50 cycles of 95 °C (15 sec), 60 °C (60 sec). Any non-target positive amplification was Sanger sequenced at AGRF to ensure assays were specific only to the target species. The efficiency of each assay was tested using synthetic oligonucleotides (gBlock Gene Fragments) (Integrated DNA Technologies) of the target species' 12S rRNA gene. Synthetic oligos were diluted in tRNA buffer (1:250 diluted in DNase/RNase water) (Sigma-Aldrich) by a factor of ten to generate concentrations between 10-2 and 10-8 ng/µL. Following annealing temperature optimisation, calibration curves were generated using eight 20 µL qPCR technical replicates with 2 µL of each concentration as template. The limit of detection (LOD) of each assay was assessed by further diluting the synthetic oligos to 50, 30, 10 and 1 copies/ µL. Sensitivity tests were carried out using qPCR reactions and conditions as above and the optimised annealing temperature for each species (Table 12.2).

Assay field assessment

Assay performance was evaluated at sites with a recent historical record of each target species. Eight water samples were filtered in situ using the eDNA sampling ANDe™ system (Smith-Root, USA). Water samples consisted of 0.5 to 2.0 L (depending on turbidity) filtered through a 5 µm polyethersulfone (PES) filter enclosed in a sterile filter housing (Smith-Root, USA) at 1.0 L/min and with a max pressure of 14.0 psi. An equipment control composed of 1.0 L of sterile water was filtered per site prior to the field samples to enable potential contamination of the equipment to be identified. Filter papers were immediately preserved in 100% ethanol and then stored in the laboratory at 4°C. All further laboratory processing then occurred in a purpose built eDNA facility at the Narrandera Fisheries Centre (NFC) (Narrandera, Australia), with separate UV-sterile rooms for DNA extraction, PCR preparation and DNA template loading. In addition, work in each room was carried out in a sterile PCR hood. DNA was extracted from the filter paper using the PureLink Genomic DNA Mini Kit (Invitrogen) with a modified protocol. In short, each filter paper was halved into two tubes using sterile scissors and forceps before incubation with 500 µL of Digestion buffer and 20 µL Proteinase K. After one hour of incubation the filter paper was removed and 400 µL of both Binding/Lysis buffer and 100% ethanol were added. Lysate was then loaded into spin columns and the protocol then followed the manufacturer's instructions. A negative extraction control consisting of 100 µL of RNase/DNase free water was also included. Prior to qPCR analysis each sample was checked in triplicate for inhibitors using the TaqMan Internal Positive Control (Exo-IPC, Applied Biosystems). Six 20 µL qPCR technical replicates were then run per sample (as described under 'Assay specificity and sensitivity assessments', including the equipment control, negative extraction control (to identify contamination at the eDNA extraction stage) and a negative PCR control (to identify contamination at the PCR stage). In addition, a positive control (containing genomic DNA from the target species to ensure the qPCR had performed correctly) was run in triplicate on each plate. Fifty percent of positive samples (i.e. a single PCR replicate from each positive sample) were Sanger sequenced at AGRF to ensure the amplicon matched the target species.

Environmental DNA field sampling methods and analysis

Sampling Sites

Six sites were selected from existing sampling locations used for Cat 1 and Cat 3 fish river methods (Figure 12.1, Appendix 12.2; sites 5-10), in addition to four sites in the Edward/Kolety River to ensure integration with other components of the Edward/Kolety River integrated research project (Figure 12.1, Appendix 12.2; sites 1-4).

Figure 12.1 Map of all sampling sites within the Edward/Kolety-Wakool system for the eDNA monitoring survey.

Field sample collection

Field sampling was carried out between the 25th and 28th of November 2019 given there is some evidence that eDNA concentrations in the water increase in the spring and summer months (de Souza *et al.*, 2016; Furlan *et al.*, 2016b). For each sampling site eight water samples were filtered in situ using the eDNA sampling ANDe™ system (a total of 80 water samples). Samples were collected from the surface, including three from each bank and two from the channel, spaced apart by at least 50 m and targeted a variety of different structural habitat and mesohabitat within each site. Water was filtered through a 5 µm PES filter enclosed in a sterile filter housing at 1.0 L/min and with a max pressure of 14.0 psi, with a target filtration volume of 2.0 L. If the volume filtered was less than 0.5 L, a second filter and filter housing was used (a maximum of two filters per sampling site). An EC composed of 1.0 L of sterile water was filtered per site prior to the field samples. Filter papers were immediately preserved in 100% ethanol and once transported stored in the laboratory at 4°C.

Laboratory eDNA analysis

All laboratory processing occurred in the purpose build eDNA facility at the Narrandera Fisheries Centre. DNA was extracted from each of the 80 filter papers using the PureLink Genomic DNA Mini Kit (Invitrogen) with a modified protocol, detailed above. A negative extraction control was included of 100 µL of RNase/DNase free water. Environmental DNA extracts were diluted to 1:2 concentration with 1 x TE buffer to provide an adequate volume of template DNA, then stored at -20°C. Prior to qPCR analysis each sample was checked in triplicate for inhibitors using the TaqMan Exo-IPC (Applied Biosystems). A sample was considered to contain inhibitors if the sample's IPC Ct value was >2 Ct higher than the IPC Ct value in the negative PCR control.

Samples were analysed using qPCR for the presence of eight target species; Murray cod, trout cod, silver perch, dwarf flathead gudgeon, freshwater catfish and Murray crayfish (using the assays developed above), as well as platypus using a targeted assay developed by Lugg et al. (2018) and redfish perch (*Perca fluviatilis*) using a targeted assay developed by Furlan & Gleeson (2016a). Redfin perch were not on the original list of target species. However, given the assay has already been developed and the species is rarely detected in the Edward/Kolety-Wakool system, it was decided to include this species to determine if this invasive species is more widely distributed in the system than is currently documented. For each species, six qPCR technical replicates were run per eDNA sample (i.e. 80 samples x 6 PCR technical replicates = 408 PCR reactions per species) to increase the chance of positive detections given eDNA is heterogeneously distributed in the water. PCR reactions consisted of 10 µL Environmental Master Mix 2.0 (Applied Biosystems), 1 µL TaqMan Gene Expression assay (Applied Biosystems), 7 µL DNase/RNase free water and 2 µL of DNA template. PCR cycling was conducted on a QuantStudio 3 PCR System (Applied Biosystems) with thermal cycling conditions set at 50 °C (2 min), 95 °C (1 min), followed by 50 cycles of 95 °C (15 sec), 60 °C (60 sec). Six technical replicates were also run for each equipment control, negative extraction control and negative PCR control and an additional positive control was run in triplicate on each plate. All PCR technical replicates were visually inspected, and technical replicates were only considered positive if there was an exponential phase at any point during the 50 reaction cycles. A single qPCR replicate was selected from twenty five percent of positive samples from for Sanger sequencing at AGRF to ensure amplicon matched the target species. A positive detection at a sampling site (e.g. Gee Gee) was achieved if either a) at least three of the eight water samples had a minimum one positive PCR replicate, or b), there were two or more positive PCR technical replicates in a single water sample. If a single PCR replicate was positive in a water sample where no other PCR technical replicates at that site were positive, a further 12 PCR technical replicates were carried out to attempt to replicate the positive result. If none of these were positive, that original positive sample was considered to be a false positive.

12.3 Results

Targeted assay development

Of the 13 target species, assay development did not progress for six species; river blackfish, unspecked hardyhead, obscure galaxias, river mussel, eastern long-necked turtle and short-necked turtle. River blackfish had too much within-species variation in the 12S gene to be a target for assay binding. Unspecked hardyhead and obscure galaxias had a high level of sequence similarity to closely related co-occurring species at the 12S gene. The river mussel also had close sequence similarity to the freshwater mussel *A. condola*. Additional sequencing of the 28S gene did not resolve this issue and the 16S gene did not amplify. The river mussel and freshwater mussels have very similar morphology and thus the specimens that we obtained may have been incorrectly identified. Further morphological assessment of this species is recommended before progressing eDNA assay development for river mussels. The eastern long-necked turtle is not suitable for eDNA primer design at any mitochondrial gene given it shares its mitochondrial genome with the closely related broad-shelled river turtle (*C. Expansa*). Progressing assay development for these species will require substantial further work that is beyond the scope of the current project.

Targeted assays were successfully developed for Murray cod, trout cod, silver perch, freshwater catfish, dwarf flathead gudgeon and Murray crayfish (Table 12.1). Occasional amplification was observed for some closely related species (Appendix 12.3), which was confirmed through Sanger sequencing. For example, the Murray cod assay amplified eastern freshwater cod (*M. ikei*) and Mary River cod (*M. mariensis*). In addition, the freshwater catfish assay amplified the Bellinger catfish (*T. bellingerensis*) and the freshwater cobbler (*T. bostocki*) (Appendix 12.3). Nevertheless, these assays were considered acceptable for use in the Edward/Kolety-Wakool system given the other species are not sympatric and thus any amplification can be attributed to the target species. We also observed unexpected amplification of the target species' DNA sequence in the DNA samples of some closely related species (Appendix 12.3), suggesting contamination had occurred at some stage during sample collection, DNA extraction or qPCR preparation. Following substantial troubleshooting, we confidently ruled out contamination at the eDNA extraction and qPCR preparation stages. Thus, it was concluded that the contamination was occurring during fin-clip collection. For example, Murray cod DNA sequences were detected in samples containing DNA extracted from golden perch (*Macquaria ambigua*) fin-clips. The golden perch fin-clips were collected from hatchery broodfish housed in a facility that also houses Murray cod. Thus, our data suggests that Murray cod eDNA on the surface of the tank or the measuring board contaminated the tissue samples as they were collected. Similarly, samples collected in the field could also be easily contaminated with DNA from the target species from the sampling equipment also contacts the target species. Thus, we considered it safe to continue to sensitivity testing and field testing of each assay.

An assessment of sensitivity showed that each assay was able to reliably detect concentrations of its target DNA down to between two and 10 copies/ µL, and all assays performed this with efficiencies of >95% (Table 12.2). The field assessment of each assay detected the presence of each target species at the two sites where the species was known to be distributed based off historical electrofishing/netting or craypot data from NSW DPI (Table 12.3). Sequencing a single replicate from 50% of positive samples confirmed amplification matched the DNA sequence of each target species. All negative control samples (equipment control, negative extraction control and negative PCR control) were clear, indicating contamination had not occurred. All assays detected their target species in an average of 93.7% of the samples collected at each test site.

Table 12.1 The primer and probe sequences and amplicon length for each targeted assay.

Target species	Label	Sequence (5'-3')	Length (bp)
Murray cod	M.pee_12S_F	CCCTTGTTCCACCAGCCTA	85
	M.pee_12S_R	GTTCTGGGTTGTACCAATTATGCT	
	M.pee_12S_probe	CCAGCTTACCCTGTGAAGGACCTA	
Trout cod	M.mac_12S_F	TCCATGACACACGGAATAC	94
	M.mac_12S_R	CCAGTTTCAGCAGAACTCT	
	M.mac_12S_probe	TGAAACACACATCTGAAGGAGG	
Silver perch	B.bid_12S_F	CGCACAGTAAGCAAAATTGG	133
	B.bid_12S_R	TCCTCCTTCATGTTGCACG	
	B.bid_12S_probe	CAGCCCAGAACGTCAGGTC	
Freshwater catfish	T.tan_12S_F	TTACCCTGTGAAGGCCCAA	133
	T.tan_12S_R	TGTTTCAGCGTGCTATTCGTTATA	
	T.tan_12S_probe	AGCCCATTTCTTCCCACTTCGTAC	
Dwarf flathead gudgeon	P.mac_12S_F	CACCCTCTCTTGTTCCACC	153
	P.mac_12S_R	ACACTGTGTCAGGGAATGTAG	
	P.mac_12S_probe	CCTATATACCGCCGTCGTCAGC	
Murray crayfish	E.arm_12S_F	GTTGGGGCTTGATAACAGC	158
	E.arm_12S_R	ACTCAATTTCACCTTTTATCCACC	
	E.arm_12S_probe	CGCGGTTAGACTTGGAGGTTAAG	

Table 12.2 Sensitivity test results, limit of detection and optimal annealing temperature for each targeted assay.

Target species and assay	Sensitivity		Limit of detection	Annealing temp
Murray cod	Efficiency (%)	96.69	2 copies / µL	59 °C
M.pee_12S_assay	R^2	0.998		
Trout cod	Efficiency (%)	96.85	10 copies / µL	57 °C
M.mac_12S_assay	R^2	1.000		
Silver perch	Efficiency (%)	97.22	10 copies / µL	57 °C
B.bid_12S_assay	R^2	1.000		
Freshwater catfish	Efficiency (%)	94.43	10 copies / µL	60 °C
T.tan_12S_assay	R^2	1.000		
Dwarf flathead gudgeon	Efficiency (%)	96.69	10 copies / µL	57 °C
P.mac_12S_assay	R^2	0.999		
Murray crayfish	Efficiency (%)	94.07	10 copies / µL	57 °C
E.arm_12S_assay	R^2	0.998		

Table 12.3 Detection results from the field test of each targeted assay. Shown is the number of positive detections at each site from eight water samples, each with six PCR technical replicates.

Target species and assay	Field test sites	Latitude	Longitude	No. positive samples	Mean no. positive PCR replicate/sample†
Murray cod	Buckingbong Boat ramp[A]	-34.80233	146.61735	8/8	6/6
M.pee_12S_assay	Narrandera Boat ramp[A]	-34.75456	146.55255	8/8	6/6
Trout cod	Buckingbong Boat ramp[A]	-34.80233	146.61735	8/8	5.87/6
M.mac_12S_assay	Narrandera Boat ramp[A]	-34.75456	146.55255	8/8	6/6
Silver perch	Buckingbong Boat ramp[A]	-34.80233	146.61735	8/8	3.25/6
B.bid_12S_assay	Narrandera Boat ramp[A]	-34.75456	146.55255	5/8	1/6
Freshwater catfish	Bindawalla[B]	-35.24995	145.97474	8/8	3.87/6
T.tan_12S_assay	Widgiewa[B]	-35.06766	146.27391	8/8	4.75/6
Dwarf flathead gudgeon	Aratula[C]	-35.81155	145.2227	8/8	6/6
P.mac_12S_assay	Broken River Lagoon[D]	-35.92899	144.45065	7/8	3.62/6
Murray crayfish	Buckingbong Boat ramp[A]	-34.80233	146.61735	8/8	1.25/6
E.arm_12S_assay	Narrandera Boat ramp[A]	-34.75456	146.55255	6/8	1.75/6

†Confirmed through sequencing a single replicate from 50% of positive samples.

[A]Murrumbidgee River, [B]Colombo Creek, [C]Aratula Creek, [D]Murray River

Environmental DNA monitoring survey

Water turbidity was between 1.4 and 102 NTU and this affected the volume of water that could be passed through the filter before it became clogged with debris. The total volume of water filtered from each site ranged from 7.26 to 11.83 L (Appendix 12.2). All qPCR equipment controls, negative extraction controls and negative PCR controls did not amplify eDNA for the target species, indicating there was no contamination of the samples. The PCR positive control and internal positive control (Exo-IPC) amplified as expected and thus confirmed that the qPCR reaction was successful and that samples were not affected by inhibition.

Murray cod were detected at 8 of 10 sites (Figure 12.2, 12.3a, Appendix 12.4). At the remaining two sites Stoney Crossing and Gee Gee Bridge there were detections in a single qPCR replicate for two water samples and one water sample respectively. A further 12 qPCRs were carried out and these detections could not be replicated, suggesting these were false positives. Trout cod were detected at 4 of 10 sites (Figure 12.2, 12.3b, Appendix 12.5), with Four Posts standing out as having the highest proportion of positive detections of all the sites sampled. Silver perch were detected in 7 of 10 sites (Figure 12.2, 12.3c, Appendix 12.6). However, there was a lower proportion of positive qPCR technical replicates per sample per site than Murray cod. Redfin perch were detected at a single site, Stoney Creek Crossing (Figure 12.2, 12.3b, Appendix 12.7). Platypus were not detected at any of the 10 sites (Appendix 12.8). At two sites Four Posts and Widgee1, there was a single positive platypus PCR replicate for one water sample however further qPCR analysis carried out for these two water samples determined them to be false positives. The two positive samples will be sequenced to determine if the qPCR product is platypus sequence. Dwarf flathead gudgeon, freshwater catfish and Murray crayfish were also not detected at any of the 10 sites (Appendix 12.9, 12.10 and 12.11, respectively). Both species had at least one single positive replicate, but further qPCRs showed these to be false positives.

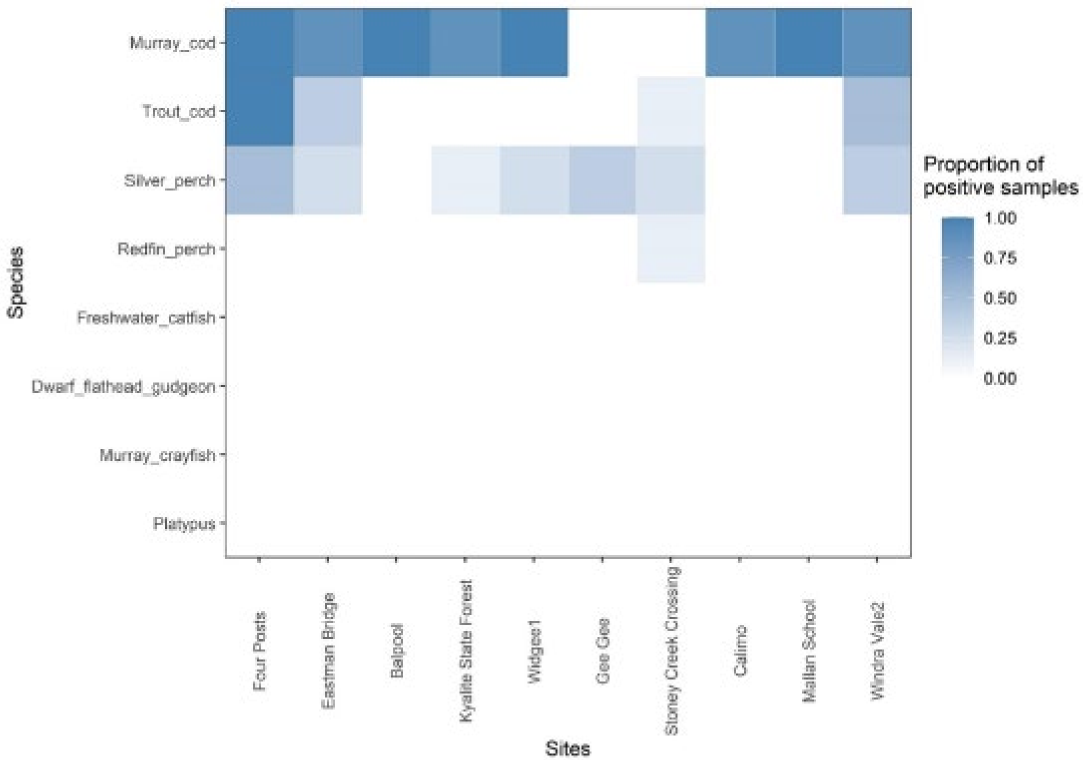


Figure 12.2 An overview of species detections represented as the proportion of positive samples for each of the target species detected across all study sites.

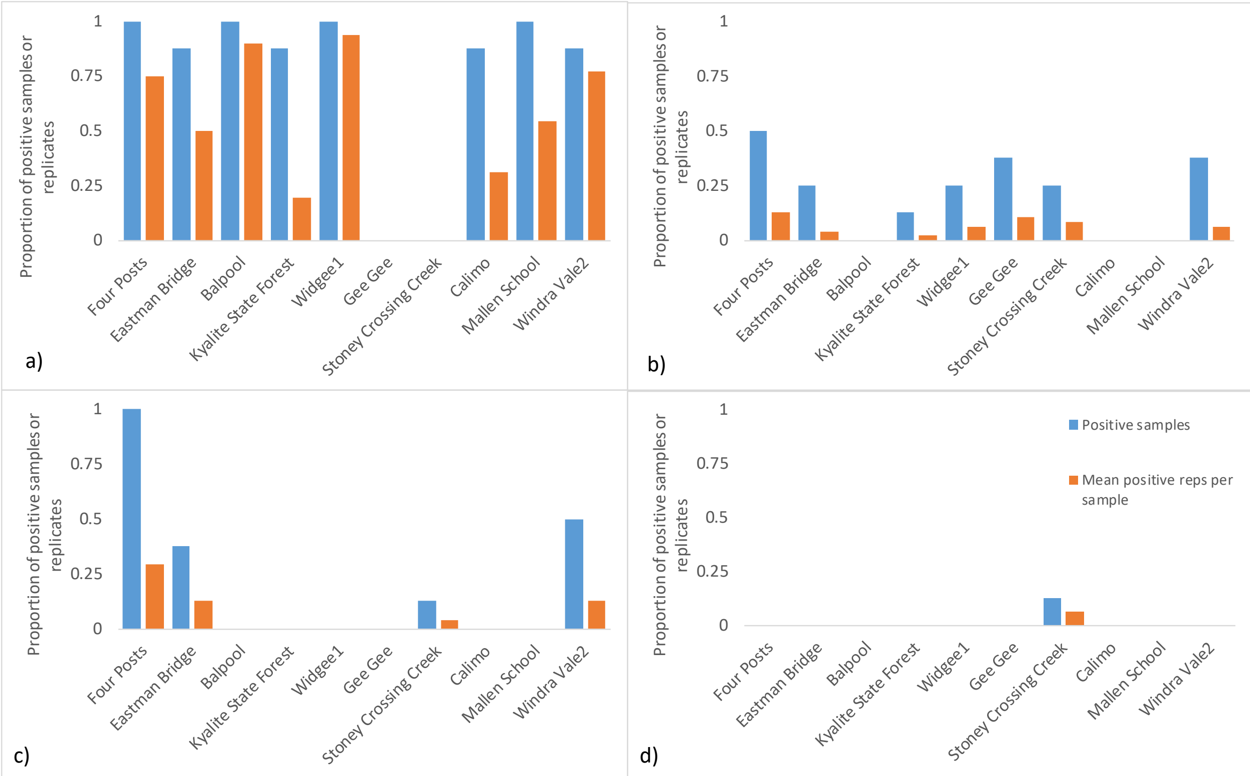


Figure 12.3 The proportion of positive samples (blue) and mean proportion of positive qPCR technical replicates per sample (orange) for a) Murray cod, b) silver perch, c) trout cod, and d) and redfin perch across all study sites.

12.4 Discussion

Suitability of assays for species monitoring in the Edward/Kolety-Wakool system

This study has successfully developed six highly sensitive and specific assays for the detection of rare and cryptic aquatic species in the Edward/Kolety-Wakool system. To our knowledge, this is the first study of its kind in Australia to develop and apply more than two targeted assays for aquatic species to a single project. The results showed that eDNA detected the recent presence of Murray cod, trout cod, silver perch and the invasive redfin perch at one or more of the ten study sites. False positives (a positive qPCR result despite the species not being present at the sampling site) can be an issue with eDNA methods. False positives can occur if samples are contaminated either in the field or laboratory, from transfer of eDNA from one location to another by other animals (such as birds) or transport from an upstream location (McColl-Gausden *et al.*, 2019). Transport of DNA from an upstream location is a possible source of false positives in the Edward/Kolety-Wakool system. Environmental DNA can persist in the water for at least 60 days (Strickler *et al.*, 2015). Thus, downstream transport could potentially result in positive detections even if the species is not present at the study location. However, in lotic systems, studies have found that eDNA is not detectable more than approximately 5km or less from its source (Deutschmann *et al.*, 2019; Shogren *et al.*, 2017; Wood *et al.*, 2020). This is due to the eDNA constantly being degraded, diluted, or settling into the substrate. The transport distance of eDNA in the Edward/Kolety-Wakool system is not known as the distance will vary depending on multiple factors including stream width, stream slope, turbulence and habitat heterogeneity (Shogren et al., 2017). Nevertheless, the precise location of the positive detection is unlikely to influence the management of a species at such a fine spatial scale in the Edward/Kolety-Wakool system given water is delivered at the scale of the river or catchment.

False positives due to sample contamination is highly unlikely in the current study given that the minimum requirements to confirm a species is detected were met; 1) no amplification in the equipment controls, negative extraction controls and negative PCR controls, 2) an eDNA appropriate laboratory was used and 3) positive detections were sequenced to confirm the species identification (Thalinger *et al.*, 2020 in review). A recent validation scale has been proposed to assess the applicability of targeted eDNA assays to be applied for species monitoring (presence/absence) that is based on 122 variables (Thalinger *et al.*, 2020 in review). Based on this scale, the assays presented here meet most of the criteria for level 4, indicating a 'substantial' level of development (out of a possible 5 levels). Of 546 assays that were reviewed to develop this scale, none reached level 5 and only around 10% reached level 4. Thus, the assays developed here are suitable for monitoring the distribution of aquatic species in the Edward/Kolety-Wakool system. These assays could be further developed by statistical modelling to enable detection probabilities to be estimated. This would allow the probability of a species being present to be calculated if it is not detected with the assay (i.e. a false negative) (Strickland *et al.*, 2019; Thalinger *et al.*, 2020 in review).

Failure to detect a species when it is present (false negatives) can occur when using eDNA (which is true for most other monitoring methods). For example, false negatives can occur if there are inhibitors in the water (tannins, humic acid, bacterial debris etc) that can disrupt the extraction or amplification of the eDNA, resulting in a negative result even if the target eDNA is present in a sufficient quantity to detect (Gibson *et al.*, 2012; Jane *et al.*, 2015). This is unlikely to be an issue for the current study given all samples were screened for inhibitors prior to being screened across the assays. False negatives could also occur if the concentration of eDNA in the water is extremely low. For example, the limit of detection (LOD) for the Trout cod assay is 10 copies per µL. If Trout cod are in low abundance, the amount of eDNA in the water is expected to be very low and possibly heterogeneously distributed. Consequently, water sampling may not capture the eDNA, or the number of copies may be too low to detect (Furlan *et al.*, 2016b; Klymus *et al.*, 2020). To confidently rule out the presence of a species using eDNA, occupancy modelling is required (Strickland *et al.*, 2019), but this is beyond the scope of the current project.

Species detected

This project was not designed to directly compare the distribution of species based on eDNA or traditional methods. Thus, eDNA sampling was not carried out simultaneously with fish river sampling (Section 8). However, three fish larval sampling sites Windra Vale2, Widgee1 and Eastman Bridge and six fish river sampling sites (Fish River Cat 3; Gee Gee, Calimo, Balpool, Kyalite State Forest and Four Posts) in 2019/20 corresponded to eDNA sampling sites. Thus, we can cautiously speculate on the likelihood that a species is present or absent at a site based on eDNA results by taking into account their presence or absence based on other methods in 2019/20 and on historical data from the last ten years, as well as the species' ecology (e.g. migratory potential).

Murray cod typically do not undertake large-scale movements and has high site fidelity (Chapter 8, Jones *et al.*, 2007; Koehn *et al.*, 2009). Thus, it is reasonable to make a comparison between sites where Murray cod were detected using traditional methods and targeted eDNA methods over recent years. Environmental DNA failed to detect Murray cod at Gee Gee (zone 5, mid-Wakool River downstream of Barbers Creek) and Stoney Creek Crossing (zone 6, lower Wakool River). Murray cod at these two locations were severely affected by fish kills in 2016-2017. Traditional monitoring data from the Edward/Kolety-Wakool LTIM and MER projects detected two Murray cod at each site prior to fish kills in 2015, but none following the fish kills in 2019. Historical records from the NSW DPI database shows that Murray cod were detected at Stoney Creek Crossing between 2010 and 2014. Taken together, these data suggest Murray cod were likely to consistently occupy Stoney Creek Crossing and Gee Gee prior to 2015. The eDNA results are consistent with other sampling methods and suggest that Murray cod have been slow to recover following the fish kills in these locations.

Environmental DNA analysis unequivocally confirmed the presence of Murray cod at the remaining eight sites. Some sites had many more positive qPCR technical replicates than other sites (Appendix 12.4), which could be indicative of population size. There is mounting evidence showing that eDNA concentration in lotic environments can be correlated with abundance or biomass (Coulter *et al.*, 2019; Doi *et al.*, 2017; Jane *et al.*, 2015). Consequently, the assays developed for the current study could be assessed for their ability to estimate population size and/or biomass through a comparison with traditional methods.

One of the most important findings of the current project was the presence of trout cod eDNA at four of the ten sampling locations; Stoney Creek Crossing (lower Wakool River), Windra Vale2 (Yallakool Creek), Eastman Bridge and Four Posts (both on the Edward/Kolety River). Across the ten sampling locations, only three trout cod have been caught using traditional methods (across all projects in the NSW DPI Fisheries database). One trout cod was captured at Calimo (Colligen-Neimur River) in 2014 and two at Four Posts (one in 2014 and one in 2015). Trout cod have been detected at one other LTIM sampling site; Yallakool/Back Creek Junction (a single fish in 2019) and they have also been detected at another DPI Fisheries site on the Edward/Kolety River upstream of Four Posts. Larval drift sampling in four sampling zones (Table 8.4) failed to detect trout cod in the four study zones. Thus, the confirmed eDNA detections of trout cod at four locations (Stoney Creek Crossing, Windra Vale2, Eastman Bridge and Four Posts) has not only confirmed the species is currently present in the upper parts of the Edward/Kolety-Wakool system, but has extended its known distribution to the lower Wakool River. These populations are likely to have been gradually recovering since the hypoxic blackwater events of 2016/17 following large-scale flooding in the system that killed large numbers of fish. Recovery is expected to be slow as trout cod display similar site fidelity and habitat overlap to Murray cod (Ebner *et al.*, 2009; Koehn *et al.*, 2014; Koehn *et al.*, 2008). The presence of trout cod across multiple locations in the Edward/Kolety-Wakool system is an important finding highlights the importance of protecting these and other high priority species from adverse effects of future hypoxic blackwater events.

Silver perch were successfully detected at seven of the ten sites using eDNA; Four Posts, Eastman Bridge, Kyalite State Forest, Widgee1, Gee Gee, Stoney Creek Crossing and Windra Vale2. Traditional sampling in 2019/20 also recorded silver perch throughout these locations (and nearby locations) but required boat electrofishing, backpack electrofishing, larval sampling, angling and baited set-lines to do so. Consequently, eDNA is appears to be as effective at documenting the distribution of silver perch in the Edward/Kolety-Wakool system as multiple fish sampling methods combined. It is important to emphasise that eDNA sampling was not concurrent with the traditional sampling, thus the data aren't directly comparable. However, historic NSW DPI Fisheries' electrofishing data from 2007 to 2014 recorded low numbers of silver perch (typically two to five fish per year across one to three sites) at six of the eDNA sites that correspond to Fish River sites (across all Fisheries' projects). In addition, silver perch recruits were recorded in every Zone of the Edward/Kolety-Wakool system from 2014-2020. (Watts *et al.*, 2019). Thus, the combined evidence suggests silver perch are likely to be present throughout the Edward/Kolety-Wakool system in most years. Therefore, targeted eDNA methods are clearly an effective method for quickly detecting this species and is likely to be useful in areas where they are in low abundance.

The invasive Redfin perch were not one of the original target species for this project. However, given a redfin perch eDNA assay is available, we considered that we could potentially provide further information on its distribution in the Edward/Kolety-Wakool system. The results showed that redfin perch are present at the Stoney Creek Crossing (lower Wakool River) site. Eggs/larval redfin were recorded during the last LTIM project in the system (Watts *et al.*, 2019), but were not recorded in the recent 2019/20 sampling (Table 8.4). The NSW DPI Fisheries database has sporadic records of this species at several sites in the Edward/Kolety-Wakool system between 1994 and 2013, including records from one eDNA sampling site (FP in 2010 and 2011). Thus, the presence of larval eggs/fish in the previous LTIM project combined with the positive eDNA results from Stoney Creek Crossing suggests that redfin perch are currently in very low numbers in the system. This is an encouraging result and indicates that the environment in the Edward/Kolety-Wakool is currently unfavourable for this species.

Species not detected

Environmental DNA did not detect dwarf flathead gudgeon, freshwater catfish and Murray crayfish. While we cannot rule out false negatives for the reasons described above, there are no records of dwarf flathead gudgeon and freshwater catfish in the NSW DPI Fisheries database at any of the ten eDNA sampling sites, nor at any surrounding site in the Edward/Kolety-Wakool system. Freshwater catfish were recorded as larvae in 2017/18 (Watts *et al.*, 2019), but none of the other fish species were recorded as eggs or larvae at any of the sampling sites in 2019/20 or in the previous LTIM project (Section 8, Watts *et al.*, 2019). Consequently, it is likely that these species are absent in the Selected Area, or if they are present, the population size is likely to be very small.

Six Murray crayfish were detected at Four Posts in August 2020; thus it was surprising that the species was not detected by the eDNA sampling given they are unlikely to have left the location prior to eDNA sampling in November 2020. NSW DPI data shows that Murray crayfish have been detected in the Edward Kolety-Wakool during annual sampling from 2012 to 2017, though the populations declined substantially after the blackwater event in 2016/17. But they were typically in low abundance below Deniliquin and most abundant around Twin Rivers Boat Ramp in town. Thus, the failure to detect Murray crayfish could be due to their absence or low abundance below Deniliquin, where all but one of our sites was located. It is possible that increasing the number of water samples collected at each site, potentially sampling lower in the water column as two of the three species are benthic) and increasing the number of qPCR technical replicates per sample could improve the chance of detecting rare species (Furlan *et al.*, 2016b). Modelling could be applied to determine the probability of detecting target DNA based on the concentration of eDNA in the water, the dispersion of eDNA in the water, DNA extraction and amplification efficiency and assay sensitivity (Furlan *et al.*, 2016b; Song *et al.*, 2020).

The failure to detect rare species at some sites (e.g. Calimo, Mallan School, Balpool and Kyalite State Forest) using eDNA collected during a single sampling event does not necessarily indicate that these sites are in poor health. This study was designed to be a proof of concept to determine if the technique was able to detect rare species that may potentially be missed by other methods. And it has succeeded in that aim by showing that rare species can indeed be detected. Nevertheless, the sampling design needs to be refined given the failure to detect crayfish at Four Posts when it was almost certainly present.

Unsuccessful eDNA assays

We abandoned development of targeted assays for six of the species of interest. In most cases, we are confident that targeted assays could be successfully developed with further laboratory work. For example, targeting a different gene for river blackfish, unspecked hardyhead and obscure galaxias is likely to result in a functional assay. Similarly, it is also likely assays could be successfully developed for eastern long-necked turtles and short-necked turtles could also be developed by targeting a nuclear gene. Other species such as the river mussel will require more work to develop functional assays. The river mussel has very similar morphology to the freshwater mussel. We found it impossible to differentiate between the two species based on morphology or sequence analysis, suggesting that the samples we collected only represented one of these species. Further morphological assessment of the *Alathyria* genus is required before progressing eDNA assay development for river mussels.

Conclusions

Through undertaking this project, we have learned that developing targeted assays is time consuming and expensive, particularly if there are any complications encountered during primer development such as a lack of variability in the target gene between closely related species. This is the reason that researchers typically focus on one or two species at a time. Consequently, the successful development of six assays is a great achievement and will allow for rapid and accurate detection of the presence of these species in the Edward/Kolety-Wakool system as well as other river systems in the Murray-Darling Basin. Furthermore, the cost of developing the assays far outweighed the cost of collecting the samples and running the assays. Therefore, if these assays are applied in the future the costs will be lower as there is only a need to collect water samples and then run the new assays against each water sample. The number of false negative detections could be reduced by taking additional water samples and running more qPCR technical replicates.

An important consideration of eDNA work is whether it is cost effective in comparison to other methods. In the case of fish, the overall cost of targeted eDNA analysis (once assays are developed) is currently similar to electrofishing the equivalent number of sites. However, the cost of eDNA analysis is likely to fall as advances are made in the technology and that reduce the total cost per sample. But it is important to note that the two methods are not directly comparable. For example, when we conduct electrofishing, we use community assessment settings to enable a broad range of species to be captured. Consequently, species such as silver perch that are more ‘pelagic’ in their behaviour may be more difficult to capture. In contrast, targeted eDNA sampling is less likely to be affected by the behaviour of the fish given it can return a positive result even if a species has recently left the immediate sampling location. Therefore, targeted eDNA methods are most suitable when the objective is to document the distribution of species inefficiently sampled by other methods. In summary, eDNA is an effective method to detect the presence of rare and threatened species in the Edward/Kolety-Wakool system. We recommend future work explores occupancy modelling to enable detection probabilities to be estimated.

12.5 Appendices

Appendix 12.1 Species and their GenBank sequence accession numbers that were included in the alignment (in Geneious 2020.0.5) for the assay development of each target species.

Target species	Species included in alignment	Accession no. (GenBank)
Murray cod (*Maccullochella peelii*)	*Maccullochella peelii*	**FJ710953.1, KY798452.1, APO14532.1, KT337330.1, KT337331.1, KT337332.1, KT337334.1, NC_023807.1, generated sequences M.PEE_12S_QS1-5 (provided below)**
	Maccullochella macquariensis	**FJ710952.1, KY798453.1**
	*Maccullochella ikei**	**HQ615495.1**
	*Maccullochella mariensis**	**HQ615496.1**
	Macquaria ambigua	**FJ710954.1, FJ710955.1, HQ615497.1, HQ615498.1**
	Macquaria australasica	**FJ710956.1, HQ615499.1, HQ615500.1, HQ615501.1, HQ615502.1**
	Gadopsis marmoratus	**FJ710927.1, FJ710928.1, FJ710929.1**
	Gadopsis bispinosus	**FJ710926.1**
	Nannoperca australis	**FJ710965.1, FJ710966.1, HQ615508.1, HQ615509.1**
	*Nannoperca obscura**	**FJ710967.1, HQ615510.1**
	*Nannoperca variegate**	**HQ615511.1, KJ774864.1**
	*Nannoperca oxleyana**	**KJ774861.1**
	*Nannoperca pygmaea**	**KJ774862.1, KJ774863.1**
	*Nannoperca vittate**	**KJ774865.1, KJ774866.1, KJ774867.1**
	*Percalates colonorum**	**FJ711001.1, FJ711002.1**
	*Percalates novemaculeata**	**HQ615503.1**
	*Bostockia porosa**	**KJ774783.1, KJ774784.1**
	*Nannatherina balstoni**	**KJ774859.1, KJ774860.1**
Trout cod (*Maccullochella macquariensis*)	*Maccullochella macquariensis*	**FJ710952.1, KY798453.1, AF295045.1, generated sequences M.MAC_12S_QS1-5 (provided below)**
	Maccullochella peelii	**FJ710953.1, KY798452.1**
	*Maccullochella ikei**	**HQ615495.1**
	*Maccullochella mariensis**	**HQ615496.1**

	Macquaria ambigua	**FJ710954.1, FJ710955.1, HQ615497.1, HQ615498.1**
	Macquaria australasica	**FJ710956.1, HQ615499.1, HQ615500.1, HQ615501.1, HQ615502.1**
	Gadopsis marmoratus	**FK710927.1, FK710928.1, FK710929.1**
	Gadopsis bispinosus	**FJ710926.1**
	Nannoperca australis	**FJ710965.1, FJ710966.1, HQ615508.1, HQ615509.1**
	*Nannoperca obscura**	**FJ710967.1, HQ615510.1**
	*Nannoperca variegate**	**HQ615511.1, KJ774864.1**
	*Nannoperca oxleyana**	**KJ774861.1**
	*Nannoperca pygmaea**	**KJ774862.1, KJ774863.1**
	*Nannoperca vittate**	**KJ774865.1, KJ774866.1, KJ774867.1**
	*Percalates colonorum**	**FJ711001.1, FJ711002.1**
	*Percalates novemaculeata**	**HQ615503.1**
	*Bostockia porosa**	**KJ774783.1, KJ774784.1**
	*Nannatherina balstoni**	**KJ774859.1, KJ774860.1**
Silver perch (*Bidyanus bidyanus*)	*Bidyanus bidyanus*	**FJ710915.1, FJ710916.1, KF999833.1, AP014539.1, NC_024854.1, generated sequences B.BID_12S_QS1-5 (provided below)**
	*Bidyanus welchi**	**HQ615466.1**
	Leiopotherapon unicolor	**FJ710951.1, HQ615491.1**
	*Amniataba percoides**	**KJ774778.1**
	*Amniataba caudavitta**	**KJ774777.1**
	*Scortum hillii**	**KJ774928.1**
	*Scortum barcoo**	**KJ774927.1**
Freshwater catfish (*Tandanus tandanus*)	*Tandanus tandanus*	**FJ710988.1, FJ710989.1, FJ710990.1, FJ710991.1, FJ710988.1, KY798489.1, generated sequences T.TAN_12S_QS1-6 (provided below)**
	Tandanus tropicanus	**HQ615524.1**
	Tandanus bellingerensis	**HQ615523.1**
	*Tandanus bostocki**	**KJ774930.1**
	Neosilurus hyrtlii	**HQ615514.1, KJ774877.1**
	Porochilus rendahli	**HQ615519.1, FJ710978.1**

Dwarf flathead gudgeon (*Phylipnodon marcrostomus*)	*Phylipnodon marcrostomus*	**HQ615518.1, FJ710976.1, KJ774997.1, KJ774002.1, KJ774000.1, KJ774998.1, generated sequences P.MAC_12S_QS1-3 (provided below)**
	Philypnodon grandiceps	**FJ710975.1, KJ774996.1, KJ774995.1, KJ774889.1, KJ774888.1, KJ774890.1**
	Mogurnda adspersa	**FJ710962.1, FJ710963.1, KJ774849.1**
	*Mogurnda larapintae**	**KJ774852.1**
	*Mogurnda mogurnda**	**KJ774989.1**
	*Mogurnda thermophlia**	**KJ774853.1**
	*Mogurnda clivicola**	**KJ774850.1**
	Gobiomorphus australis	**KJ774831.1**
	Gobiomorphus coxii	**KJ774832.1**
	Hypseleotris klunzingeri	**KJ774972.1, KJ774971.1, KJ774973.1, KJ774976.1, KJ774974.1, KJ774977.1, HQ615482.1**
	Hypseleotris galii	**HQ615479.1, HQ615478.1, KJ774970.1, KJ774835.1, KJ774836.1, KJ774969.1**
	Hypseleotris compressa	**KJ774834.1**
	Hypseleotris sp. 'Lake's carp gudgeon'	**KJ784396.1, HQ615483.1, HQ615486.1**
	Hypseleotris 'Midgleys carp gudgeon'	**KJ774839.1, KJ774838.1**
	Hypseleotris sp. 'Murray-Darling carp gudgeon'	**KJ774984.1**
	Hypseleotris hybrid 'Midgleys-Lakes'	**KJ774980.1**
	Hypseleotris sp. hybrid 'Midgleys-Murray-Darling'	**KJ774982.1, KJ774983.1**
	Oxyeleotris lineolata	**HQ615516.1**
Murray cray (*Euastacus armatus*)	*Euastacus armatus*	**NC_026575.1, KP294310.1, DQ006424.1, DQ006425.1, DQ006426.1**
	Euastacus crassus	**DQ006453.1, DQ006455.1,**
	Euastacus rieki	**DQ006495.1, DQ006496.1**
	Cherax destructor	**KJ573468.1, KJ573469.1, AY191737.1, AY191738.1, AY191739.1, AY191740.1**

* Closely related species that do not co-occur with the target species but were still included in the alignment.

Appendix 12.1 continued: Newly generated sequences – Sequences that were included in the alignments for each target species but not yet published on GenBank. The location provided reflects the sample's origin.

Target species	Sequence (5'-3')
Murray cod (*Maccullochella peelii*) Wakool River, NSW	M.PEE_12S_QS1 TTAGCCTTAAAAATTGATAATACACTACACCTATTATCCGCCTGGGCACTACGAGCATCAGCTTAAAACCCAAAG GACTTGGCGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCCCC TTGTTCCACCAGCCTATATACCGCCGTCGCCAGCTTACCCTGTGAAGGACCTATAGTAAGCATAATTGGTACAAC CCAGAACGTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATAACACATGAAATACG AACGATGTATTGAAAAACACATCCGAAGGAGGATTTAGTAGTAAGTAGAAAAAAGAGTATTCTACTGAAACCG GCCCTGAAGCGCGT
Lachlan River, NSW	M.PEE_12S_QS2 GCTTAGCCTTAAAAATTGATAATACACTACACCTATTATCCGCCTGGGCACTACGAGCATCAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCC CCTTGTTCCACCAGCCTATATACCGCCGTCGCCAGCTTACCCTGTGAAGGACCTATAGTAAGCATAATTGGTACA ACCCAGAACGTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATAACACATGAAATA CGAACGATGTATTGAAAAACACATCCGAAGGAGGATTTAGTAGTAAGTAGAAAAAAGAGTATTCTACTGAAAC CGGCCCTGAAGCGCGT
Murrumbidgee River, NSW	M.PEE_12S_QS3 GCTTAGCCTTAAAAATTGATAATACACTACACCTATTATCCGCCTGGGCACTACGAGCATCAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCC CCTTGTTCCACCAGCCTATATACCGCCGTCGCCAGCTTACCCTGTGAAGGACCTATAGTAAGCATAATTGGTACA ACCCAGAACGTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATAACACATGAAATA CGAACGATGTATTGAAAAACACATCCGAAGGAGGATTTAGTAGTAAGTAGAAAAAAGAG
Edward River, NSW	M.PEE_12S_QS4 CGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCCCCTTGTTCCA CCAGCCTATATACCGCCGTCGCCAGCTTACCCTGTGAAGGACCTATAGTAAGCATAATTGGTACAACCCAGAAC GTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATAACACATGAAATACGAACGATG TATTGAAAAACACATCCGAAGGAGGATTTAGTAGTAAGTAGAAAAAAGAGTATTCTACTGAAACCGGCCCTGAA GCGCGT
Neimur River, NSW	M.PEE_12S_QS5 GGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCCCCTTGTTCCAC CAGCCTATATACCGCCGTCGCCAGCTTACCCTGTGAAGGACCTATAGTAAGCATAATTGGTACAACCCAGAACGT CAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATAACACATGAAATACGAACGATGTA TTGAAAAACACATCCGAAGGAGGATTTAGTAGTAAGTAGAAAAAAGAGTATTCTACTGAAACCGGCCCTGAAG CGCGT
Trout cod (*Maccullochella macquariensis*) Murray River, NSW	M.MAC_12S_QS1 GCTTAGCCCTAAACATTGATAATACACTACAATTATTATCCGCCCGGGTACTACGAGCATCAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCC CCTTGTTCTCCTAGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTTATAGTAAGCATAATTGGCATAA CCCAGAACGTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATGACACACGGAATAC GAACGATGTCCTGAAACACACATCTGAAGGAGGATTTAGTAGTAAGCAGAAAACAGAGAGTTCTGCTGAAACT GGCCCTGAAGCGCGT

Murray River, NSW	M.MAC_12S_QS2 GCTTAGCCCTAAACATTGATAATACACTACAATTATTATCCGCCCGGGTACTACGAGCATCAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCC CCTTGTTCTCCTAGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTTATAGTAAGCATAATTGGCATAA CCCAGAACGTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATGACACACGGAATAC GAACGATGTCCTGAAACACACATCTGAAGGAGGATTTAGTAGTAAGCAGAAAACAGAGAGTTCTGCTGAAACT GGCCCTGAAGCGCGT
Mary Ada Creek, NSW	M.MAC_12S_QS3 GCTTAGCCCTAAACATTGATAATACACTACAATTATTATCCGCCCGGGTACTACGAGCATCAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCC CCTTGTTCTCCTAGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTTATAGTAAGCATAATTGGCATAA CCCAGAACGTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATGACACACGGAATAC GAACGATGTCCTGAAACACACATCTGAAGGAGGATTTAGTAGTAAGCAGAAAACAGAGAGTTCTGCTGAAACT GGCCCTGAAGCGCGT
Mary Ada Creek, NSW	M.MAC_12S_QS4 GCTTAGCCCTAAACATTGATAATACACTACAATTATTATCCGCCCGGGTACTACGAGCATCAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCC CCTTGTTCTCCTAGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTTATAGTAAGCATAATTGGCATAA CCCAGAACGTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATGACACACGGAATAC GAACGATGTCCTGAAACACACATCTGAAGGAGGATTTAGTAGTAAGCAGAAAACAGAGAGTTCTGCTGAAACT GGCCCTGAAGCGCGT
Mary Ada Creek, NSW	M.MAC_12S_QS5 GCTTAGCCCTAAACATTGATAATACACTACAATTATTATCCGCCCGGGTACTACGAGCATCAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCCCCTAGAGGAGCCTGTCCTAGAACCGATAATCCCCGTTAAACCTCACCCCC CCTTGTTCTCCTAGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGACTTATAGTAAGCATAATTGGCATAA CCCAGAACGTCAGGTCGAGGTGTAGCTTATGGGAGGGAAAGAGATGGGCTACATTCCATGACACACGGAATAC GAACGATGTCCTGAAACACACATCTGAAGGAGGATTTAGTAGTAAGCAGAAAACAGAGAGTTCTGCTGAAACT GGCCCTGAAGCGCGT
Silver perch (*Bidyanus bidyanus*)	
Murray River, NSW	B.BID_12S_QS1 GCTTAGCCCTAAACATAAATAGTACAATACACCCACTATTCGCCCGGGGACTACGAGCATTAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCCTT CCTTGCTCTTTTCCGCCTATATACCGCCGTCGCCAGCTTACCCTATGAAGGACGCACAGTAAGCAAAATTGGCAC AGCCCAGAACGTCAGGTCGACGTGTAGCGTATGGAAGGGGAAGAAATGGGCTACATTCCCTAACACAGTGAAT ACGAAAGATGCACTGAAACGTGCAACATGAAGGAGGATTTAGCAGTAAGCGGGAAATAGAGTGTCCCGCTGA AACCGGCCCTGAAGCGCGC
Murray River, NSW	B.BID_12S_QS GCTTAGCCCTAAACATAAATAGTACAATACACCCACTATTCGCCCGGGGACTACGAGCATTAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCCTT CCTTGCTCTTTTCCGCCTATATACCGCCGTCGCCAGCTTACCCTATGAAGGACGCACAGTAAGCAAAATTGGCAC AGCCCAGAACGTCAGGTCGACGTGTAGCGTATGGAAGGGGAAGAAATGGGCTACATTCCCTAACACAGTGAAT ACGAAAGATGCACTGAAACGTGCAACATGAAGGAGGATTTAGCAGTAAGCGGGAAATAGAGTGTCCCGCTGA AACCGGCCCTGAAGCGCGC

Darling River, NSW	B.BID_12S_QS3 GCTTAGCCCTAAACATAAATAGTACAATACACCCACTATTCGCCCGGGGACTACGAGCATTAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCCTT CCTTGCTCTTTTCCGCCTATATACCGCCGTCGCCAGCTTACCCTATGAAGGACGCACAGTAAGCAAAATTGGCAC AGCCCAGAACGTCAGGTCGACGTGTAGCGTATGGAAGGGGAAGAAATGGGCTACATTCCCTAACACAGTGAAT ACGAAAGATGCACTGAAACGTGCAACATGAAGGAGGATTTAGCAGTAAGCGGGAAATAGAGTGTCCCGCTGA AACCGGCCCTGAAGCGCGC
Murray River, NSW	B.BID_12S_QS4 GCTTAGCCCTAAACATAAATAGTACAATACACCCACTATTCGCCCGGGGACTACGAGCATTAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCCTT CCTTGCTCTTTTCCGCCTATATACCGCCGTCGCCAGCTTACCCTATGAAGGACGCACAGTAAGCAAAATTGGCAC AGCCCAGAACGTCAGGTCGACGTGTAGCGTATGGAAGGGGAAGAAATGGGCTACATTCCCTAACACAGTGAAT ACGAAAGATGCACTGAAACGTGCAACATGAAGGAGGATTTAGCAGTAAGCGGGAAATAGAGTGTCCCGCTGA AACCGGCCCTGAAGCGCGC
Murray River, NSW	B.BID_12S_QS5 GCTTAGCCCTAAACATAAATAGTACAATACACCCACTATTCGCCCGGGGACTACGAGCATTAGCTTAAAACCCAA AGGACTTGGCGGTGCTTTAGATCCACCTAGAGGAGCCTGTTCTAGAACCGATAACCCCCGTTCAACCTCACCCTT CCTTGCTCTTTTCCGCCTATATACCGCCGTCGCCAGCTTACCCTATGAAGGACGCACAGTAAGCAAAATTGGCAC AGCCCAGAACGTCAGGTCGACGTGTAGCGTATGGAAGGGGAAGAAATGGGCTACATTCCCTAACACAGTGAAT ACGAAAGATGCACTGAAACGTGCAACATGAAGGAGGATTTAGCAGTAAGCGGGAAATAGAGTGTCCCGCTGA AACCGGCCCTGAAGCGCGC
Freshwater catfish (*Tandanus tandanus*)	
Lachlan River, NSW	T.TAN_12S_QS1 GCTTAGCCTTAAACCTAGGTGTATTTTTACACATGCACCCGCCCGGGCACTACGAGCACAGCTTAAAACCCAAAG GACTTGGCGGTGCCTCAGACCCACCTAGAGGAGCCTGTTCTAGAACCGATAATCCTCGTTAAACCTCACCACTTC TTGTTTTACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCCAACAGTAAGCAAAACTGGTCAGCC CAAAAACGTCAGGTCGAGGTGTAGCGTACGAAGTGGGAAGAAATGGGCTACATTTTCTAATAATAGACTATAA CGAATAGCACGCTGAAACACGTGCTTAAAGGTGGATTTAGCAGTAAAAAGTAAATAGAGAGTTCTTTTGAACCC GGCTCTGAAGCGCGC
Lachlan River, NSW	T.TAN_12S_QS2 GCTTAGCCTTAAACCTAGGTGTATTTTTACACATGCACCCGCCCGGGCACTACGAGCACAGCTTAAAACCCAAAG GACTTGGCGGTGCCTCAGACCCACCTAGAGGAGCCTGTTCTAGAACCGATAATCCTCGTTAAACCTCACCACTTC TTGTTTTACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCCAACAGTAAGCAAAACTGGTCAGCC CAAAAACGTCAGGTCGAGGTGTAGCGTACGAAGTGGGAAGAAATGGGCTACATTTTCTAATAATAGACTATAA CGAATAGCACGCTGAAACACGTGCTTAAAGGTGGATTTAGCAGTAAAAAGTAAATAGAGAGTTCTTTTGAACCC GGCTCTGAAGCGCGC
Lachlan River, NSW	T.TAN_12S_QS3 GCTTAGCCTTAAACCTAGGTGTATTTTTACACATGCACCCGCCCGGGCACTACGAGCACAGCTTAAAACCCAAAG GACTTGGCGGTGCCTCAGACCCACCTAGAGGAGCCTGTTCTAGAACCGATAATCCTCGTTAAACCTCACCACTTC TTGTTTTACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCCAACAGTAAGCAAAACTGGTCAGCC CAAAAACGTCAGGTCGAGGTGTAGCGTACGAAGTGGGAAGAAATGGGCTACATTTTCTAATAATAGACTATAA CGAATAGCACGCTGAAACACGTGCTTAAAGGTGGATTTAGCAGTAAAAAGTAAATAGAGAGTTCTTTTGAACCC GGCTCTGAAGCGCGC

Colombo Creek, NSW	T.TAN_12S_QS4 GCTTAGCCTTAAACCTAGGTGTATTTTTACACATGCACCCGCCCGGGCACTACGAGCACAGCTTAAAACCCAAAG GACTTGGCGGTGCCTCAGACCCACCTAGAGGAGCCTGTTCTAGAACCGATAATCCTCGTTAAACCTCACCACTTC TTGTTTTACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCCAACAGTAAGCAAAACTGGTCAGCC CAAAAACGTCAGGTCGAGGTGTAGCGTACGAAGTGGGAAGAAATGGGCTACATTTTCTAATAATAGACTATAA CGAATAGCACGCTGAAACACGTGCTTAAAGGTGGATTTAGCAGTAAAAAGTAAATAGAGAGTTCTTTTGAACCC GGCTCTGAAGCGCGC
Colombo Creek, NSW	T.TAN_12S_QS5 GCTTAGCCTTAAACCTAGGTGTATTTTTACACATGCACCCGCCCGGGCACTACGAGCACAGCTTAAAACCCAAAG GACTTGGCGGTGCCTCAGACCCACCTAGAGGAGCCTGTTCTAGAACCGATAATCCTCGTTAAACCTCACCACTTC TTGTTTTACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCCAACAGTAAGCAAAACTGGTCAGCC CAAAAACGTCAGGTCGAGGTGTAGCGTACGAAGTGGGAAGAAATGGGCTACATTTTCTAATAATAGACTATAA CGAATAGCACGCTGAAACACGTGCTTAAAGGTGGATTTAGCAGTAAAAAGTAAATAGAGAGTTCTTTTGAACCC GGCTCTGAAGCGCGC
Colombo Creek, NSW	T.TAN_12S_QS6 GCTTAGCCTTAAACCTAGGTGTATTTTTACACATGCACCCGCCCGGGCACTACGAGCACAGCTTAAAACCCAAAG GACTTGGCGGTGCCTCAAACCCACCTAGAGGAGCCTGTTCTAGAACCGATAATCCTCGTTAAACCTCACCACTTC TTGTTTTACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGTGAAGGCCCAACAGTAAGCAAAACTGGTCAGCC CAAAAACGTCAGGTCGAGGTGTAGCGTACGAAGTGGGAAGAAATGGGCTACATTTTCTAATAATAGACTATAA CGAATAGCACGCTGAAACACGTGCTTAAAGGTGGATTTAGCAGTAAAAAGTAAATAGAGAGTTCTTTTGAACCC GGCTCTGAAGCGCGC
Dwarf flathead gudgeon (*Phylipnodon marcrostomus*)	
Aratula Creek, NSW	P.MAC_12S_QS1 GCCTTGCCCTAAACAAAAGTAGCAAAGTATGTCTGCTACTCGCCCGGGAACTACGAGCAAAAGCTTAAAACCCA AAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTTCTGGAACCGATAACCCCCGTTCAACCTCACCCT CTCTTGTTCCACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGCAAAGGGCACACAGTAAGCATAATTGGCAT AGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGAGAGGGGAAGAAATGGGCTACATTCCCTGACACAGTGTAC ACGAACGATGTGATGAAATAAACATCTAAAGGAGGATTTAGCAGTAAGAAGGAAAGCAGAGCGTTCCTCTGAA AATGGCCCTGAAGCGCGC
Aratula Creek, NSW	P.MAC_12S_QS2 GCCTTGCCCTAAACAAAAGTAGCAAAGTATGTCTGCTACTCGCCCGGGAACTACGAGCAAAAGCTTAAAACCCA AAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTTCTGGAACCGATAACCCCCGTTCAACCTCACCCT CTCTTGTTCCACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGCAAAGGGCACACAGTAAGCATAATTGGCAT AGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGAGAGGGGAAGAAATGGGCTACATTCCCTGACACAGTGTAC ACGAACGATGTGATGAAATAAACATCTAAAGGAGGATTTAGCAGTAAGAAGGAAAGCAGAGCGTTCCTCTGAA AATGGCCCTGAAGCGCGC
Aratula Creek, NSW	P.MAC_12S_QS3 GCCTTGCCCTAAACAAAAGTAGCAAAGTATGTCTGCTACTCGCCCGGGAACTACGAGCAAAAGCTTAAAACCCA AAGGACTTGGCGGTGCTTTAGACCCACCTAGAGGAGCCTGTTCTGGAACCGATAACCCCCGTTCAACCTCACCCT CTCTTGTTCCACCCGCCTATATACCGCCGTCGTCAGCTTACCCTGCAAAGGGCACACAGTAAGCATAATTGGCAT AGCCCAAAACGTCAGGTCGAGGTGTAGCGTATGAGAGGGGAAGAAATGGGCTACATTCCCTGACACAGTGTAC ACGAACGATGTGATGAAATAAACATCTAAAGGAGGATTTAGCAGTAAGAAGGAAAGCAGAGCGTTCCTCTGAA AATGGCCCTGAAGCGCGC

Table 12.2 eDNA sampling sites and the volume of water filtered at each site.

Site number	Waterway	Sampling site (ID)	Sampling date	Total volume filtered (L)
1	Edward/Kolety River	Four Posts (FP)	27/11/2019	10.19
2	Edward/Kolety River	Eastman Bridge (EB)	27/11/2019	10.99
3	Edward/Kolety River	Balpool (BP)	26/11/2019	10.55
4	Edward/Kolety River	Kyalite State Forest (KY)	25/11/2019	9.08
5	Wakool River	Widgee1 (WG)	28/11/2019	9.39
6	Wakool River	Gee Gee (GG)	26/11/2019	7.26
7	Wakool River	Stoney Creek Crossing (SC)	25/11/2019	9.08
8	Colligen-Neimur River	Calimo (CL)	27/11/2019	7.85
9	Colligen-Neimur River	Mallan School (MS)	25/11/2019	11.83
10	Yallakool Creek	Windra Vale2 (WV)	28/11/2019	9.30

Appendix 12.3 The closely related species (at the family level) that co-occur with the target species that were included in the specificity test for each assay. In some cases, amplification was detected in the non-target species during PCR that was the result of contaminating DNA from the target species (PCR test result) that occurred at the time of fin-clip sampling of the non-target species. To confirm any non-target amplification, Sanger sequencing was conducted (Sanger sequencing test result). The final column details the final result of any non-target amplification success.

Target species	**Species tested**	**Was there amplification of the DNA from the species tested?**	**Did sequencing confirm that the DNA was a match to the species tested?**	**Final amplification result**
Murray cod (*Maccullochella peelii*)	*Maccullochella macquariensis*	Yes	No	**No**
	Macquaria ambigua	Yes	No	**No**
	Macquaria australasica	Yes	No	**No**
	Gadopsis marmoratus	Yes	No	**No**
	Gadopsis bispinosus	Yes	No	**No**
	Nannoperca australis	No	-	**No**
	Maccullochella ikei †	Yes	Yes	**Yes**
	Maccullochella mariensis †	Yes	Yes	**Yes**
Trout cod (*Maccullochella macquariensis*)	*Maccullochella peelii*	No	-	**No**
	Macquaria ambigua	Yes	No	**No**
	Macquaria australasica	Yes	No	**No**
	Gadopsis marmoratus	Yes	No	**No**
	Gadopsis bispinosus	Yes	No	**No**
	Nannoperca australis	No	-	**No**
	Maccullochella ikei	No	-	**No**
	Maccullochella mariensis	No	-	**No**
Silver perch (*Bidyanus bidyanus*)	*Leiopotherapon unicolor*	No	-	**No**
Freshwater catfish (*Tandanus tandanus*)	*Neosilurus hyrtlii*	No	-	**No**
	Tandanus bellingerensis†	Yes	Yes	**Yes**
	Tandanus bostocki†	Yes	Yes	**Yes**
Dwarf flathead gudgeon†(*Phylipnodon marcrostomus*)	*Philypnodon grandiceps*	Yes	No	**No**
	Hypseleotris klunzingeri	No	-	**No**
	Mogurnda adspersa	No	-	**No**
Murray cray (*Euastacus armatus*)	*Cherax destructor*	No	-	**No**

† *Maccullochella ikei* and *Maccullochella mariensis* both amplified using the *M. peelii* assay, however both species have geographically distinct distributions to *M. peelii*. *Tandanus bellingerensis* and *Tandanus bostocki* both amplified using the *T. tandanus* assay, however, both have geographically distinct distributions to *T.tandanus* and thus the *T. tandanus* assay is suitable for application in the Murray-Darling Basin. *P. macrostomus* is comprised of a number of distinct genetic lineages (Hammer et al., 2019). This assay has not been tested on individuals belonging to lineages outside of the MDB. Thus, this assay is only applicable for use in the MDB.

Appendix 12.4 Positive detections of Murray cod at the ten sampling locations. Red crosses are negative for the target species, green ticks are positive for the target species and yellow ticks are false positives (based on a further 12 qPCR technical replicates). No amplification was detected in equipment controls, negative extraction controls and negative PCR controls.

			Technical replicates					
Sample ID	Waterway	Pos reps	n1	n2	n3	n4	n5	n6
Four Posts 1	Edward/Kolety River	6/6	✓	✓	✓	✓	✓	✓
Four Posts 2	Edward/Kolety River	6/6	✓	✓	✓	✓	✓	✓
Four Posts 3	Edward/Kolety River	4/6	✓	✓	×	✓	✓	×
Four Posts 4	Edward/Kolety River	4/6	×	✓	×	✓	✓	✓
Four Posts 5	Edward/Kolety River	4/6	✓	✓	×	✓	×	✓
Four Posts 6	Edward/Kolety River	4/6	✓	✓	×	×	✓	✓
Four Posts 7	Edward/Kolety River	2/6	✓	✓	✓	✓	×	×
Four Posts 8	Edward/Kolety River	6/6	✓	✓	✓	✓	✓	✓
Eastman Bridge 1	Edward/Kolety River	1/6	✓	×	×	×	×	×
Eastman Bridge 2	Edward/Kolety River	6/6	✓	✓	✓	✓	✓	✓
Eastman Bridge 3	Edward/Kolety River	4/6	×	✓	✓	✓	✓	×
Eastman Bridge 4	Edward/Kolety River	4/6	✓	×	✓	×	✓	✓
Eastman Bridge 5	Edward/Kolety River	2/6	✓	×	✓	×	×	×
Eastman Bridge 6	Edward/Kolety River	6/6	✓	✓	✓	✓	✓	✓
Eastman Bridge 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 8	Edward/Kolety River	1/6	×	×	✓	✓	×	×
Balpool 1	Edward/Kolety River	6/6	✓	✓	✓	✓	✓	✓
Balpool 2	Edward/Kolety River	6/6	✓	✓	✓	✓	✓	✓
Balpool 3	Edward/Kolety River	5/6	×	✓	✓	✓	✓	✓
Balpool 4	Edward/Kolety River	5/6	✓	×	✓	✓	✓	✓
Balpool 5	Edward/Kolety River	5/6	✓	×	✓	✓	✓	✓
Balpool 6	Edward/Kolety River	6/6	✓	✓	✓	✓	✓	✓
Balpool 7	Edward/Kolety River	5/6	×	✓	✓	✓	✓	✓
Balpool 8	Edward/Kolety River	5/6	×	✓	✓	✓	✓	✓
Kyalite State Forest 1	Edward/Kolety River	2/6	×	×	×	×	✓	✓
Kyalite State Forest 2	Edward/Kolety River	1/6	×	×	✓	×	×	×
Kyalite State Forest 3	Edward/Kolety River	2/6	✓	×	×	×	✓	×
Kyalite State Forest 4	Edward/Kolety River	2/6	×	✓	✓	×	×	×
Kyalite State Forest 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 6	Edward/Kolety River	1/6	✓	×	×	×	×	×
Kyalite State Forest 7	Edward/Kolety River	1/6	×	✓	×	×	×	×
Kyalite State Forest 8	Edward/Kolety River	1/6	×	✓	×	×	×	×
Widgee 1	Wakool River	6/6	✓	✓	✓	✓	✓	✓
Widgee 2	Wakool River	6/6	✓	✓	✓	✓	✓	✓
Widgee 3	Wakool River	6/6	✓	✓	✓	✓	✓	✓
Widgee 4	Wakool River	4/6	✓	✓	×	×	✓	✓
Widgee 5	Wakool River	6/6	✓	✓	✓	✓	✓	✓
Widgee 6	Wakool River	6/6	✓	✓	✓	✓	✓	✓
Widgee 7	Wakool River	5/6	✓	✓	✓	×	✓	✓

Widgee 8	Wakool River	6/6	✓	✓	✓	✓	✓	✓
Gee Gee 1	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 2	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 3	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 4	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 5	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 6	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 7	Wakool River	0/6	×	×	×	×	×	✓
Gee Gee 8	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 1	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 2	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 3	Wakool River	0/6	×	×	×	×	✓	×
Stoney Creek Crossing 4	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 5	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 6	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 7	Wakool River	0/6	×	×	✓	×	×	×
Stoney Creek Crossing 8	Wakool River	0/6	×	×	×	×	×	×
Calimo 1	Colligen-Neimur River	1/6	×	×	✓	×	×	×
Calimo 2	Colligen-Neimur River	2/6	✓	×	×	×	×	✓
Calimo 3	Colligen-Neimur River	3/6	✓	✓	×	×	✓	×
Calimo 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 5	Colligen-Neimur River	3/6	×	✓	✓	✓	×	×
Calimo 6	Colligen-Neimur River	4/6	✓	✓	✓	×	✓	×
Calimo 7	Colligen-Neimur River	1/6	×	×	✓	×	×	×
Calimo 8	Colligen-Neimur River	1/6	×	×	×	×	×	✓
Mallan School 1	Colligen-Neimur River	2/6	✓	×	×	×	×	✓
Mallan School 2	Colligen-Neimur River	6/6	✓	✓	✓	✓	✓	✓
Mallan School 3	Colligen-Neimur River	3/6	×	×	✓	✓	✓	×
Mallan School 4	Colligen-Neimur River	3/6	×	✓	✓	×	×	✓
Mallan School 5	Colligen-Neimur River	1/6	✓	×	×	×	×	×
Mallan School 6	Colligen-Neimur River	3/6	✓	×	✓	✓	×	×
Mallan School 7	Colligen-Neimur River	5/6	✓	✓	✓	✓	✓	×
Mallan School 8	Colligen-Neimur River	3/6	✓	×	✓	×	✓	×
Windra Vale2 1	Yallakool Creek	6/6	✓	✓	✓	✓	✓	✓
Windra Vale2 2	Yallakool Creek	6/6	✓	✓	✓	✓	✓	✓
Windra Vale2 3	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 4	Yallakool Creek	6/6	✓	✓	✓	✓	✓	✓
Windra Vale2 5	Yallakool Creek	4/6	✓	✓	✓	✓	×	×
Windra Vale2 6	Yallakool Creek	4/6	✓	✓	×	×	✓	✓
Windra Vale2 7	Yallakool Creek	5/6	✓	✓	✓	✓	✓	×
Windra Vale2 8	Yallakool Creek	6/6	✓	✓	✓	✓	✓	✓

Appendix 12.5 Positive detections of trout cod at the ten sampling locations. Red crosses are negative for the target species, green ticks are positive for the target species and yellow ticks are false positives (based on a further 12 qPCR technical replicates). No amplification was detected in equipment controls, negative extraction controls and negative PCR controls.

			Technical replicates					
	Waterway	Pos reps	n1	n2	n3	n4	n5	n6
Four Posts 1	Edward/Kolety River	1/6	✓	×	×	×	×	×
Four Posts 2	Edward/Kolety River	1/6	×	✓	×	×	×	×
Four Posts 3	Edward/Kolety River	1/6	×	×	×	×	✓	×
Four Posts 4	Edward/Kolety River	3/6	×	✓	×	✓	×	✓
Four Posts 5	Edward/Kolety River	1/6	×	✓	×	×	×	×
Four Posts 6	Edward/Kolety River	5/6	✓	✓	×	✓	✓	✓
Four Posts 7	Edward/Kolety River	1/6	×	×	×	×	×	✓
Four Posts 8	Edward/Kolety River	1/6	✓	×	×	×	×	×
Eastman Bridge 1	Edward/Kolety River	2/6	✓	×	×	✓	×	×
Eastman Bridge 2	Edward/Kolety River	3/6	✓	✓	×	×	×	✓
Eastman Bridge 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 8	Edward/Kolety River	1/6	×	×	×	×	×	✓
Balpool 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 5	Edward/Kolety River	0/6	×	×	×	×	×	✓
Kyalite State Forest 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Widgee 1	Wakool River	0/6	×	×	×	×	×	×
Widgee 2	Wakool River	0/6	×	×	×	×	×	×
Widgee 3	Wakool River	0/6	×	×	×	×	×	×
Widgee 4	Wakool River	0/6	×	✓	×	×	×	×
Widgee 5	Wakool River	0/6	×	×	×	×	×	×
Widgee 6	Wakool River	0/6	×	×	×	×	×	×
Widgee 7	Wakool River	0/6	×	×	×	×	×	×

Widgee 8	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 1	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 2	Wakool River	0/6	×	✓	×	×	×	×
Gee Gee 3	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 4	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 5	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 6	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 7	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 8	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 1	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 2	Wakool River	2/6	×	✓	×	×	×	✓
Stoney Creek Crossing 3	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 4	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 5	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 6	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 7	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 8	Wakool River	0/6	×	×	×	×	×	×
Calimo 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 4	Colligen-Neimur River	0/6	✓	×	×	×	×	×
Calimo 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 6	Colligen-Neimur River	0/6	×	×	×	✓	×	×
Calimo 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 3	Colligen-Neimur River	0/6	×	×	×	×	✓	×
Mallan School 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Windra Vale2 1	Yallakool Creek	3/6	✓	×	✓	×	×	✓
Windra Vale2 2	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 3	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 4	Yallakool Creek	1/6	×	×	✓	×	×	×
Windra Vale2 5	Yallakool Creek	1/6	×	✓	×	×	×	×
Windra Vale2 6	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 7	Yallakool Creek	1/6	✓	×	×	×	×	×
Windra Vale2 8	Yallakool Creek	0/6	×	×	×	×	×	×

Appendix 12.6 Positive detections of silver perch at the ten sampling locations. Red crosses are negative for the target species, green ticks are positive for the target species and yellow ticks are false positives (based on a further 12 qPCR technical replicates). The boxed green tick (Eastman Bridge) was detected in two of the 12 additional qPCR technical replicates and thus is considered a true positive. No amplification was detected in equipment controls, negative extraction controls and negative PCR controls.

			Technical replicates					
	Waterway	Pos reps	n1	n2	n3	n4	n5	n6
Four Posts 1	Edward/Kolety River	1/6	×	×	×	×	×	✓
Four Posts 2	Edward/Kolety River	1/6	×	×	✓	×	×	×
Four Posts 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 5	Edward/Kolety River	3/6	✓	✓	×	✓	×	×
Four Posts 6	Edward/Kolety River	1/6	×	×	×	✓	×	×
Four Posts 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 3	Edward/Kolety River	1/6	×	✓	×	×	×	×
Eastman Bridge 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 8	Edward/Kolety River	1/6	×	☑	×	×	×	×
Balpool 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 3	Edward/Kolety River	1/6	×	×	×	×	×	✓
Kyalite State Forest 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Widgee 1	Wakool River	0/6	×	×	×	×	×	×
Widgee 2	Wakool River	0/6	×	×	×	×	×	×
Widgee 3	Wakool River	0/6	×	×	×	×	×	×
Widgee 4	Wakool River	1/6	×	✓	×	×	×	×
Widgee 5	Wakool River	0/6	×	×	×	×	×	×
Widgee 6	Wakool River	0/6	×	×	×	×	×	×
Widgee 7	Wakool River	0/6	×	×	×	×	×	×

Widgee 8	Wakool River	2/6	×	✓	×	✓	×	×
Gee Gee 1	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 2	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 3	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 4	Wakool River	1/6	×	×	×	×	×	✓
Gee Gee 5	Wakool River	2/6	×	×	✓	✓	×	×
Gee Gee 6	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 7	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 8	Wakool River	2/6	✓	✓	×	×	×	×
Stoney Creek Crossing 1	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 2	Wakool River	3/6	×	×	✓	✓	✓	×
Stoney Creek Crossing 3	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 4	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 5	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 6	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 7	Wakool River	1/6	×	✓	×	×	×	×
Stoney Creek Crossing 8	Wakool River	0/6	×	×	×	×	×	×
Calimo 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Windra Vale2 1	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 2	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 3	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 4	Yallakool Creek	1/6	×	×	✓	×	×	×
Windra Vale2 5	Yallakool Creek	1/6	×	×	×	×	✓	×
Windra Vale2 6	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 7	Yallakool Creek	1/6	✓	×	×	×	×	×
Windra Vale2 8	Yallakool Creek	0/6	×	×	×	×	×	×

Appendix 12.7 Positive detections of redfin perch at the ten sampling locations. NA, lack of available assay resulted in four samples not yet being analysed (NV8, CL8, FP8, WG7) and one sample only being analysed for three technical replicates (WG8). Red crosses are negative for the target species, green ticks are positive for the target species and yellow ticks are false positives (based on a further 12 qPCR technical replicates). No amplification was detected in equipment controls, negative extraction controls and negative PCR controls.

			Technical replicates					
	Waterway	Pos reps	n1	n2	n3	n4	n5	n6
Four Posts 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Widgee 1	Wakool River	0/6	×	×	×	×	×	×
Widgee 2	Wakool River	0/6	×	×	×	×	×	×
Widgee 3	Wakool River	0/6	×	×	×	×	×	×
Widgee 4	Wakool River	0/6	×	×	×	×	×	×
Widgee 5	Wakool River	0/6	×	×	×	×	×	×
Widgee 6	Wakool River	0/6	×	×	×	×	×	×
Widgee 7	Wakool River	0/6	×	×	×	×	×	×

Widgee 8	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 1	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 2	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 3	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 4	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 5	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 6	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 7	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 8	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 1	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 2	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 3	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 4	Wakool River	0/6	✓	✓	×	×	✓	×
Stoney Creek Crossing 5	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 6	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 7	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 8	Wakool River	0/6	×	×	×	×	×	×
Calimo 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Windra Vale2 1	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 2	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 3	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 4	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 5	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 6	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 7	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 8	Yallakool Creek	0/6	×	×	×	×	×	×

Appendix 12.8 Positive detections of Platypus at the ten sampling locations. Red crosses are negative for the target species, green ticks are positive for the target species and yellow ticks are false positives (based on a further 12 qPCR technical replicates). No amplification was detected in equipment controls, negative extraction controls and negative PCR controls.

			Technical replicates					
Sample ID	Waterway	Pos reps	n1	n2	n3	n4	n5	n6
Four Posts 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 4	Edward/Kolety River	0/6	×	×	×	×	×	✓
Four Posts 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Widgee 1	Wakool River	0/6	×	✓	×	×	×	×
Widgee 2	Wakool River	0/6	×	×	×	×	×	×
Widgee 3	Wakool River	0/6	×	×	×	×	×	×
Widgee 4	Wakool River	0/6	×	×	×	×	×	×
Widgee 5	Wakool River	0/6	×	×	×	×	×	×
Widgee 6	Wakool River	0/6	×	×	×	×	×	×
Widgee 7	Wakool River	0/6	×	×	×	×	×	×

Widgee 8	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 1	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 2	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 3	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 4	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 5	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 6	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 7	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 8	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 1	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 2	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 3	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 4	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 5	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 6	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 7	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 8	Wakool River	0/6	×	×	×	×	×	×
Calimo 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Windra Vale2 1	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 2	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 3	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 4	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 5	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 6	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 7	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 8	Yallakool Creek	0/6	×	×	×	×	×	×

Appendix 12.9 Positive detections of dwarf flathead gudgeon at the ten sampling locations. Red crosses are negative for the target species, green ticks are positive for the target species and yellow ticks are false positives (based on a further 12 qPCR technical replicates). No amplification was detected in equipment controls, negative extraction controls and negative PCR controls.

Sample ID	Waterway	Pos reps	n1	n2	n3	n4	n5	n6
Four Posts 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Widgee 1	Wakool River	0/6	×	×	×	×	×	×
Widgee 2	Wakool River	0/6	×	×	×	×	×	×
Widgee 3	Wakool River	0/6	×	×	×	×	×	×
Widgee 4	Wakool River	0/6	×	×	×	×	×	×
Widgee 5	Wakool River	0/6	×	×	×	×	×	×
Widgee 6	Wakool River	0/6	×	×	×	×	×	×
Widgee 7	Wakool River	0/6	×	×	×	×	×	×
Widgee 8	Wakool River	0/6	×	×	×	×	×	×

Gee Gee 1	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 2	Wakool River	1/6	×	×	×	✓	×	×
Gee Gee 3	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 4	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 5	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 6	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 7	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 8	Wakool River	1/6	✓	×	×	×	×	×
Stoney Creek Crossing 1	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 2	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 3	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 4	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 5	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 6	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 7	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 8	Wakool River	0/6	×	×	×	×	×	×
Calimo 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Windra Vale2 1	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 2	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 3	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 4	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 5	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 6	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 7	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 8	Yallakool Creek	0/6	×	×	×	×	×	×

Appendix 12.10 Positive detections of freshwater catfish at the ten sampling locations. Red crosses are negative for the target species, green ticks are positive for the target species and yellow ticks are false positives (based on a further 12 qPCR technical replicates). No amplification was detected in equipment controls, negative extraction controls and negative PCR controls.

			Technical replicates					
	Waterway	Pos reps	n1	n2	n3	n4	n5	n6
Four Posts 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 5	Edward/Kolety River	0/6	×	×	×	×	✓	×
Four Posts 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Widgee 1	Wakool River	0/6	×	×	×	×	×	×
Widgee 2	Wakool River	0/6	×	×	×	×	×	×
Widgee 3	Wakool River	0/6	×	×	×	×	×	×
Widgee 4	Wakool River	0/6	×	×	×	×	×	×
Widgee 5	Wakool River	0/6	×	×	×	×	×	×
Widgee 6	Wakool River	0/6	×	×	×	×	×	×
Widgee 7	Wakool River	0/6	×	×	×	×	×	×

Widgee 8	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 1	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 2	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 3	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 4	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 5	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 6	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 7	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 8	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 1	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 2	Wakool River	0/6	×	×	×	✓	×	×
Stoney Creek Crossing 3	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 4	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 5	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 6	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 7	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 8	Wakool River	0/6	×	×	×	×	×	×
Calimo 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Windra Vale2 1	Yallakool Creek	0/6	×	×	×	✓	×	×
Windra Vale2 2	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 3	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 4	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 5	Yallakool Creek	0/6	×	×	✓	×	×	×
Windra Vale2 6	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 7	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 8	Yallakool Creek	0/6	×	×	×	×	×	×

Appendix 12.11 Positive detections of Murray crayfish at the ten sampling locations. Red crosses are negative for the target species, green ticks are positive for the target species. No amplification was detected in equipment controls, negative extraction controls and negative PCR controls.

			Technical replicates					
	Waterway	Pos reps	n1	n2	n3	n4	n5	n6
Four Posts 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Four Posts 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Eastman Bridge 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Balpool 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 1	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 2	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 3	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 4	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 5	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 6	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 7	Edward/Kolety River	0/6	×	×	×	×	×	×
Kyalite State Forest 8	Edward/Kolety River	0/6	×	×	×	×	×	×
Widgee 1	Wakool River	0/6	×	×	×	×	×	×
Widgee 2	Wakool River	0/6	×	×	×	×	×	×
Widgee 3	Wakool River	0/6	×	×	×	×	×	×
Widgee 4	Wakool River	0/6	×	×	×	×	×	×
Widgee 5	Wakool River	0/6	×	×	×	×	×	×
Widgee 6	Wakool River	0/6	×	×	×	×	×	×
Widgee 7	Wakool River	0/6	×	×	×	×	×	×
Widgee 8	Wakool River	0/6	×	×	×	×	×	×

Gee Gee 1	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 2	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 3	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 4	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 5	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 6	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 7	Wakool River	0/6	×	×	×	×	×	×
Gee Gee 8	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 1	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 2	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 3	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 4	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 5	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 6	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 7	Wakool River	0/6	×	×	×	×	×	×
Stoney Creek Crossing 8	Wakool River	0/6	×	×	×	×	×	×
Calimo 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Calimo 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 1	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 2	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 3	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 4	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 5	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 6	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 7	Colligen-Neimur River	0/6	×	×	×	×	×	×
Mallan School 8	Colligen-Neimur River	0/6	×	×	×	×	×	×
Windra Vale2 1	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 2	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 3	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 4	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 5	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 6	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 7	Yallakool Creek	0/6	×	×	×	×	×	×
Windra Vale2 8	Yallakool Creek	0/6	×	×	×	×	×	×

13 RECOMMENDATIONS FOR FUTURE MANAGEMENT OF ENVIRONMENTAL WATER

Summary of recommendations from previous reports and progress made to date

A summary of recommendations from the Edward/Kolety-Wakool LTIM annual reports (Watts et al. 2015, 2016, 2017b, 2018, 2019b) and the extent to which they have been implemented to improve the planning and delivery of Commonwealth environmental water are summarised in Table 13.1.

Table 13.1 Summary of recommendations from Edward/Kolety-Wakool 2014-15, 2015-16, 2016-17 and 2017-18 and 2018-19 LTIM annual reports, showing year implemented and details of actions undertaken. EWEWRG = Edward/Kolety-Wakool Environmental Water Reference Group, EWSC=Edward/Kolety-Wakool Stakeholder Committee, EWOAG= Edward/Kolety-Wakool Operations Advisory Group. R = recommendation number

Recommendation	Year(s) recommended	Year(s) implemented	Details of actions undertaken to implement the recommendation
Small in-channel freshes (within normal river operating rules)			
1. Consider a trial to increase the delivery of environmental water to the upper Wakool River	2014-15 (R3) 2015-16 (R6) 2016-17 (R5)	2018-19	In most years a small volume of environmental water has been delivered to the upper Wakool River. However the regulator limits the delivery of larger volumes of environmental water to this zone. Water can be delivered to part of this zone from the Wakool escape. **2018-19:** Environmental water was delivered from the Wakool escape to add to the total discharge during the 800 ML/d flow trial. The use of the escape was not included in the original plan for this action, but delivery was adapted during the action when the required discharge for the trial could not be delivered from the Yallakool regulator. In 2020-21 the increased use of CEW in the upper Wakool was implemented and will be continued into 2021-22 to enable outcomes from changes in the flow regime to be monitored.
2. Consider the implementation of an environmental watering action in the Edward/Kolety River to target golden perch and silver perch spawning.	2014-15 (R8) 2015-16 (R4) 2016-17 (R4) 2017-18 (R3)	Not yet implemented	This recommendation has not yet been implemented
In-channel freshes (higher than current normal operating rules to connect additional in-channel habitats)			
3. In collaboration with stakeholders explore options to implement a short duration environmental flow trial in late winter/spring 2016 at a higher discharge than the current constraint of 600 ML/d at the Wakool-Yallakool confluence. This would facilitate a test of the hypothesis that larger in-channel environmental watering action will result in increased river productivity. Implement a second flow trial in-channel fresh in late winter or early spring that	2014-15 (R7) 2015-16 (R3) 2017-18 (R4) 2018-19 (R3)	2018-19	**2016-17:** CEWO and Wakool River Association facilitated discussions with stakeholders to trial flows above current operational constraints, up to ~ 800 ML/d at the Wakool/Yallakool confluence. **2017-18:** Discussions continued and flow trial proposal was planned to proceed in Autumn 2018. However, due to poor water quality in the system the Autumn flow trial was postponed until 2018-19. **2018-19:** A flow trial up to 800 ML/d was implemented in Spring 2020.

exceeds the current normal operating rules, to increase the lateral connection of in-channel habitats and increase river productivity. The earlier timing of flows would help to prime the system and thus increase the outcomes of subsequent watering actions delivered later in spring or early summer.			
4. Each year plan to deliver at least one flow event with higher than normal operating discharge to the upper Wakool River. This may include delivery of water through the Wakool offtake regulator or via the Wakool escape from Mulwala Canal.	2018-19 (R1)	2018-19	**2018-19** – higher flows were delivered to the upper Wakool River from the Wakool escape during the 800 ML/d flow trial
Flows that contribute to flow recession			
5. Increase the duration of the recession of environmental watering actions relative to the Yallakool Creek environmental watering actions in 2012-13 and 2013-14	2014-15 (R1) 2015-16 (R8)	2015-16 2016-17 2017-18	Environmental water has consistently been used to increase the duration of recession of small in-channel freshes in the Edward/Kolety-Wakool system
Winter flows			
6. Consider the delivery of continuous base environmental flows during autumn and winter to promote the temporal availability and continuity of instream habitat	2014-15 (R4) 2015-16 (R2) 2016-17 (R3)	Winter 2017	**2016-17:** CEWO held discussions with stakeholder groups and management agencies **2017:** A continuous winter flow was implemented in Yallakool Creek,-Mid & Lower Wakool River and the Colligen - Niemur system.
7. Implement a second trial of continuous base winter environmental flow (no winter cease to flow) in the tributaries of the Edward/Kolety-Wakool system to promote the temporal availability and continuity of instream habitat to benefit fish and other aquatic animals and assist the recovery of submerged aquatic plants in the system.	2017-18 (R2)	Winter 2019	**2018:** Winter watering was discussed during planning for 2018-19 but could not be delivered in winter 2018 due to maintenance of Stevens Weir. **2019:** Second inter flow trial was implemented in winter 2019 commencing on 16 May 2019. This action will continue into the 2019-20 water year and will be evaluated in the 2019-20 annual report. **2020:** Winter flows occurred during winter 2020 due to operational flows and unregulated flows (no environmental water). This action will continue into 2020-21 water year and will be evaluated in the 2020-21 annual report
Flow variability			
8. Avoid long periods of constant flows by introducing flow variability into environmental watering actions. Include variation in the timing of environmental watering actions among water years to promote the temporal availability and continuity of instream habitat to benefit fish and other aquatic animals and assist the recovery of submerged aquatic plants in the system.	2014-15 (R2) 2015-16 (R5) 2018-19 (R2)	2015-16 2016-17 2018-19	**2015-16** Flow variability was provided the river operator with an ‘operational range’. **2016-17 and 2017-18** this has been applied by including variability in the watering plan.
9. Implement environmental watering actions for freshes in spring and early	2017-18 (R1)		Watering actions were planned for spring 2018 that include multiple pulses in

summer (October to December) that include flow variability up to a magnitude of + 125 to 150 ML/d. Undertake trials to improve understanding of the magnitude of variability that provides beneficial ecosystem outcomes.			Yallakool Creek with discharge ranging from 430 to 550 ML/d, over a range of approximately 20 cm change in water level. However, this was not implemented because during spring 2018 CEWO actions were suspended due to lack of operational capacity to deliver environmental water in the system.
Timing of flows			
10. Explore options to implement in-channel pulses at any time of the year to connect additional in-channel habitats and increase river productivity.	2018-19 (R4)	Not yet implemented	The opportunity to action this recommendation has not yet arisen.
Flows to mitigate poor water quality events			
11. Continue to include a water use option in water planning that enables environmental water to be used to mitigate adverse water quality events	2014-15 (R5) 2015-16 (R7)	2014-15 2015-16 2016-17 2017-18 2018-19	Contingency flows have been made available to contribute to responses to hypoxic blackwater events or other poor water quality events should they occur. This allowance has been used on several occasions to deliver flows.
12. If there is an imminent hypoxic blackwater event during an unregulated flow and the quality of source water is suitable, water managers in partnership with local landholder and community representatives should take action to facilitate the earlier release of environmental water on the rising limb of the flood event to create local refuges prior to DO concentrations falling below 2 mgL^{-1}.	2016-17 (R1)	Not yet implemented	Over time the CEWO has implemented a greater variety of timing of flows, including series of flows from late winter/early spring through to autumn
Flows through forests and/or floodplains			
13. Trial a carefully managed environmental watering action through Koondrook-Perricoota Forest via Barbers Creek to improve the productivity of the mid and lower Wakool River system.	2017-18 (R5)	Not yet implemented via Barbers Creek	**2018-19:** An environmental flow (NSW e-water) through Koondrook-Perricoota Forest via Thule Creek is underway as art of the 2019 southern spring flow This recommendation can not be implemented with CEW until NSW water management planning allow outflows from Koondrook-Perricoota Forest
Flows during drought			
14. Explore and develop a range of options for the delivery of environmental water during times of drought to ensure connectivity of habitat and avoid damage to key environmental assets. Inform the community of the factors limiting water delivery in extreme drought.	2018-19 (R5)	Not yet implemented	The discussion around flows during drought is ongoing, but no specific plans yet developed
Other flow related recommendations			
15. Set watering action objectives that identify the temporal and spatial scale at which the response is expected and are realistic given the magnitude of watering actions proposed	2014-15 (R6)	ongoing	Water managers have improved objective setting in their water planning.
16. Undertake a comprehensive flows assessment for the tributaries of the Edward/Kolety-Wakool system to better	2014-15 (R9) 2015-16 (R1)	Partly undertaken	Some flow assessments have been undertaken by MDBA and NSW OEH but there are still limitations of models in

inform future decisions on environmental watering in this system.			parts of the Edward/Kolety-Wakool system. These assessments contribute management decisions and long-term water planning by OEH.
17. Collaborate with other management agencies and the community to maximise the benefits of Commonwealth environmental watering actions	2014-15 (R10)	ongoing	**2014-16:** Engagement through the Edward/Kolety-Wakool Stakeholder Group (chair Murray LLS). **2016 - ongoing**: EWEWRG established **2014 - ongoing**: Edward/Kolety-Wakool Operations Advisory Group
18. The installation of a DO logger on a gauge downstream of Yarrawonga and upstream of Barmah-Millewa Forest should be considered a priority. Consideration should also be given to installing DO loggers, both upstream and downstream of other forested areas that influence water quality in the Edward/Kolety-Wakool system	2016-18 (R2)	Not yet implemented	This action is not the responsibility of the CEWO. It is a matter for NSW agencies. The CEWO is continuing to be involved in discussions requesting better flow data for key sites.
19. Undertake in-channel habitat mapping for key reaches of the Edward/Kolety-Wakool system, which could then be combined with existing hydraulic modelling to facilitate learning about this system	2016-17 (R6)	Implemented in part by NSW DPI	This is not the responsibility of the CEWO and has been undertaken in part by NSW DPI Fisheries.
20. The CEWO and other relevant agencies undertake a review of the 2016 flood and subsequent hypoxic blackwater event in the Murray system and support further research into understanding these events	2016-17 (R7)	2017	A review of blackwater events was undertaken in 2017

Flow Recommendations from 2019-20 report

We continue to endorse the recommendations from previous LTIM reports as summarised in Table 13.1. In addition, we outline the following 15 recommendations to improve the planning and delivery of Commonwealth environmental water in the Edward/Kolety-Wakool system. These recommendations are underpinned by monitoring and evaluation results from the Edward/Kolety-Wakool system.

Recommendation for small in-channel freshes

Recommendation 1: Although small watering actions have provided a beneficial outcome for the riverine ecosystem productivity, it is highly probable that reconnecting backwaters and the floodplain to the river channel would result in much larger positive outcomes. It is recommended that, when possible, consideration be given to providing a more variable flow regime in the Edward/Kolety-Wakool system in future years.

Recommendation 2: Deliver a series of freshes to all rivers in all major tributaries of the Edward/Kolety-Wakool system to increase the wetted area of the bank. Late winter/early spring freshes that inundate slackwater areas, in-channel benches or low lying areas of riverbank within the channel will trigger emergence of river bank vegetation. Following the recession of flows, these damp banks provide ideal conditions for plants to establish and grow prior to the onset of hotter weather in summer that can quickly dry out the river banks.

Recommendation 3: In years with high water availability, consider a late spring/early summer pulse, immediately after Murray cod larvae have left the nest, to support food resources for Murray cod larvae while at the same time providing opportunities for spawning to occur in silver perch and golden perch.

Recommendation 4: Consider adaptive use of water to coincide with high Murray River flows to maximise attraction/immigration of upstream migrating juvenile golden perch and silver perch in late summer. The probability of silver perch moving into and then staying in other more upstream tributaries of the Murray River (Goulburn and Campaspe rivers) is elevated in March-May (Koster et al. 2020), so delivering attraction flows in the Edward/Kolety-Wakool river system at this time or before (e.g. January-March) may be optimal for this more downstream tributary.

CEWO Adaptive Management Response:
The CEWO agrees that late winter/early spring pulses are important for a range of outcomes, including vegetation, native fish and connectivity. When flows in the Murray River may focus on late spring/early summer pulses, the CEWO will examine the delivery of two pulses into the Edward/Kolety system – one in late winter/early spring and another synchronised with Murray River flows in late spring/early summer.

Recommendations for flows to mitigate poor water quality events

Recommendation 5: In watering years where risk of hypoxic blackwater events is probable, consider how CEW watering actions could be used to mitigate effects on fish populations. One option to explore could be use of flows to encourage movement out of high risk reaches.

Recommendations for winter flows

Recommendation 6: The median total contribution of Commonwealth environmental water to carbon production was higher during watering action 3 in spring/summer than watering action 1 in winter. These results reflect the higher overall rates of GPP during summer and the greater probability that there will be days with high rates of carbon production. However, delivery of environmental water had the greatest proportional effect during winter low-flow periods. Maintaining discharge and wetted area during low flow periods helps to maintain zooplankton and other invertebrates that feed on phytoplankton and periphyton, and in turn this increases food availability for fish and other higher order consumers during periods in which food availability might otherwise be low.

Recommendation 7: Prevent negative impacts of a-seasonal cease-to-flow events by delivering winter base flows to promote temporal availability and continuity of instream habitat for aquatic vegetation. This will have minimise damage from damage from frost and livestock if the system is shut down during the winter, and result in positive benefits for the survival and maintenance of aquatic and riverbank vegetation.

Recommendation 8: Prevent negative impacts of a-seasonal cease-to-flow events by delivering winter base flows to promote temporal availability and continuity of instream habitat for fish. Evidence from 2019-20 monitoring indicates this has positive benefits for the survival and local retention of juvenile fish.

CEWO Adaptive Management Response: The ability to prevent winter cease-to-flow conditions in the Yallakool-Wakool and Colligen-Niemur systems is not controlled by the CEWO. The opportunity to provide winter base flows is determined by the need to undertake annual maintenance on Stevens Weir. The CEWO will continue to work with WaterNSW to identify when the opportunities arise to deliver winter base flows in the Edward/Kolety River system.

Flow recommendations for the upper Wakool River

Recommendation 9: Undertake watering actions to improve the aquatic and riverbank vegetation outcomes in the Upper Wakool River. Deliver larger freshes with increased variability to enable riverbank vegetation to establish and be maintained.

Recommendation 10: Deliver elevated base flows to the Upper Wakool River from September-December to maximise nesting and spawning opportunities for Murray cod. Record catches of larvae have been recorded when this type of watering action is delivered. This type of flow delivery should be supported with subsequent winter base flows throughout the Selected Area to maximise retention and survival of YOY in the region.

CEWO Adaptive Management Response: The CEWO increased flows into the upper Wakool River system during summer and autumn 2021, primarily to improve water quality in this reach. The CEWO is interested to see if the monitoring also shows any change in vegetation and fish outcomes as a result of these increased flows.

CEWO Adaptive Management Response:

- A number of the recommendations above are linked to recommendations for aquatic and riverbank vegetation outcomes. The CEWO will seek to implement these recommendations via multi-objective watering actions, as it has done so in the past.

Flow recommendations for Edward/Kolety River downstream of Stevens Weir

Recommendation 11: We recommend that options for a high flow event downstream of Stevens Weir (>2700 ML/day) that inundates low lying part of Werai forest and is likely to return flows to either Colligen Creek or the Edward/Kolety River are explored.

CEWO Adaptive Management Response: Options for delivering environmental water to Werai Forest are being explored. There are issues around delivery and gauging of water that need to be resolved.

Recommendations for monitoring and research

Recommendation 12: We recommend that a campaign/intervention monitoring type of study be undertaken during a high flow event (>2700 ML/day) that inundates low lying part of Werai forest and is likely to return flows to either Colligen Creek or the Edward/Kolety River. The evaluation of primary productivity associated with the event would be enhanced by the installation of temporary gauges to collect data on the inflows to the forest. Analysis of Sentinel images would also quantify extent of inundation within Werai Forest.

Recommendation 13: Targeted eDNA methods are most suitable when the objective is to document the distribution of species inefficiently sampled by other methods. This research has shown that eDNA is an effective method to detect the presence of rare and threatened species in the Edward/Kolety-Wakool system. We recommend future work explores occupancy modelling to enable detection probabilities to be estimated.

Recommendation 14: Although there were no golden or silver perch eggs or larvae detected in the Edward/Kolety River in 2019-20, further monitoring over a longer period of time is warranted. The growing appreciation of large spatial scales at which these species operate highlights the need for continued monitoring of spawning and recruitment indicators across key main channel and off-channel environments in both the southern and northern Murray-Darling Basin. Ongoing monitoring and analysis of the pattern of flow delivery and water velocities across multiple years will be able to better inform a discussion about spawning of silver perch and golden perch in the Edward/Kolety River.

Recommendation for communication

Recommendation 15: Consider developing communication products and contribute to engagement programs in collaboration with other agencies (e.g. Local Land Services) to support projects that reduce risks to recovery and maintenance of aquatic and riverbank plants by carp, pigs and livestock. Disturbance of the riverbank caused by carp, pigs and livestock has a high potential to undo the positive outcomes of environmental watering actions.

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