Monitoring the effect of the Clayton Regulator on the biota of the Goolwa Weir Pool: 18-month Synthesis

A report prepared for the South Australian Department of Environment and Natural Resources

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Executive Summary

Acid sulfate soils, which are naturally-sulfidic sediments, are a by-product of microbial activity in many aquatic environments. Large amounts of acid-sulfate soils are present in the Lower Murray Lakes, but usually do not pose any ecological hazard if saturated with water. The recent declines in water levels in the Lower Lakes exposed much of this material to the air, leading to oxidation of the soils, production of sulfuric acid and release of soluble metal salts. A regulator was constructed in mid 2009 across the Goolwa Channel at Clayton as a part of a management strategy to maximise inundation of acidic soils in Currency Creek, Finniss River and the Goolwa Channel, thereby limiting the production and transport of acid in the region. Following the closure of the regulator, water was pumped into the Goolwa Channel from Lake Alexandrina to maintain water levels above 0 m AHD over the following summer.

The construction of the regulator, the pumping and subsequently higher water levels all had the potential to affect the biota of the Goolwa Channel and so monitoring was undertaken to measure this effect on phytoplankton, zooplankton, benthic macroinvertebrates, plants, birds, fish and southern bell frog assemblages. This report synthesises the findings across these taxonomic groups to identify ecosystem-wide changes and interactions among the groups. We also investigate the impact of the available physico-chemical conditions on each biotic group. This report covers monitoring undertaken in the 22 months after the closure of the regulator.

Time was the main factor found to explain the structure of the individual taxonomic assemblages, indicating that there were shifts in most assemblages over the course of the monitoring. It was significant in structuring five of the seven individual biotic assemblages investigated. The location of a site inside or outside the weir pool was not a significant factor in explaining the patterns observed for any of the individual biotic groups. It is possible that time was acting as a surrogate for the changes in flow regimes and water levels (i.e. the study spanned a substantial change from very dry conditions at the beginning to much wetter conditions at the close of the study), which were likely to have influenced the biota present. These broad-scale changes may have overwhelmed the smaller-scale effects as a result of the Clayton regulator.

Few ecosystem-wide patterns were detected during this study. There was a significant correlation between patterns observed in fish and zooplankton assemblages. This was particularly interesting, as the individual fish and zooplankton assemblages were found to be best correlated with different subsets of the available environmental data. This suggests that perhaps there may be a direct influence between the two groups, such as predation pressure of fish structuring zooplankton assemblages. Further targeted investigation could highlight important links across the two assemblages. There were marked differences among the remaining taxonomic groups, with few relationships detectable among groups, and different groups best-correlated with different subsets of the available environmental data.

We identified four ‘syndromes’ within the available environmental data. These represented suites of measured variables that appeared to respond in similar ways in space and time. Based on the variables best correlated with each of the syndromes, we have offered an interpretation for each. In decreasing order of importance, these interpretations include a syndrome related to marine water influence, one associated with alkalinity and high pH, and one related to ammonia and ion balance.

The fourth syndrome explained a significant segment of the variation in the environmental data set, but was not particularly well correlated with any of the variables measured, suggesting that there may be another factor that is not currently measured driving some patterns. The various syndromes were all found to be best-correlated with at least one of the taxonomic assemblages investigated, and thus all appear to be important in structuring the patterns observed. We recommend that a chemical expert review the groupings we found and confirm or modify the explanations for each syndrome that we have offered as preliminary interpretations.

After much of the monitoring had been undertaken, it became apparent that two sites that were originally expected to fall outside the weir pool (i.e. Upper Finniss River and Upper Currency Creek) were likely to be hydrologically affected by the weir pool. Investigation indicated that they were not acting as a part of the weir pool proper, but were unlikely to be independent of that system year-round. Thus, while we have treated them as being located outside the weir pool for these analyses, in line with the original project design, there may have been some effects on the findings. The small number of other sites located outside the weir pool means that these effects cannot be quantified.

Overall, there was a relatively low level of overlap in the location of sites and the timing of sampling across the different taxonomic groups, which significantly limited our ability to detect any ecosystem-scale patterns. Better coordination across different monitoring groups is needed for any future attempts at synthesis to be successful. We also recommend that data be collected before an intervention occur and that a larger number of control sites (i.e. here, outside the Goolwa Channel) be included, to ensure that the effects of monitoring can be untangled from background variation. However, there was significant improvement in the quality and consistency of data generated by each group during the course of the monitoring, suggesting that greater coordination may be developing across the various research groups involved. The creation of a standard template for the provision of data to DENR was a major improvement arising from this project.

Introduction

Acid sulfate soils are sediments that contain natural sulfide minerals, which are a by-product of microbial activity in coastal, estuarine and freshwater environments (DENR 2009). Acid sulfate soils do not pose an ecological hazard whilst saturated, but recent water-level declines as a consequence of drought and excessive water-extraction levels exposed acid sulfate soils in the Lower Lakes to atmospheric oxygen, allowing the highly-soluble oxidised sulphuric acid and other soluble metal salts (DENR 2009) to be released with a return of river flow and rainfall. A primary management strategy for acid sulfate soils is to minimise exposure of acid sulfate soils to atmospheric oxygen (DENR 2009), and thus a regulator was constructed in mid 2009 across the Goolwa Channel at Clayton as a part of the Goolwa Channel Water Level Management Project (GCWLMP). The regulator was designed to inundate the existing acid sulfate soils in Currency Creek, Finniss River and the Goolwa Channel west of Clayton, creating the Goolwa Weir Pool (hereafter GWP) to prevent the acidity that was produced by these soils entering the Lake Alexandrina water body and other aquatic environments.

Construction of the regulator was completed on August 12, 2009 and following closure of the regulator, pumping of water commenced on August 17, 2009 from Lake Alexandrina to increase the water level in the GWP to a maximum of +0.7 m AHD, such that water levels did not drop below 0.0m AHD during the following summer. Good tributary inflows in spring 2009 meant that less water was required (27.5GL estimated versus 26.9 GL actually pumped) from Lake Alexandrina than had been anticipated to achieve water-level targets. This pumping ceased on November 8, 2009 after water levels were maintained above a rolling average of +0.7 m AHD for 5 days. The regulator was breached on the 25th of September 2010, after larger-than-expected flows coming from the Murray River triggered regulatory decision points. Higher-than-average flows continued through to winter 2011.

The impact that the regulator and the subsequent re-filling would have on the biotic assemblages of the GWP was unknown and thus monitoring to understand these effects has occurred since the regulator was completed, including following the breaching of the regulator. In addition to understanding the effect on the surrounding biota as a result of the regulator, this study provides an excellent opportunity to obtain data about the effect of inundating lake sediments on biotic assemblages in the region, which can then inform management in the wider region where it is likely that lake levels will be more variable in the future. Targeted monitoring occurred for phytoplankton, zooplankton, benthic macroinvertebrate, plant, bird, fish and (in 2010-11 only) southern bell frog assemblages. Where possible, monitoring was linked to existing monitoring programs (e.g. bird monitoring for The Living Murray initiative).

The objectives of the monitoring program (Lester & Fairweather 2009) were to:

- gain a detailed understanding of how benthic macroinvertebrate assemblages in the GWP respond to the presence of the blocking bank, and the re-filling process; and
- gain an understanding of the relative responses of
    - likely faster responders, including:
        - macroinvertebrates;
        - zooplankton;
        - phytoplankton and

- southern bell frogs

Versus

- likely slow responders, including:
  - fishes;
  - birds; and
  - vegetation;

The design adopted for this monitoring sought to contrast sites located within the GWP with those located outside its influence (Lester & Fairweather 2009, Lester *et al.* 2010). The allocation of sites between those within and outside the GWP occurred before the arrival of water and subsequent changes in the water levels and the management actions (i.e. the Finniss regulator was never constructed) made this division contentious as the GWP may have influenced additional sites than originally expected (see Discussion). However, such a design is necessary to examine the overall effects of such management actions and not just focus upon the delivery of some water to overlie and stabilise acid sulfate soils.

Individual components of the ecosystem were monitored by specialists for each taxonomic group. Thus this report attempts to synthesise the findings across the different groups, based on the data collected during the twenty-one months following the closure of the regulator at Clayton (see Lester *et al.* 2010 and Hamilton *et al.* 2011 for syntheses after 6 and 12 months). Therefore, we provide a brief summary of the individual taxonomic findings, as they have been provided by the original authors, and then outline the methods used for, and results of, a synthetic analysis combining datasets across the different taxonomic groups. We conclude by discussing the findings and making recommendations for future cross-disciplinary ecological monitoring.

Summary of findings relating to individual taxonomic groups

Reports outlining the findings of the post-construction monitoring within the GWP and/or associated data were provided for:

- benthic macroinvertebrates (Dittmann *et al.* 2011);
- zooplankton (data collected by Shiel, no report provided);
- phytoplankton (data collected by the EPA, no report provided);
- southern bell frog (Mason 2011);
- fish (Bice & Zampatti 2011);
- birds (Paton 2011; no data provided); and
- vegetation (Gehrig & Nicol 2011).

These reports are summarised below to provide context for the subsequent analyses. Data were sourced for water level, salinity, pH and numerous other water quality variables at corresponding sites from the website EPA (2011).

Monitoring of macroinvertebrate assemblages (Dittmann *et al.* 2011)

Benthic macroinvertebrate community structure, environmental condition and habitat quality were monitored throughout the Goolwa Channel to determine whether trends in macroinvertebrate communities could be identified after recent significant freshwater inflows (Dittmann *et al.* 2011).

Six sites throughout the Goolwa Channel were sampled in December 2010, April and February 2011, where 10 core and 10 Ekman grab samples were taken from intertidal habitats. Grain size, organic matter, and chlorophyll-*a* of the sediments were also sampled.

PRIMER software was used to run permutation-based analyses of variance (PERMANOVA), principal coordinate (PCO) and analysis of similarities (ANOSIM) analyses on the resultant data.

Nineteen species were recorded throughout the entire Goolwa Channel, with insect larvae providing the greatest taxonomic contribution (14 species).

Diversity indices calculated (including Shannon-Wiener diversity, Pielou's index for equitability and total species number) revealed low diversity overall (i.e. few dominant species). The authors also tested macroinvertebrate abundance (as well as the abundance of several major phyla) using PERMANOVA, but included 'site' as the only factor (not using the 'inside' versus 'outside' comparisons used in this synthesis). They found that the abundance of each major phylum was significantly different across study sites. The authors went on to mention that there was no significant difference in macroinvertebrate assemblages across the six sites or between waterbodies (tested with ANOSIM), but it is not clear whether waterbody was included as a separate factor.

The authors concluded by discussing the continuing shift away from an estuarine benthic community to a more freshwater macroinvertebrate fauna, as discovered in previous sampling efforts, with only sites closer to the Murray Mouth inhabited by typical estuarine species.

Monitoring of zooplankton assemblages

No report has been provided to date. Raw data was provided by Russell Shiel from the University of Adelaide, and these were used in subsequent analyses.

Monitoring of phytoplankton assemblages and water quality (Aldridge & Brookes 2011)

No report has been provided to date. Raw data was provided by the EPA through Alec Rolston, and these were used in subsequent analyses.

Monitoring of the southern bell frog population (Mason 2011)

The southern bell frog (also known as the growling grass frog, *Litoria raniformis*) is a large ground-dwelling frog, once common throughout the south-east of Australia. It is listed as vulnerable in both South Australia and at a national level, with the current study addressing key populations in Lakes Alexandrina and Albert and the lower reaches of the tributaries, including Finniss River, Currency and Tookayerta Creeks (Mason 2011).

Recent historical drought conditions have contributed to receding water levels, and drying of lake beds and fringing wetlands, causing the loss of aquatic and riparian habitat. The southern bell frog requires permanent water with dense vegetative structure for successful breeding and recruitment. So with recent freshwater inflows from the Murray Darling Basin having inundated wetlands, a rapid response in local frog communities (including the southern bell frog) has provided an opportunity for breeding (although no data from prior to the recent flows were included in the current report).

The objective of the study was to conduct targeted broad-scale surveys to identify the location and habitat conditions of extant and key breeding populations of the southern bell frog, *L. raniformis*.

Twenty-six sites were chosen based upon occupation during a 2009 and 2010 inventory, historic records, or the presence of suitable habitat associations. Nocturnal surveys, which included call recording, recognition and response, and active searching were undertaken from early nightfall (8pm – 12am) during October, November and December 2010. Relative abundance measures were estimated based on a scoring system. Tadpole surveys were also undertaken during December 2010 and February 2011, timed to occur one and three months after the peak in calling recorded during nocturnal studies. Two fyke nets and five baited traps were set overnight at each survey location across a 50-m fringe of wetland vegetation. Frogs, as well as fish and crustaceans, were recorded. Water quality monitoring was also undertaken at each survey location, recording salinity, pH, turbidity and temperature.

Peak male calling was October (at Pelican Lagoon) and December 2010 (at the remaining occupied sites), where manual searching discovered the majority of frogs utilizing partially-submerged and emergent vegetation. With no formal statistics included, the author suggested that peak calling could be explained by prevailing weather conditions, but concluded that water levels and inundation were a more likely explanation. Calling could have begun earlier than October 2010, and this was acknowledged in the report. Atmospheric conditions, including temperature and humidity, did not demonstrate clear trends in the detection of the southern bell frog; however, the author later stated that higher abundances were detected during sampling events with low temperatures, with more moderate abundances detected during higher temperatures. This hypothesis was not tested using any formal statistical tests.

The south-west area of the study region, including the Finniss River, Goolwa Channel, and Hindmarsh Island, contained a greater number of occupied sites, with the greatest abundance of calling males recorded at the northern point of Lake Alexandrina (Pelican Lagoon). The abundance of calling males remained low at all other locations.

Out of the seventeen sites sampled for recruitment, tadpoles were only recorded at Pelican Lagoon, where a total of fourteen were caught. Size and diversity of habitat was touted as a reason for greater catchability. Occupied sites appeared to be also distinguished by the presence of lignum (*Muehlenbeckia florulenta*) shrubland, where calling males seemed to prefer semi-open water bodies with a physical structure to call from and high densities of organic matter.

The author concluded that small changes in water levels played a vital role in the provision of suitable breeding habitats, invoking a response in the southern bell frog. Seasonal shifts in water level would allow plants to colonize exposed sediments, in turn providing physical structure for the southern bell frog when water levels rise.

No formal statistical analyses have been undertaken to confirm any observations, with the above observations and associated conclusions based on raw data alone.

Monitoring of fish assemblages(Bice & Zampatti 2011)

During the drought, insufficient inflow through the Murray Darling Basin failed to regulate water levels, leading to a loss of off-channel wetland habitats, submerged vegetation and the disconnection of fringing emergent vegetation. However, a significant increase in flow through the Murray Darling Basin during 2010 resulted in an increase in water levels throughout Lake Alexandrina, resulting in the return of hydraulic connectivity between the Lower Lakes, Coorong, and Southern Ocean.

Bice & Zampatti (2011) aimed to determine the response of fish to the GCWLMP in 2010/11 by comparing fish assemblages to those of previous years. Seven sites were used in two sampling events, December 2010 and March/April 2011. Of the seven sites, four were inside the GWP and three were on the eastern side of the Clayton Regulator.

Sites were sampled with single-winged fyke and multi-panel gill nets set perpendicular to the shore overnight. Fish were identified, counted, and length measurements taken for up to 50 individuals per species per gear type. Fish condition (including the presence of parasites, lesions, disease etc.) was assessed for each fish measured. The otoliths of 25 common galaxias (*Galaxias maculatus*) and Australian smelt (*Retropinna semoni*) were collected from within and outside the GWP in December for ageing.

No formal statistical analyses were performed on the 15,109 fish that were caught during the study, of which seven species (including redfin perch [*Perca fluviatilis*], bony herring [*Nematalosaerebi*], flat-headed gudgeon [*Philypnodon grandiceps*], small-mouthed hardyhead [*Artherinosoma microstoma*], Australian smelt [*Retropinna semoni*], and common carp [*Cyprinus carpio*]) contributed over 90%.

Authors concluded that the higher abundance of redfin perch (*P. fluviatilis*) during December 2010 was due to young-of-year recruitment, whilst common carp (*C. carpio*) remained abundant but

moderately less so than previous years. Partial re-connection of the Coorong and Goolwa Channel/Lake Alexandrina was cited as a reason for the increase in abundance of Congolli (*Pseudaphritis urvillii*), following the increasing freshwater flow.

Monitoring of bird assemblages (Paton 2011)

Significant rainfall returned large flows to the Murray-Darling Basin, flooding many key inland wetlands (e.g. Macquarie Marshes). Waterbirds using wetlands are likely to respond to flows such as these by dispersing into the highly-productive but short-lived ecosystems nearby, or adjusting their distribution, abundance and behavior to local changes in habitat or food availability. The study by Paton (2011) focused on changes in the abundance of birds within the Coorong relative to previous years, bird distributions near the barrages during different flow regimes, and bird performance (e.g. foraging effort and success rates) at different locations over time.

The author used a census, completed annually since January 2000 that systematically counted bird species (along with their associated activity) along a 113-km stretch from the southernmost point of the South Lagoon to the Goolwa Barrage.

The author based his observations in waterbird abundances (summarised below) on comparisons between the average number of birds observed between three time periods, 2000 to 2007 (eight years of declining flows), 2008 to 2010 (three years of drought), and 2011 (returning flows). This method is an informal assessment of the effect of different flows, but was not adjusted for inter-annual variability or differing numbers of years in the three time periods.

The author stated that, of fifteen predominantly fish-eating bird species, four, including the hoary-headed (*Poliocephalus poliocephalus*) and great-crested (*Podiceps cristatus*) grebes, little pied cormorant (*Phalacrocorax melanoleucos*) and the whiskered tern (*Chlidonias hybridus*), were notably absent in January 2011. The great egret (*Ardea alba)* and little egret (*Egretta garzetta)*continued to decline over each subsequent time period, whilst the common greenshank (*Tringa nebularia*) and fairy tern (*Sterna nereis*)were substantially less abundant in later time periods when compared to previous years. Australian pelican (*Pelecanus conspicillatus*) and crested tern (*Sterna bergii*) populations were similar to previous years, possibly driven by their habit of feeding exclusively outside the Coorong, whilst the white-faced heron (*Egretta novaehollandiae*) maintained its population due to a history of foraging in adjacent habitats, according to the author. The author believed there was no overall increase in any predominantly fish-eating species in the Coorong associated with returning flows.

Of the largely non-piscivorous species, only three (pied oystercatcher *Haematopus longirostris*, Australian shelduck*Tadorna tadornoides* and chestnut teal *Anas castanea*) maintained the same average population number in January 2011 compared to previous years. Species that largely vacated the Coorong (or at least were in very low abundances compared to previous years) during January 2011 included the grey teal (*Anas gracilis*), black swan (*Cygnus atratus*), musk duck (*Biziura lobata*), red-necked stint (*Calidris ruficollis*), sharp-tailed sandpiper (*Calidris acuminate*), curlew sandpiper (*Calidris ferruginea*), black-winged stilt (*Himantopus himantopus*), red-necked avocet (*Recurvirostra novaehollandiae*) and the red-capped plover (*Charadrius ruficapillus*). Only two species were dominant in the Coorong during January 2011, the banded stilt (*Cladorhynchus leucocephalus*) and silver gull (*Larus novaehollandiae*).

The author reported anecdotal evidence that extremely-high water levels in the Coorong during January 2011 covered much of the surrounding mudflats, samphire (*Sarcocornia* spp.) habitat, and terrestrial vegetation, potentially preventing most species from accessing foraging areas, whilst increased turbidity of the incoming freshwater may have disrupted visual foraging. Data on these abiotic (including salinity, water levels and turbidity) and biotic (including the distribution and abundance of available food) variables were not collected in this study, so were unable to be analysed to corroborate the findings (or not).The author also suggested another possible explanation, that some species may have dispersed to inland or distant wetlands.

The author concluded by stating that the bird assemblage response to freshwater releases is likely ongoing, and not limited to the first initial release.

Data from this study were not provided, and so have not been included in the analyses presented in this report.

Monitoring of vegetation assemblages (Gehrig & Nicol 2011)

Below average River Murray flows, as a result of abstraction and river regulation, resulted in reduced freshwater inflow into the Lower Lakes and Coorong. To mitigate acid sulfate soils caused by decreasing water levels, two regulators were constructed in August 2009. The effect that this had on the diverse emergent and submergent fringing wetland vegetation communities in the Goolwa Channel and throughout Lake Alexandrina was studied by Gehrig & Nicol (2011).

Ten sites were sampled during October (2009), March (2010) and November (2010) within the GWP, whilst six sites were sampled upstream in Lake Alexandrina (during the same periods). At each site, a single transect (running perpendicular to the shoreline) was used. Three 1 x 3-m quadrats, separated by 1 m were established at each of six elevation intervals (+0.8, +0.6, +0.4, +0.2, 0 and -0.5 m AHD). Cover and abundance of each species present was estimated visually. The authors did not use any statistical analyses to test patterns and observations.

Increased water levels in the region (including both Goolwa Channel and Lake Alexandrina) resulted in distinctive plant communities forming between -0.5m and +0.8m AHD in both waterbodies. Zonation of the plant community between water levels was apparent in the Goolwa Channel, with high elevations dominated by emergent and amphibious species (including *Phragmites australis*, *Muehlenbeckia florulenta*, *Typha domingensis* and *Calystegia sepium*), intermediate elevations dominated by emergent species adapted to deeper water (*Typha domingensis* and *Schoenoplectus validus*) and only submergent species (*Potamogeton pectinatus* and *Myriophyllum salsugineum*) only found at low elevations. Numerous submerged species (*Vallisneria australis*, *Ruppia megacarpa*, *Ceratophyllum demersum*, and *Ruppia polycarpa*) were observed, but not recorded during sampling. This was in contrast to Lake Alexandrina, which was dominated by terrestrial species at all elevations during spring 2009 and autumn 2010, with adjacent land use likely contributing to the dominance of exotic species.

Although plant assemblages showed little change in response to the partial removal of the Clayton regulator and a return to historical water levels, submergent species in the Goolwa Channel, Finniss River and Currency Creek colonized ground in response to the regulated inundation, indicating the importance of a seedbank in the recovery of plant assemblages after drought conditions.

Methods for the synthetic analysis

Detailed methods are presented here for the analyses undertaken to synthesise the individual (taxonomic) data sets. Please refer to the individual reports for each taxonomic group for collection and processing methods (Bice & Zampatti 2011; Dittmann et al. 2011; Gehrig & Nicol 2011;Mason 2011). Analyses investigating patterns of response for each individual taxonomic group, where included, can also be found in the reports cited. Datasets that were available for inclusion in this synthesis are outlined in Table 1.

The survey design for monitoring the GWP to determine the effect of the Clayton Regulator originally included a suite of sites that were intended to be monitored by each group for different taxa, at roughly the same timeframes (although some taxa were to be sampled more frequently than others due to a faster likely response, see Lester & Fairweather 2009). However, subsequent changes by different researchers to both the exact sites sampled and the times at which they were sampled have meant that only limited synthesis is now possible (Table 1). A map (including regulator position) is shown below (Figure 1), and includes sites (blue triangles inside the GWP, red circles outside the GWP) that are indicative of the region sampled (several sites may have been consolidated where appropriate), but do not show exact sampling locations for each taxonomic group. More detail on exact sampling locations can be found in the respective reports (Bice &Zampatti2011; Dittmann *et al.* 2011; Gehrig &Nicol 2011; Mason 2011; Paton 2011).

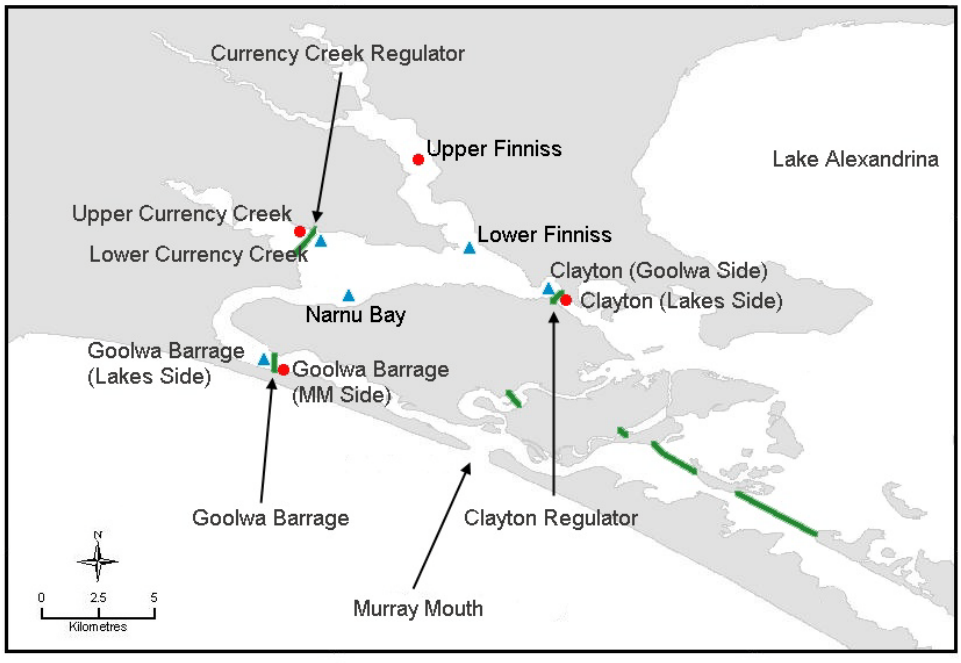

Figure 1: A map of the study region. Blue triangles represent sites designated 'Inside' the GWP and red dots indicate sites designated 'Outside' the GWP. Green lines indicate the various barrages and regulators. Sites are indicative of the original monitoring design only, and do not represent exact sampling locations for each taxonomic group over time.

There was some question as to whether the Upper Currency Creek and Upper Finniss River sites should be classified as 'Inside' or 'Outside' the GWP. In the initial study design, the two sites were expected to fall outside the influence of the weir pool itself, so had been identified *a priori* as being 'Outside' (Lester and Fairweather 2009). Anecdotal evidence suggested that these sites may have been affected to some extent by the weir pool, and so water levels from the closest gauging stations were graphed to inform a *post hoc* assessment of that allocation (Figure 2). The gauging stations did not coincide precisely with the study sites surveyed here, and the Finniss River site (Figure 2) was much further downstream than the Upper Finniss sampling station. The Currency Creek gauging station was closer to the sampling site. Both the gauging stations in the tributaries show continued declines in water level (in April through to July, for example) when compared with the stabilised water levels that are unequivocally within the weir pool (i.e. Hindmarsh Island and Clayton; Figure 2). This difference in water levels between both Currency Creek and Finniss River and the rest of the weir pool suggests that neither site is a part of the same water body (Figure 2), particularly at times of low flow. This is not to say that there acid sulfate soils were not covered in the tributaries, with higher than average flows inundating soils at high risk of acidification. However, this amelioration was unrelated to the effect of the GWP and would have occurred in the absence of the regulator, so cannot be considered to be as a result of this management action. Thus, we have retained the *a priori* classification of the Upper Finniss and Upper Currency sites as being 'Outside' the GWP. The implications of this decision are presented in the Discussion below.

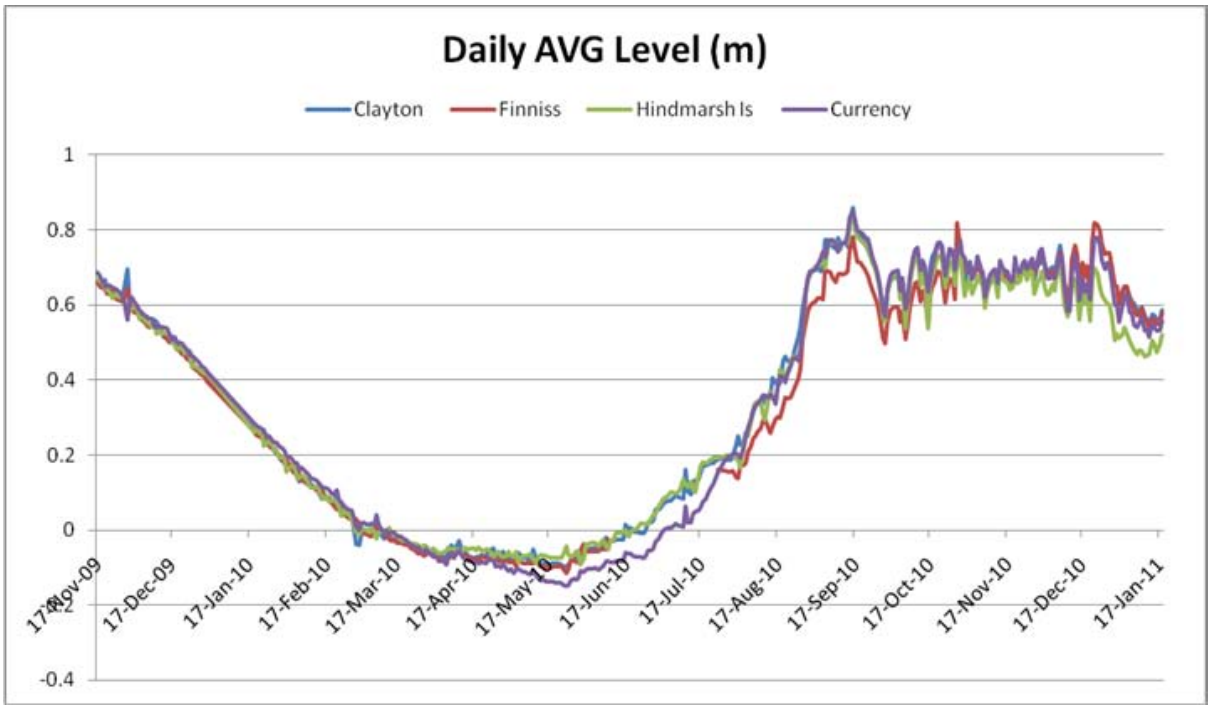

Figure 2: Average daily water levels for Clayton (Blue), Finniss River (Red), Hindmarsh Island (Green), and Currency Creek (Purple), from the Department of Water (2010).

Table 1: Summary of monitoring sites and times per data set supplied. Not all sites were sampled at every time in all data sets, hence, E = Early (First 6 months), M = Middle (7-12 months), L = Late (13-18 months) and R = Recent (19+ months), representing time classes after the closure of the Clayton Regulator. Aliasing of locations and times was necessary to maximise the amount of data able to be included in further analyses. The 'Water body' column indicates whether sites were designated as inside or outside the Goolwa Weir Pool.

Sampling site used in analyses	**Waterbody**	**Macroinvertebrates**	**Zooplankton**	**Phytoplankton**	**Southern Bell Frog**	**Fish**	**Birds**	**Vegetation**	**Wa Qua**
Clayton (Lakes side)	Outside	E M L R	M L		L	E M	L R	E M	E
Clayton (Goolwa side)	Inside	E M L R	M L R	E M	L			E M	E
Lower Finniss	Inside	E M L R	E	E M L		E M		E M	E
Upper Finniss	Outside	E M L	E M L R		L			E M	E
Narnu Bay	Inside	E M L	E M L R						L
Lower Currency	Inside	E M L R	E M L	M L	L	E M		E M	E
Upper Currency	Outside	E M L	E M L R		L		L R	E M	E
Goolwa Barrage (Lakes side)	Inside	E M L R	E M L R	M L		E M	L R	E M	E
Goolwa Barrage (MM side)	Outside	E M L R	E M L				M L R		E
Data source:		Dittmann *et al.* (2011)	***	Aldridge & Brookes (2011)	Mason (2011)	Bice &Zampatti (2011)	*	Gehrig &Nicol (2011)	**

* A report was provided for the bird assemblages (see Paton 2011) but raw data was unavailable. Data used in subsequent analyses was provided by David Dadd.

** Raw data only. Monthly reports provided by the EPA for monthly water quality data were not summarised (for further detail see http://www.epa.sa.gov.au/environmental_info/water_quality/lower_lakes_water_quality_monitoring).

*** Raw data only (collected by R. Shiel), no report provided.

All data was supplied by the Department of Environment and Natural Resources.

The continued lack of alignment of sampling sites and times across the taxonomic groups severely limited the syntheses that were possible. Macroinvertebrates, fish and vegetation were analysed together, maximising the number of sites (including Goolwa Barrage [Lakes side], Clayton [both Lakes & Goolwa sides] and Lower Currency Creek) and times (First 6 months, 7-12 months and 19+ months) which could be used. In order to achieve the synthesis, some aliasing of data collected at slightly-different times and locations was necessary (which may include the loss of some level of variability at individual location or sampling time levels).

For each individual taxonomic group, the effect of waterbody ('inside' versus 'outside') and time (first six months, 7-12 months, 13-18 months, and 19+ months) were assessed using PERMANOVA (Anderson *et al.* 2008). This format was used to reflect the study design, so removing factors such as site or time could lead to potentially misleading results, by attributing variation among samples to the wrong factor. Either time or site, or both, have significant levels of variability associated with them in different analyses (see detailed results below), and so removing either term could attribute smaller-scale variability (e.g. purely site-to-site variability or temporal change) to the effect of the weir pool, or impute larger-scale variability (e.g. differences based on the effect of the weir pool) when it is in fact due solely to smaller-scale variability.

Graphical representations of these differences were constructed using non-metric multidimensional scaling ordination (MDS plots). MDS plots were based on Bray-Curtis similarity matrices of standardised, square root or log(x+1)-transformed abundance data using 25 restarts.

BEST analyses (Clarke & Gorley 2006) were undertaken comparing each individual taxonomic group with the available environmental data. Environmental values were obtained from the EPA (2011) and subsequently averaged across each of the four time periods. BEST analysis identifies which environmental variable (or combination of variables) is most highly correlated with the patterns observed in the biotic data. Principal component analysis (PCA) was used as data-reduction technique (Norman & Streiner 1998) to reduce the 20 environmental water quality variables to fewer principal components. Based on their correlation structure, SYSTAT sorts highly-correlated raw variables into fewer but uncorrelated principal components (with VARIMAX rotation to maximise loadings and retaining only eigenvalues > 1). This allows the interpretation of each principal component by their constituent variables (Norman & Streiner 1998; SYSTAT 2004). Thus four principal components were retained and the scores from those 'syndromes' were used as independent variables in the BEST analysis described above.

The RELATE routine in the PRIMER software package was also used to measure the relatedness of any two matching sets of multivariate biological data (Clarke & Gorley 2006).

PERMANOVA, MDS, RELATE and BEST analyses were undertaken using PRIMER v.6.0 with the PERMANOVA+ add-on (Clarke and Gorley 2006; Anderson *et al.* 2008). PCA analysis was undertaken using SYSTAT v.13 (SYSTAT 2004).

Results

Any routine synthesis of information across different combinations of taxonomic groups was hindered by a lack of temporal and spatial overlap among data sets. Data have therefore been aliased across space (i.e. nearby sites combined) and time (i.e. similar times combined) to maximise the data available to test differences 'inside' versus 'outside' the weir pool. One synthesis was achievable, as macroinvertebrate, fish and vegetation assemblages had sufficient sampling that overlapped in time and space, as the original experimental design intended. This allowed meaningful analyses across these multiple taxa.

We therefore present here findings from synthetic analyses that maximise the potential power of the test, that span taxonomic groups where applicable, and relate environmental data to biotic data.

Assessing the effect of water body and time on individual biotic assemblages

When macroinvertebrate assemblages were investigated in isolation (Figure 3), waterbody (i.e. inside vs. outside the weir pool) was not a significant factor (pseudo-F = 1.24, P = 0.23). Time and site nested within waterbody were significant factors, however (pseudo-F = 3.37, P = 0.001 and pseudo-F = 2.38, P = 0.0001, respectively). This illustrated that macroinvertebrate assemblages were changing with time and exhibited small-scale site-to-site variability, but that this was not related to whether they were inside or outside of the Goolwa Weir Pool. These factors (i.e. time and site nested within waterbody) explained an order-of-magnitude more variability in macroinvertebrate assemblages than waterbody itself. This was apparent from the MDS plot, whereby points did not tend to be grouped by waterbody (Figure 3),although some grouping by time and site nested within waterbody was apparent (not shown). There was no significant difference in the dispersion of samples within waterbodies (F = 0.36, P = 0.61) or among sampling times (F = 0.69, P = 0.88).

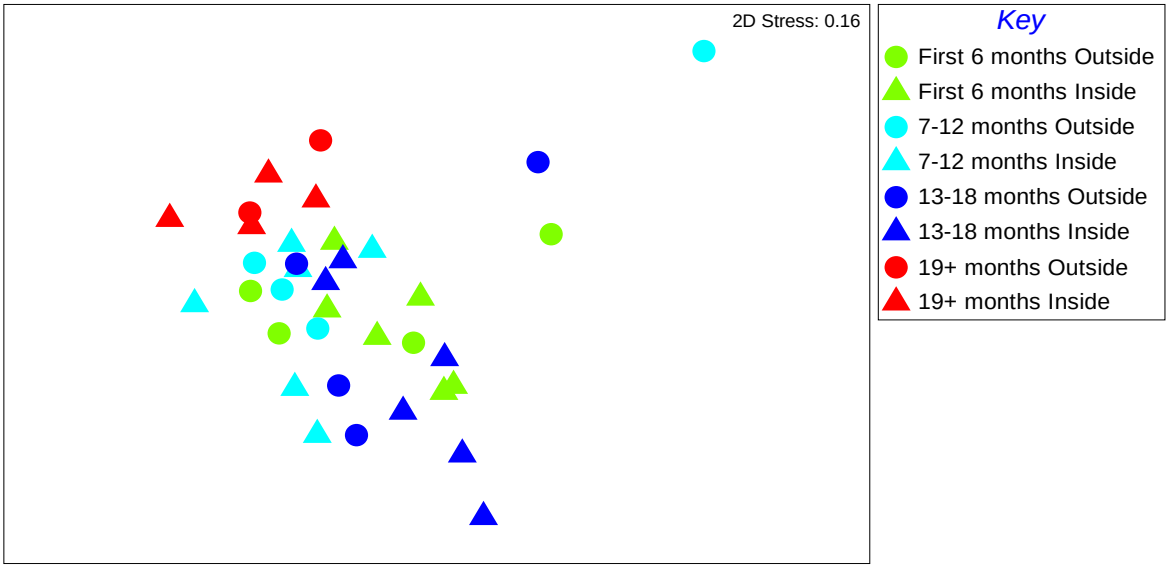

Figure 3: An MDS ordination plot showing the difference in assemblage composition for macroinvertebrates inside and outside the Goolwa Weir Pool across four time periods. Each point represents a single sample (i.e. a replicate from a single site per sampling time). The distance between points represents their relative dissimilarity of the assemblage composition between those samples. A 2-D stress value of 0.16 indicates that the plot is a reasonable two-dimensional representation of the multi-dimensional data. The plot was based on a Bray-Curtis similarity matrix of log(x+1)-transformed abundance data (n = 36). The number of cases varied across categories because not all sites were sampled at each time within the data set.

Time of sampling was the only significant factor explaining patterns in zooplankton assemblages (pseudo-F = 4.42, P = 0.001; Figure 4). None of waterbody, site nested within waterbody nor the interaction of waterbody and time were significant factors. This suggested that zooplankton assemblages changed with time, but in a consistent manner across sites and between waterbodies. Sites inside the GWP changed in a similar manner to those outside the weir pool. The MDS plot (Figure 4) shows clear grouping by sampling times, but not by waterbody, nor site (not shown).

There was also a significant difference in the dispersion of samples taken at different sampling times (F = 4.44, P = 0.05), but not between samples for the two waterbodies (F = 0.58, P = 0.43). This suggests that samples taken at some times were more variable than those taken at other times, with samples taken at 19+ months being the least variable of the categories.

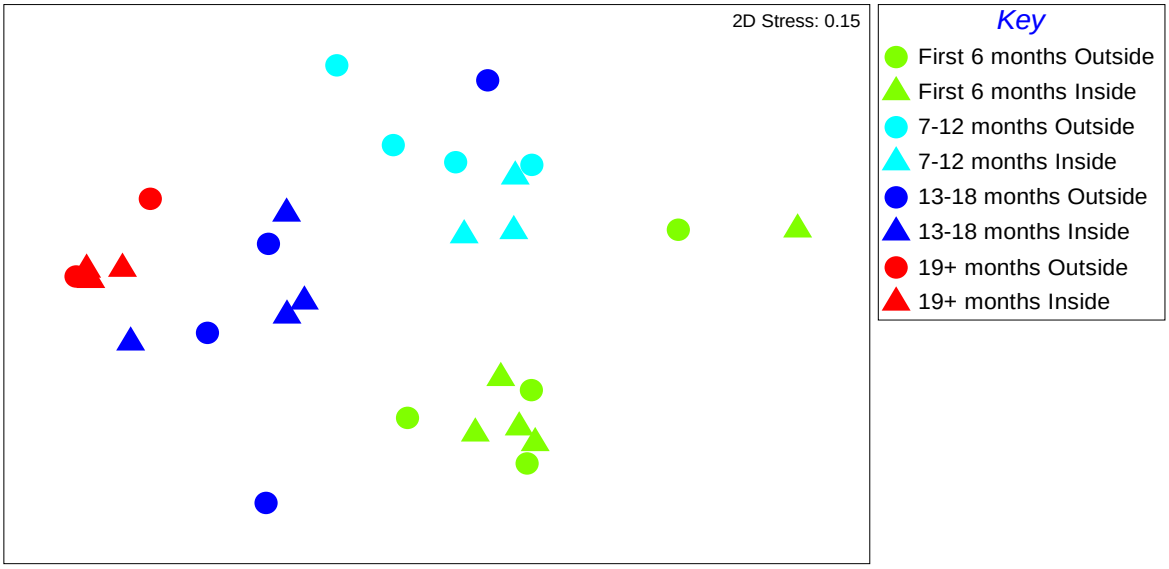

Figure 4: An MDS subset plot showing the difference in assemblage composition for zooplankton inside and outside the Goolwa Weir Pool across four time periods. Each point represents a single sample (i.e. a replicate from a single site per sampling time). The distance between points represents their relative dissimilarity of the assemblage composition between those samples. A 2-D stress value of 0.15indicates that the plot is a reasonable two-dimensional representation of the multi-dimensional data. The plot was based on a Bray-Curtis similarity matrix of square root-transformed abundance data (n = 30). The number of cases varied across categories because not all sites were sampled at each time within the dataset.

Phytoplankton samples were consistently collected only from sites within the Goolwa Weir Pool. This means that no inference can be drawn as to whether there were differences associated with assemblages being located within or outside the GWP. However, time was a significant factor in explaining the patterns observed within the GWP (pseudo-F = 14.39, P = 0.0001; Figure 5), explaining an order of magnitude more variance than the site factor. No significant difference in dispersion was found with time of sampling.

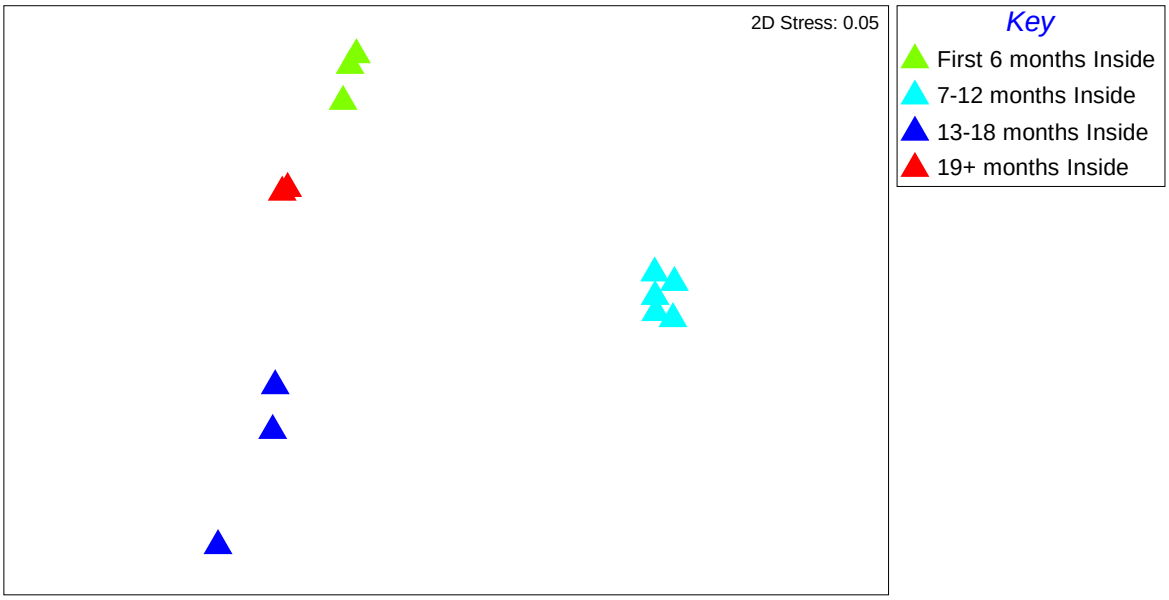

Figure 5: An MDS plot showing the difference in assemblage composition for phytoplankton inside the Goolwa Weir Pool across four time periods. Each point represents a single sample (i.e. a replicate from a single site per sampling time). The distance between points represents their relative dissimilarity of the assemblage composition between those samples. A 2-D stress value of 0.05 indicates that the plot is a good two-dimensional representation of the multi-dimensional data. The plot was based on a Bray-Curtis similarity matrix of log(x+1)-transformed abundance data (n = 13). The number of cases varied across categories because not all sites were sampled at each time within the dataset.

Clear patterns were detectable in the MDS plot for fish assemblages (Figure 6) but the waterbody factor was not significant (pseudo-F = 2.51, P = 0.06; using Monte Carlo simulated probability levels, due to a small number of permutations available for analysis because of having few samples). Time was a significant factor in structuring fish assemblages (pseudo-F = 5.17, P = 0.008), but site nested within waterbody and the interaction between time and site nested within waterbody were not. Time explained twice as much variance as waterbody. The level of dispersion among samples did not vary significantly with time or waterbody.

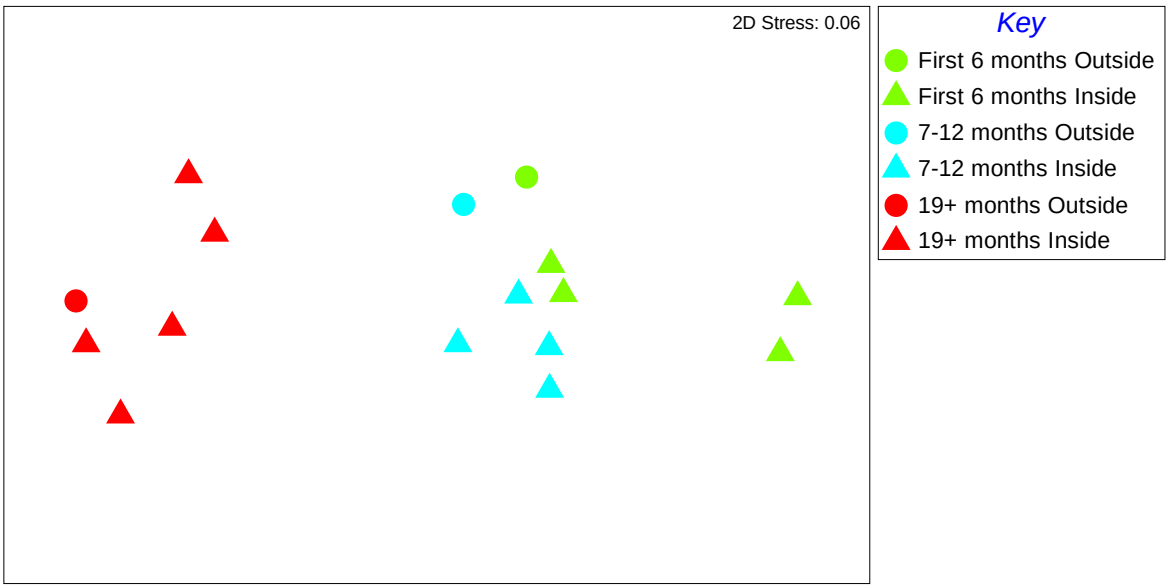

Figure 6: An MDS plot showing the difference in assemblage composition for fish inside and outside the Goolwa Weir Pool across three time periods. Each point represents a single sample (i.e. a replicate from a single site per sampling time). The distance between points represents their relative dissimilarity of the assemblage composition between those samples. A 2-D stress value of 0.06 indicates that the plot is a good two-dimensional representation of the multi-dimensional data. The plot was based on a Bray-Curtis similarity matrix of square root-transformed abundance data (n = 16). The number of cases varied across categories because not all sites were sampled at each time within the dataset.

None of the factors investigated (i.e. waterbody, time of sampling, site nested within waterbody nor any interaction) was able to significantly explain the patterns observed in bird assemblages. This is evident from the lack of grouping of the relatively few samples observed on the MDS plot (Figure 7). Given the relatively small size of the GWP (from a bird perspective), the mobility of bird assemblages and the relatively few samples collected, it is not particularly surprising that this was the case. The level of dispersion among samples did differ with sampling time (F = 30.27, P = 0.02), but this result should be treated with caution due to the small number of replicate samples for each time.

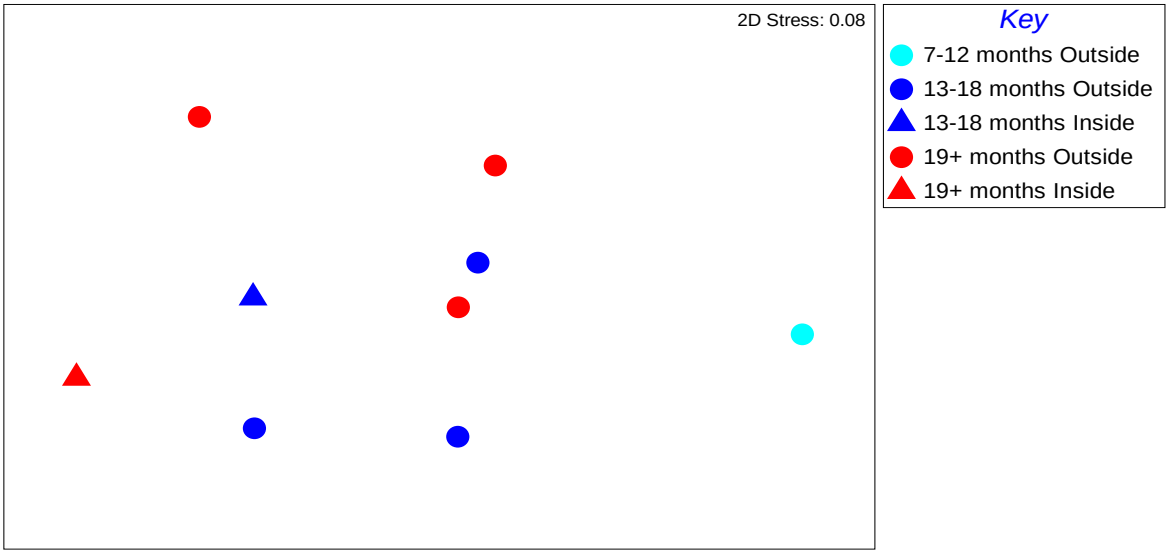

Figure 7: An MDS plot showing the difference in assemblage composition for birds inside and outside the Goolwa Weir Pool across three time periods. Each point represents a single sample (i.e. a replicate from a single site per sampling time). The distance between points represents their relative dissimilarity of the assemblage composition between those samples. A 2-D stress value of 0.08 indicates that the plot is a good two-dimensional representation of the multi-dimensional data. The plot was based on a Bray-Curtis similarity matrix of square root-transformed abundance data (n = 9). The number of cases varied across categories because not all sites were sampled at each time for this dataset.

As was observed for macroinvertebrate assemblages, vegetation assemblages were explained by the time of sampling (pseudo-F = 3.68, P = 0.002) and the site nested within waterbody (pseudo-F = 5.41, P = 0.0001). Waterbody was not a significant factor. Small-scale, site-to-site variability explained the majority of the variance, with time explaining approximately only one-third as much. Waterbody explained an order of magnitude less variance than site nested within waterbody. No obvious pattern was detectable from the MDS plot, consistent with the idea that site nested within waterbody (not shown with these symbols) was structuring assemblages (Figure 8).

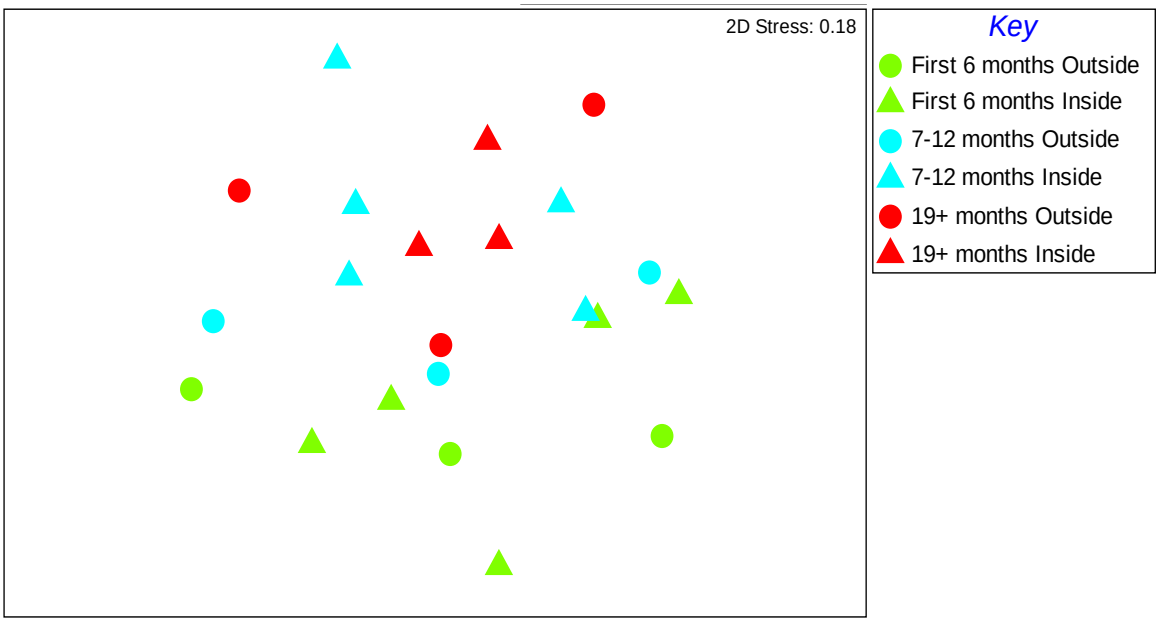

Figure 8: An MDS plot showing the difference in assemblage composition for vegetation inside and outside the Goolwa Weir Pool across three time periods. Each point represents a single sample (i.e. a replicate from a single site per sampling time). The distance between points represents their relative dissimilarity of the assemblage composition between those samples. A 2-D stress value of 0.2 indicates that the plot is a reasonable two-dimensional representation of the multi-dimensional data. The plot was based on a Bray-Curtis similarity matrix of non-transformed abundance data (n = 22). The number of cases varied across categories because not all sites were sampled at each time for this dataset.

No significant difference was detected between waterbody for the southern bell frog (pseudo-F = 0.80, P = 0.45 using Monte Carlo simulated probability levels, due to a small number of permutations available for analysis) for the single time period sampled, but the study suffered from low replication (n = 6) at both the site and time level, and therefore no other tests (including time, site nested in waterbody and any interaction) were possible.

For the combined synthesis, which included macroinvertebrates, fish and vegetation, none of the factors (time, site, site nested within waterbody and the interaction of waterbody and time) explained the observed patterns. For these components, no significant ecosystem-scale patterns were apparent.

Relating environmental conditions to observed biotic patterns

A principal component analysis (PCA) was conducted on the available physico-chemical data in order to identify groups of factors that may have influenced biotic assemblages. PCA combines sets of individual variables into axes that represent combinations of those variables that act independently from one another (i.e. are orthogonal). We identified four principal components (PC's termed 'syndromes' here) acting in the physico-chemical data for the GWP (Table 2). The first syndrome (Syndrome 1) was highly correlated with soluble calcium, chloride, potassium and sulfate concentrations, electrical conductivity and total dissolved solids, and highly but negatively correlated with total and extractable aluminium, total iron and total phosphorus concentration and turbidity.

This may be explained as a marine water influence (given the high salinity but low turbidity). Syndrome 2 may have been related to the buffering effect of water, with high correlations with alkalinity, bicarbonate and carbonate concentrations and pH. This syndrome was also highly correlated with TKN, a measure of organic nitrogen. Syndrome 3 was highly correlated with ammonia concentration, ion balance and negatively correlated with phosphorus, while Syndrome 4 was not strongly correlated with any of the individual variables. This may indicate that the driver of syndrome 4 is a variable that was not included in the analysis, but that some hint is provided by the variables that were included. The strongest associations for Syndrome 4 were a negative correlation with ammonia concentration and a positive correlation with pH; however, both these variables were more-strongly correlated with other syndromes. It should be noted that the explanations of the various syndromes require additional testing by chemical experts and should be considered preliminary at this stage, although the PCs themselves (that is the combination of individual variables) are more robust.

Table 2: Results of a PCA analysis, separating 20 independent environmental variables into four principal components. Results are indicated by a positive or negative symbol (depending on the direction of the correlation) where the correlation was strongest (i.e. a rotated loading > 0.7) across all principal components. Principal component four is not included below, as it was not strongly correlated with any environmental variables. Raw data were collected by the EPA (2011).

Principal Component (Syndrome)	**PC1**	**PC2**	**PC3**
Interpretation based on rotated loadings **Eigenvalue** **Raw variables**	Marine influence 10.819	Alkaline, high pH 3.821	Ammonia & Ion balance 1.448
Alkalinity		+	
Aluminium (acid extractable)	-		
Aluminium (total)	-		
Ammonia			+
Bicarbonate		+	
Calcium (soluble)	+		
Carbonate		+	
Chloride	+		
Conductivity	+		
Fluoride	+		
Ion Balance			+
Iron (total)	-		
pH		+	
Phosphorus (soluble P)			-
Phosphorus (total P)	-		
Potassium	+		
Sulfate	+		
Total Kjeldahl nitrogen (TKN)		+	
Total dissolved solids	+		
Turbidity	-		

The macroinvertebrate assemblage was analysed relative to the physico-chemical data (using the results of the PCA) to determine whether any of the syndromes identified could account for patterns observed in that dataset. Syndrome 3 (related to ammonia and ion balance) was most strongly correlated (*rho* = 0.26, *P*> 0.05; Figure 9) but this was not a statistically significant result, and there was not an obvious pattern of similar macroinvertebrate samples (i.e. points close together on Figure 9) having similar values for Syndrome 3 (as represented by the size of the 'bubble') which would be expected if the relationship were particularly strong. While the sample size was relatively small (*n* = 18), it is relatively unlikely that this weak correlation would be ecologically significant even if additional statistical power were available.

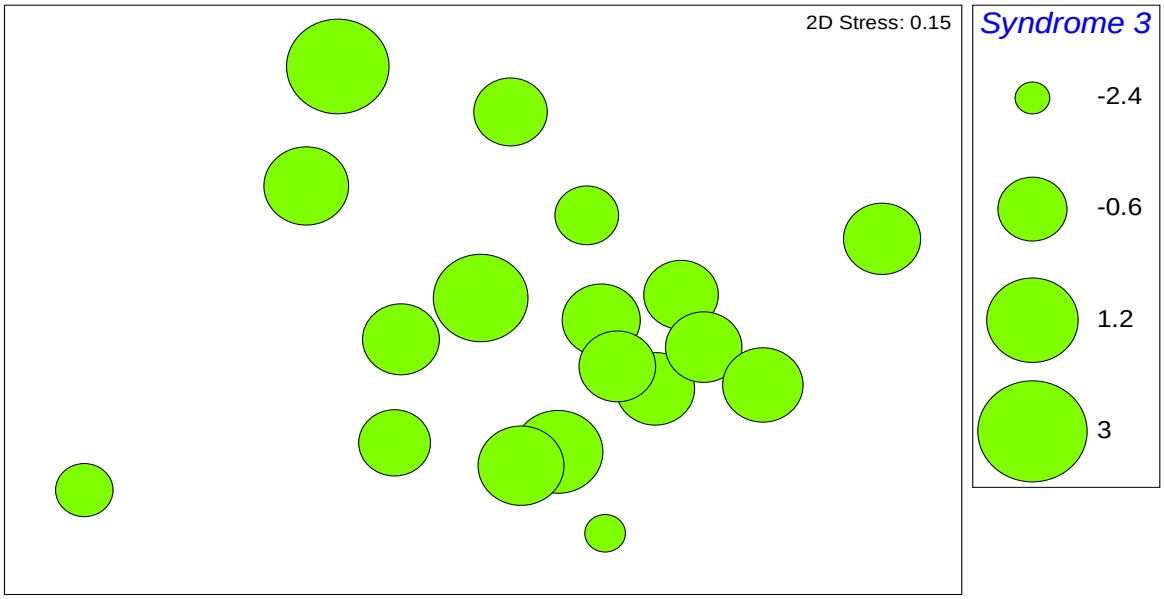

Figure 9: An MDS plot of macroinvertebrates (*n* = 18) with superimposed circles representing Syndrome 3 Principal Component scores. The size of the circle indicates the Principal Component score for Syndrome 3 for that sample. Some circles overlap where samples are similar. Larger circles indicate stronger positive associations with that syndrome while smaller circles indicate stronger negative associations with that syndrome. This plot is the same as in Figure 3, so the locations and times of samplings are consistent, but here the values of the best-correlated PC score are illustrated as the size of the bubble.

Zooplankton assemblages were somewhat better explained by the various syndromes identified, but this correlation was again not statistically significant. Here, Syndrome 2 had the strongest correlations with patterns in the zooplankton data (*rho* = 0.23, *P*> 0.05; Figure 10) but the number of samples was relatively small (*n* = 14), so an increase in statistical power may yield a significant correlation. The greater strength in relationship between zooplankton assemblages and the identified syndrome is more apparent from the grouping of samples that had similar conditions (i.e. so the value for Syndrome 2 was similar; Figure 10).

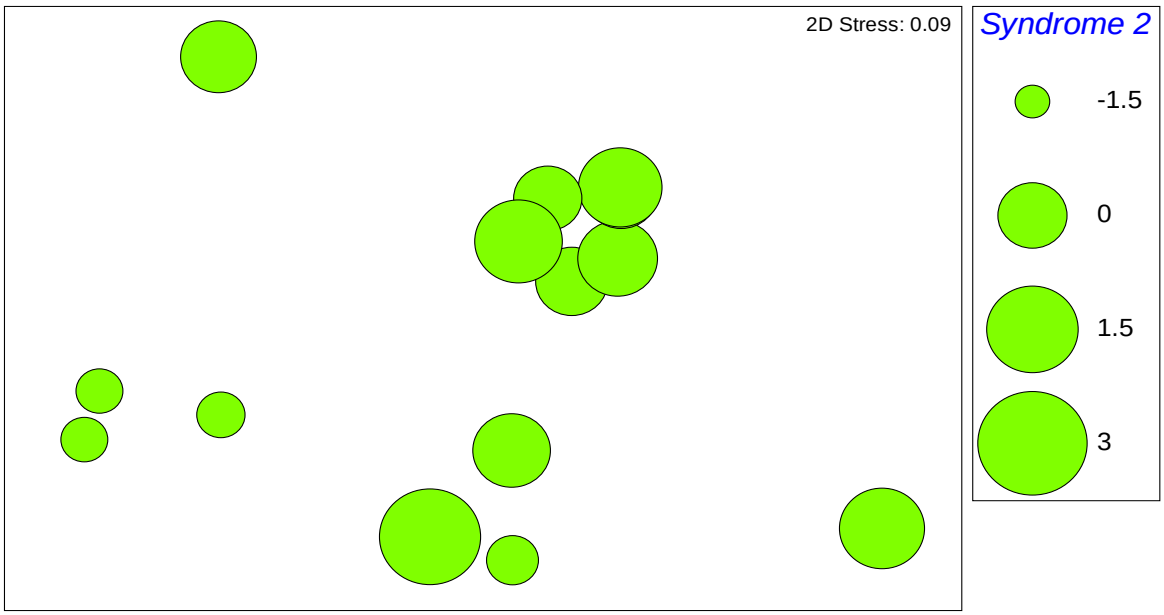

Figure 10: An MDS plot of zooplankton (*n* = 14) with superimposed circles representing Syndrome 2 Principal Component scores. The size of the circle indicates the Principal Component score for Syndrome 2 for that sample. Some circles overlap where samples are similar. Larger circles indicate stronger positive associations with that syndrome while smaller circles indicate stronger negative associations with that syndrome. This plot is the same as in Figure 4, so the locations and times of samplings are consistent, but here the values of the best-correlated PC score are illustrated as the size of the bubble.

Few samples represented phytoplankton assemblages when patterns were analysed for correlations with the available physico-chemical data (*n* = 8). Despite this small sample size, there was a significant correlation with Syndrome 1 (which included relationships with pH, electrical connectivity and the concentration of a range of compounds including potassium, fluoride, sulfate and calcium) (*rho* = 0.67, $P \leq 0.05$; Figure 11). This is apparent by the grouping of different-sized bubbles in Figure 11 with similar phytoplankton assemblages (i.e. bubbles of the same size tend to appear in the same area of the plot).

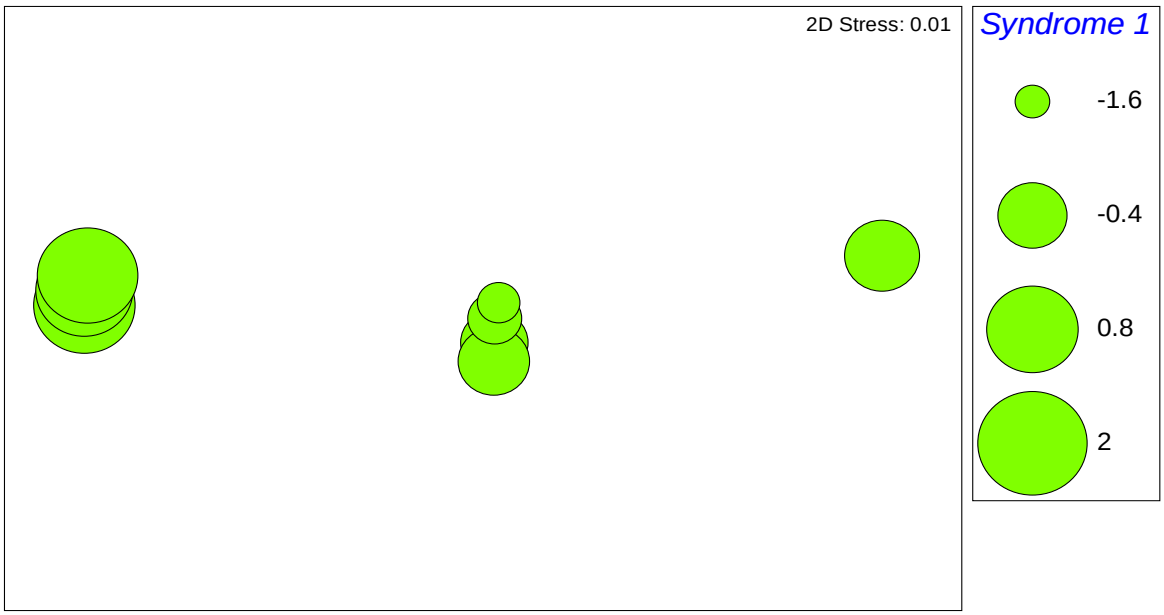

Figure 11: An MDS plot of phytoplankton (*n* = 8) with superimposed circles representing Syndrome 1 Principal Component scores. The size of the circle indicates the Principal Component score for Syndrome 1 for that sample. Some circles overlap where samples are similar. Larger circles indicate stronger positive associations with that syndrome while smaller circles indicate stronger negative associations with that syndrome. This plot is the same as in Figure 5, so the locations and times of samplings are consistent, but here the values of the best-correlated PC score are illustrated as the size of the bubble.

Fish assemblages could also only be represented by a small number of samples (*n* = 9), which limits the inference that can be drawn from an analysis of the relationship of those assemblages and the available physico-chemical data. However, there was a strong correlation between patterns in fish assemblages and Syndrome 4 (*rho* = 0.56, *P*> 0.05; Figure 12) with all high values associated with Syndrome 4 clustered on the right-hand side of figure 12. Again, this correlation was not statistically significant, but this is likely to be due to a lack of statistical power.

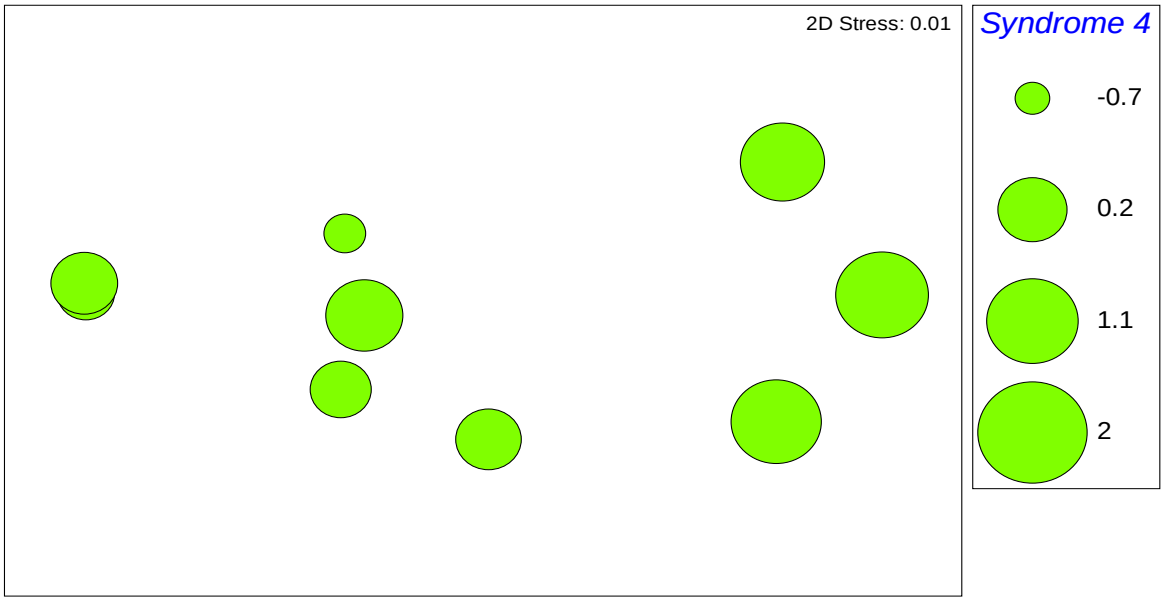

Figure 12: An MDS plot of fish (*n* = 9) with superimposed circles representing Syndrome 4 Principal Component scores. The size of the circle indicates the Principal Component score for Syndrome 4 for that sample. Some circles overlap where samples are similar. Larger circles indicate stronger positive associations with that syndrome while smaller circles indicate stronger negative associations with that syndrome. This plot is the same as in Figure 6, so the locations and times of samplings are consistent, but here the values of the best-correlated PC score are illustrated as the size of the bubble.

Patterns in vegetation assemblages were best explained by a combination of Syndromes 3 and 4 (*rho* = 0.29, *P*> 0.05; Figure 13). Once again, this correlation was not statistically significant, although the sample size was relatively small (*n* = 14) suggesting this may be due to a lack of statistical power. Syndrome 4 was the strongest individually-correlated syndrome, so that has been shown in Figure 13; however, no pattern was evident relating to the values for that Syndrome, illustrating the relatively weak relationship between the individual syndrome and vegetation assemblages.

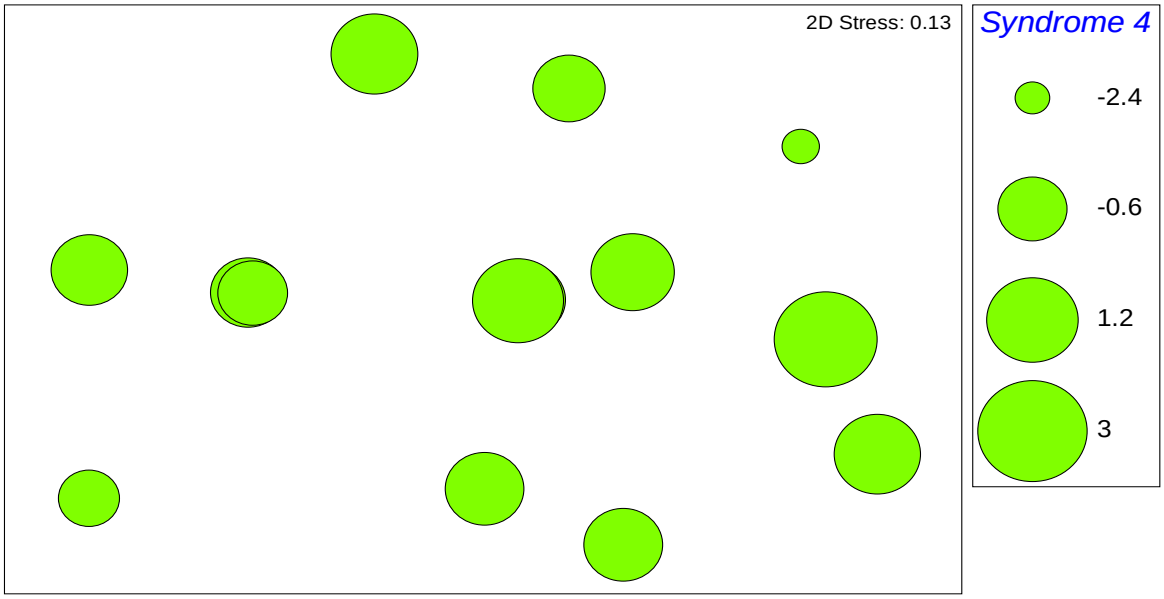

Figure 13: An MDS plot of vegetation (*n* = 14) with superimposed circles representing Syndrome 4 Principal Component scores. The size of the circle indicates the Principal Component score for Syndrome 4 for that sample. Some circles overlap where samples are similar. Larger circles indicate stronger positive associations with that syndrome while smaller circles indicate stronger negative associations with that syndrome. This plot is the same as in Figure 8, so the locations and times of samplings are consistent, but here the values of the best-correlated PC score are illustrated as the size of the bubble.

There were even fewer samples available to represent bird (*n* = 5) and the southern bell frog (*n* = 2) assemblages, so no analyses were undertaken.

Table 3 summarises the results of the above analyses relating syndromes to individual taxonomic groups. Again, only phytoplankton was strongly correlated, in that case with Syndrome 1.

Table 3: Results of BEST analyses on four principal components (Syndromes) on each taxonomic group, including the strongest correlated syndrome accounting for patterns observed in that dataset. Syndrome 1 is related to a marine water influence, syndrome 2 related to alkaline and high pH and syndrome 3 is related to ammonia and ion balance. Syndrome 4 was excluded, as it was not correlated with any environmental variables. The P-value, *rho* value and total sample size is shown. The southern bell frog and bird assemblages had insufficient sample sizes for meaningful analyses. Significant values are shown in bold.

	Strongest correlated syndrome	*P*-value	*rho* value	Sample size
Macroinvertebrates	Syndrome 3	>0.05	0.26	18
Zooplankton	Syndrome 2	>0.05	0.23	14
Phytoplankton	Syndrome 1	**≤0.05**	**0.67**	8
Fish	Syndrome 4	>0.05	0.56	9
Vegetation	Syndrome 3	>0.05	0.29	14

Relating biotic responses to the response for other taxonomic groups

Various combinations of taxonomic groups were investigated in an attempt to determine whether patterns in one group were correlated with those observed in other groups. Combinations of taxa were limited to those where there would be an ecological reason for such a relationship to exist (e.g. vegetation assemblages provide habitat for fish, and so fish and vegetation may be related).

No statistically-significant associations were detected among macroinvertebrate and zooplankton assemblages (*rho* = -0.11, *P* = 0.88). There were no statistically-significant relationships between patterns in macroinvertebrate and fish assemblages (*rho* = 0.19, *P* = 0.08), suggesting that they were responding to different environmental cues, and that fish assemblages were not reliant on the macroinvertebrate assemblages measured. No statistically-significant association was detected among fish and phytoplankton assemblages (*rho* = 0.21, *P* = 0.11). No relationship was detected among fish and vegetation or macroinvertebrate and vegetation assemblages from the available data (*rho* = -0.07, *P* = 0.66 and *rho* = 0.032, *P* = 0.41), suggesting that vegetation structure was not driving observed patterns in fish or macroinvertebrate assemblages.

There was a statistically significant correlation in patterns observed in fish and zooplankton (*rho* = -0.73, *P* = 0.003), but given that fish and zooplankton were best-associated with different environmental syndromes (4 and 2, respectively), the correlation in observed patterns may suggest fish assemblages could be responding to patterns in zooplankton assemblages, perhaps as prey items, for example. This presents an interesting hypothesis which could be tested during future monitoring.

Discussion

This report is the third and final in a series that investigated responses across taxonomic groups within the Goolwa Weir Pool after the closure of the Clayton Regulator (see also Lester *et al.* 2010, Hamilton *et al.* 2011). Here, we investigate responses up to more than 22 months after the closure including post-breaching of the regulator, building on the syntheses that were undertaken six and 12-months after closure (Lester *et al.* 2010 and Hamilton *et al.* 2011, respectively). Responses for vegetation assemblages, benthic macroinvertebrates, phytoplankton, zooplankton, birds, southern bell frogs and fish were assessed and compared to one another, and to the available environmental data.

As was the case for previous syntheses, the general lack of overlap in the locations and timing of sampling, and inconsistencies in the number of sites that were sampled at each sampling occasion, seriously limited the synthesis that was able to be undertaken. This is largely a result of a lack of re-alignment following earlier syntheses and the lack of additional sites outside the GWP, in particular, made differences within the water body difficult to detect. There is evidence of a lack of statistical power in many of the analyses undertaken here, so caution should be used when interpreting non-significant results as there may be many Type II errors (where the null hypothesis is rejected as a conclusion of a study, even though there is, in truth, a significant effect) due to this relative lack of replication. Consistency in sampling locations and in sampling times (allowing for differences in response times) remain critical for deriving maximum value from syntheses such as this. As previously, the lack of baseline data from prior to the construction of the regulator also limited the inference that was possible. Had that been undertaken, a full Before-After, Control-Impact (BACI) assessment would have been possible (see Downes *et al*. 2002 for discussion of options). Therefore, as per the previous syntheses, we strongly recommend that any future monitoring programs be designed to allow for more comparison across taxonomic groups, which would allow for better comparison, with a more even spilt of sites within and outside the influence of the action being investigated, and some sampling both before and after the intervention occurs.

Another factor which may have limited our ability to detect the impact of waterbodies was the previously recorded changes in management actions, following the initial design of the study (Lester & Fairweather 2009). Sites that were expected to be outside the influence of the GWP may not have been wholly uninfluenced, as a regulator in the Finniss River was not constructed and the Upper Currency site was further downstream than initially intended due to access issues. Thus, both sampling sites in the upper reaches of the tributaries (Upper Finniss and Upper Currency) may have been connected to the GWP, particularly when water levels were high. At times of low flow, water-level data indicated that this was not the case, with water levels falling well below the levels observed in the weir pool proper (as observed in Figure 2); however, it is possible that these sites constituted a third category of 'sometimes within' the GWP and so were not an independent control for the 'Inside' sites. Were the study to be designed in hindsight, particularly following the decision not to construct the Finniss regulator, then the number and location of study sites would have been different. Additional sites, along a gradient of likely influence (i.e. up- versus down-stream of a regulator), would be more appropriate to untangle the decreasing effects of the weir pool upstream than the current design. The relatively few sites that were outside the GWP (either occasionally or completely) did not allow for the untangling of this potential effect based on the data currently

available. Thus, we have remained faithful to the original study design, but not that there may be any confusion about the effect of waterbody in the current study as a result of this effect.

Time was factor that was most commonly found to be structuring individual assemblages. Of the seven individual taxonomic datasets analysed, time alone was found to be a significant factor for three (phytoplankton, zooplankton and fish) and was found to be a significant factor along with site (nested within waterbody) for another two (macroinvertebrates and vegetation), although where site was a significant factor, it tended to explain more of the variability detected than time. The prevalence of time as a significant factor is not surprising given the large changes in flows and water levels that occurred through time in the region. The study commenced immediately after the closure of the Clayton regulator, at a time of very low flows. Water was then pumped into the GWP and held higher than had been the case for several years over a summer, when the return of tributary and then River Murray flows provided a source of fresh water and resulted in the breaching of the regulator. This sequence of events is best represented by the temporal factor in the analysis. It is interesting that the combined analysis of macroinvertebrates, fish and vegetation communities did not also identify time as a significant factor, as it had been significant for all of the individual components of that combination. Time was the factor that explained the most variation in that analysis, although it was not statistically-significant. This suggests that either the temporal patterns observed in the individual analyses counteracted one another, so were not apparent in the combined analysis, or that statistical power may have been lacking, due to the relatively small number of replicates in that combined analysis (n = 9).

Waterbody, on the other hand, was never a significant factor for any of the individual or combined analyses. Lack of replication, particularly outside the GWP *per se*, and the potential issues associated with two of the 'outside' sites may have contributed to this pattern. It is also possible that the Clayton regulator did not sufficiently disrupt connectivity within the region (i.e. over and above the effect of the drought itself) to have an influence, and the return of tributary flows likewise may have masked any effects as a result of higher water levels, given that water levels outside the weir pool also rose (albeit somewhat later). A finer temporal resolution of sampling may have allowed these effects to have been identified.

Four syndromes were identified in the available environmental data, simplifying the task of identifying patterns among those data and the biotic assemblages. The explanations relating to each of the syndromes should be further tested with chemical water-quality experts, but it appeared the variables which may be associated with a marine influence and with a buffering capacity explained the majority of variability in the environmental data set. Further investigation into Syndrome 4 is certainly warranted, given that it currently represents unexplained variability in the environmental data, which may suggest that there is an important driver (particularly of fish and vegetation) that is not captured by the current EPA water-quality sampling. Interestingly, of the five analyses that were possible relating the individual taxonomic groups to those syndromes, each of the four were best-correlated in one instance, with a combination of Syndromes 3 and 4 best-correlated in the fifth instance. This suggests that different biotic groups are responding differently to their environment and may need to be managed differently in times of future stress.

When investigating relationships between pairs of individual taxonomic datasets, few significant relationships were identified. The notable exception was a strong negative correlation between patterns associated with fish and zooplankton assemblages. Given that the two groups were most strongly correlated with different environmental syndromes, it is less likely that this correlation is a result of both groups reacting to the same driving factor, and more likely that one is directly affecting the other. A first attempt at a hypothesis would suggest that zooplankton assemblages are influenced by the presence and/or diversity of fish assemblages. We suggest that this relationship may indicate a predation effect, with zooplankton assemblages known to be more diverse in areas without planktivorous fish (Donald *et al.* 2001), and suspect that this may be operating in and around the Goolwa channel. Further investigation and experimentation could confirm (or otherwise) this hypothesis and provide important information relating to the likely zooplankton assemblages when different fish species are dominant. Important dietary information regarding planktivorous fish may also arise from such an investigation.

Finally, it should be noted that since the previous synthesis, there was been a marked improvement in the consistency and format of the data received as a part of this study, with all groups following the new format stipulated by DENR. This allowed much easier comparison of datasets and represents a significant achievement on behalf of DENR. This consistent format should be used in the future, and will facilitate comparison of data among year, sites and groups.

Conclusions

- **A lack of overlap and consistency in the timing and location of sampling again limited the analyses possible and made differences between water bodies difficult to detect. We recommend future monitoring include more overlap, replication, control sites and data collected before the action under investigation.**

- **Time was the main factor explaining the structure of individual assemblages, but the position within or outside the weir pool did not explain the patterns of either individual taxa or combined assemblages. Time may have acted as a surrogate for changes in flow regimes and water levels, suggesting that broad-scale patterns may have overwhelmed the effect of the smaller-scale intervention of the Clayton regulator.**

- **An additional investigation into the interaction between fish and zooplankton patterns is recommended. Both fish and zooplankton responded to different environmental syndromes, so it is hypothesised that one may be directly influencing the other, possibly through predation pressure. Few additional interactions between taxonomic groups were identified and different taxonomic groups appeared to respond differently to their environment.**

- **Consultation with water quality experts would build on the interpretation of relationships between syndromes and taxonomic groups. Key environmental syndromes appear to be driving patterns of individual taxonomic groups, and specific monitoring or experiments could be designed accordingly.**

- **The creation of a standard template for the provision of data to DENR resulted in a major improvement in the usability of data provided, compared with previous syntheses.**

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