Introduced and native fishes in the Vasse-Wonnerup Wetland System and its rivers

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Frontispiece: the Vasse floodgates and a large Goldfish removed from the lower Vasse River

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A native Gilgie *Cherax quinquecarinatus* from the Abba River.

Executive Summary

The Vasse-Wonnerup Ramsar Wetland System (VWWS) has international ecological significance due to its designation as a Ramsar site. However, the water quality is poor due in large part to high levels of nutrients which, in conjunction with other stressors such as regulation of flow, have caused numerous algal blooms and fish deaths. Surprisingly, there has been no quantitative research or monitoring of the fish communities in the VWWS despite fishes being key indicator group of ecosystem health and being a critical component in foodwebs and particularly for birds which give the system its Ramsar status.

Although there has never been a comprehensive fish survey of the entire wetland, the adjoining rivers were known contain a number of introduced fish and crayfish species. As found elsewhere in south-western Australia, these introduced fishes and crayfishes are known to impact directly on native fish species by predation and competition and indirectly by introducing exotic diseases. In addition, one of the key invasive species in the VWWS, Goldfish, is known to exacerbate algal blooms by re-suspending nutrients and stimulating algal growth through their feeding processes. Other ecological impacts include alteration of natural food webs and habitat destruction through feeding and burrowing activities.

The current project aimed to provide a comprehensive quantitative understanding of the fishes and crayfishes of the VWWS across the full range of habitats in order to assess the current and future health of the ecosystem and guide effective management decisions and monitoring programs. Given the impact of introduced fishes, the study had a specific simultaneous aim to document, prioritise and control introduced fishes and crayfishes to directly address a major known impact on aquatic ecosystems. Finally, it aimed to provide management recommendations that could be implemented to mitigate the impacts of existing and future introduced species.

The report is separated into two major sections; ‘riverine’ and ‘wetland’ (including estuarine) that together form the VWWS. This was undertaken as these habitats are obviously linked yet exhibit different hydrological regimes and thus require different sampling techniques and generally house distinct suites of species. However, at all stages of the project, these activities were carefully coordinated (e.g. such as the timing of sampling)

to ensure compatible seasonal comparisons, and to enable the findings and recommendations of the report to be framed in a ‘whole-off-system’ setting. This study provides an overall baseline dataset of the fishes and crayfish communities of the entire system and will enable a more coordinated management approach to the issue of introduced species in the VWWS.

Fish faunas of the rivers of the Vasse-Wonnerup Wetland System

A total of 17 sites were chosen for seasonal sampling in the rivers of the VWWS including five each in the Ludlow, Abba, and Sabina rivers, and two in the Vasse River. Six seasonal sampling trips were conducted in total, commencing in March 2012 and concluding in June 2013. A range of sampling techniques were deployed designed to quantity the density and also movement patterns of native species whilst minimising impacts of sampling on those species and importantly removing large numbers of introduced species. Level of fin damage by the invasive Eastern Gambusia was also assessed throughout the study to determine which native fishes are most susceptible and explore the factors that influence the degree of impact of its aggressive behaviour.

A suite of environmental variables (including habitat, riparian vegetation, and water quality) were also recorded at each site to enable calculation of the Pen-Scott foreshore condition index at each site, and also relationships between native and introduced species and water quality and habitat variables to be explored. Fixed position photography occurred at each site to provide a visual representation of seasonal changes in site characteristics.

The first ever acoustic tracking study of Goldfish was conducted as part of the project in the Vasse River. An array of eight acoustic receiver units (‘listening stations’) was deployed in a 5.8 km stretch of the lower Vasse River and acoustic transmitter tags were implanted in 21 individual Goldfish. These Goldfish were passively tracked from 12 months from December 2012 – December 2013 to provide information on movement patterns and spawning locations.

A total of 66,944 individual fishes and decapod crustaceans representing 12 species were captured during riverine sampling. These included self-maintaining populations of three native freshwater fishes (Western Minnow *Galaxias occidentalis*, Western Pygmy Perch *Nannoperca vittata*, and the Nightfish *Bostockia porosa*), three native estuarine fishes that are known to utilise riverine habitats (i.e. Bluespot Goby *Pseudogobius olorum*, Sea Mullet *Mugil cephalus*, and Western Hardyhead *Leptatherina wallacei*, two introduced fishes Goldfish *Carassius auratus*, Eastern Gambusia *Gambusia holbrooki,* three native decapod crustaceans Gilgie *Cherax quinquecarinatus*, Koonac *Cherax preissii*, Glass Shrimp *Palaemonetes australis*, and one introduced decapod crustacean, the eastern Australian Yabby *Cherax destructor*.

The highest aquatic biodiversity sites were in the lower reaches of the river catchments. These sites tended to hold permanent water and act as vital refuge for aquatic species during the dry summer/autumn baseflow period. Sites located further upstream in the rivers only held water in winter/spring and housed fewer aquatic species. However, native fishes were found to utilise upstream sites, indicating that seasonal migration is an important life history trait and longitudinal stream connectivity must be considered to effectively manage native freshwater fish populations in the VWWS. Stream habitats were generally degraded throughout the catchments, except in the headwater reaches located on the Darling/Whicher range which were near pristine. Habitats were most highly degraded in the middle reaches of rivers, in the agricultural and grazing land lying between Bussell Highway and the foot of the Darling/Whicher Range.

The project resulted in the removal of 26,081 introduced Eastern Gambusia and 842 Goldfish from rivers of the Vasse-Wonnerup catchment. The removal of large numbers of mature Goldfish during this project is likely to significantly reduce the subsequent rate of recruitment to the population. Importantly, the exhaustive seasonal sampling did not reveal any unexpected pest species incursions in the rivers of the Vasse-Wonnerup.

In the 12 month duration of the Goldfish tracking study, 15 Goldfish were tracked for the entire year. A high site fidelity (in terms of percentage of days visited) was recorded at the receiver upstream of the Boat Ramp near the Shire (93% of fish detected each on average 27% of the days), followed by the New River Wetlands (80% of fish, 22% of days). 47% of Goldfish were detected in the Lower Vasse River Wetland at least once during the study.

Tagged Goldfish were far from being sedentary, and movements of at least 5.4 km in a 24 hour period were recorded for some individuals, indicating that the species is highly mobile in the Vasse River. There were considerable seasonal patterns in habitat use and the key sites utilised during the known spawning period of Goldfish were the site upstream of the Boat Ramp and the New River Wetland (60% of fish detected) suggesting they were the key spawning habitats. Furthermore, 80% of Goldfish were detected in the New River Wetland at some stage during the study. Almost half of tagged Goldfish were detected in the Ramsar listed Lower Vasse River Wetland and the salinity tolerance of Goldfish determined during the study (i.e. 11 ppt acute, and 21 ppt gradual tolerance) suggests that it is plausible that the species can use the estuary as a dispersal pathway into other rivers of the VWWS. This was also supported by the findings of the Wetland component of the current study that detected large numbers of juveniles using the Vasse axis of the wetland system. However, rigorous seasonal sampling in both wetland and riverine waters throughout the Vasse-Wonnerup failed to detect the species outside of the lower Vasse River and upper Vasse Estuary, indicating that such dispersal has not yet taken place.

As outlined in the Table of Management and Monitoring Recommendations at the rear of the report, the information gathered during this study will be critical to future management. Efforts should be made to protect the high conservation habitats in the lower reaches of the rivers particularly those that provide permanent refuge pools. Protection and rehabilitation of riparian vegetation and continuing to address nutrient loads will greatly benefit riverine fishes in these systems. Other actions including addressing instream barriers to migrations and ensuring future water abstractions are carefully monitored to offset projected declines in rainfall and river flow.

The current study also allows clarity and confidence in the allocation of resources for ongoing introduced species control efforts in the VWWS. Investment should be made in continued control of the Goldfish population using the information gathered from the tracking study. Importantly, priority can also be focused on preventing future species incursions with the confidence in knowing that urgent control of other pest species is not required at this time. Ongoing monitoring should occur to detect, as early as possible, any future introductions into the rivers of the Vasse-Wonnerup (see the **Table of Management and Monitoring Recommendations** at the rear of this report).

Fish fauna of the Wetlands of the Vasse-Wonnerup Wetland System

The study in the wetland and estuarine habitats of the VWWS was conducted simultaneously and in coordination with the riverine survey. It was also expanded to assess the impact of a large fish kill in April 2013 on the fish fauna of the Vasse-Wonnerup that was made possible by the regular sampling of the fish in the nearshore waters of the system that had been occurring since February 2012 as part of the current project. This thus provided a timely example as to the value of the current study in providing a baseline upon which to assess these sorts of stochastic events. The results of the fish kill investigation are presented in the current study but also in a stand-alone companion report (see Tweedley et al., 2014).

Analysis of the nearshore fish fauna collected every three months over a two year period between February 2012 and November 2013 indicated the presence of two introduced fishes, the Eastern Gambusia and the Goldfish existing in the VWWS; consistent with the findings in the riverine sampling with no new species being detected. The composition of the fish fauna changed markedly both among seasons and regions and that this change was accompanied by large changes in water quality, most notably salinity. Both the number of species and individuals were greatest in the lower reaches of the system, *i.e.* the Deadwater and Wonnerup Inlet, and declined in an upstream direction through both the lower and upper Vasse and Wonnerup estuaries, before increasing in the Lower Vasse River Wetlands. The diversity in the lower reaches reflects the presence of juveniles of commercially and recreational important fish species, while the high numbers of fish is a reflection of the relatively stable water and salinity levels. These levels changed markedly in the estuaries, with the upper estuaries becoming dry and the lower estuaries markedly hypersaline, reaching 132 (around four times full strength seawater) during summer and autumn, before freshwater discharge in winter lowers the salinity considerably (*i.e.* to < 10).

The density of fishes in the wetlands of the VWWS -Wonnerup changed dramatically over the course of a year, with those changes being consistent across the two years. High densities of fish were recorded during November and February, before declining rapidly in May and remaining at those levels in August. This trend is a reflection of i) the breeding cycle of many of the estuarine species, which typically live for one year and spawn over the summer period and ii) the reduction in water levels, increase in salinity and temperature and consequently lower dissolved oxygen concentrations at night. The decline in water levels in the upper estuaries also traps fish in shallow pools making them easy prey for the many birds that inhabit the Vasse-Wonnerup at that time of year.

With regard to the nearshore fish community as a whole (*i.e.* including all species), no significant change in faunal composition was detected as a result of the fish kill in April 2013. This is due mainly to the fact that there is a 'natural' decrease in the fish fauna at this time of year and that a similar suite of species inhabit the wetlands of the VWWS in all seasons. Had the fish kill occurred earlier in the year, a stronger signal would likely have been detected. However, this event did highlight the importance of the Deadwater, Wonnerup Inlet and the Lower Vasse River Wetlands as refuges for fish and as sources for recolonisation of areas where severe depletions in fish stock occurred either due to 'natural' stressors or fish kills.

The Deadwater and Wonnerup Inlet should be prioritised for protection from environmental degradation, as these regions act as fish refuge areas during times of poor water quality. Annual monitoring of the nearshore fish community in February is recommended to assess the condition of the fish fauna and would also help track long-termer changes, such as climate change and efforts to remediate the VWWS and its catchment. The disturbing trends in the biology and ecology of Black Bream also warrant further investigation. If another fish kill occurs the information in this report, together with any annual monitoring data, can be used to assess the effects and implications. Such analysis would be greatly helped by the presence of sound, quantitative data of the species and number of fish that die as a result of the event. Such events, although unwelcome, also represent the chance to collect biological data that can be used to help elucidate the causes and effects of the kill. Immediately following any fish kill an independent assessment of its effects (e.g. fish mortality and subsequent ecological effects) should also be undertaken (**see the Table of Management and Monitoring Recommendations** at the rear of this report (see also the recommendations in Tweedley et al., 2014)).

Fish faunas of the rivers of the Vasse-Wonnerup Wetland System


Nighfish *Bostockia porosa* from the lower Abba River.

Introduction

Introduced freshwater fishes are one of the major global threats to aquatic biodiversity. In south-western Australia, the number of introduced freshwater fishes in wild systems (i.e. 13) has recently surpassed the number of native species (i.e. 11) (Beatty and Morgan, 2013). Of great concern is the increased rate of recent introductions with a 44% increase in known introductions over past decade with 80% of those being aquarium species (Beatty and Morgan, 2013). In this region as in others, introduced fish and crayfish are known to directly impact ecosystems by predating on and competing with native species (Morgan et al., 2004; Beatty et al., 2005) and indirectly by altering habitat, water quality and introducing exotic diseases and parasites (Marina et al., 2008). While investment in preventing new introductions is the most cost-effective approach to mitigating impacts, monitoring for early detection of newly introduced species is crucial to increase the chance of containment or eradication.

Although no comprehensive survey has yet been conducted on the rivers of the Vasse-Wonnerup Wetland System (VWWS), a number of introduced fish and crayfish species are known to be present in the region (Morgan and Beatty, 2004). In addition, one of the key invasive species in the VWWS, Goldfish, is known to exacerbate algal blooms by re-suspending nutrients and stimulating algal growth through their feeding processes (Kolmakov and Gladyshev, 2003). Other ecological impacts include alteration of natural food webs and habitat destruction through feeding and burrowing activities.

Reducing or eradicating populations of introduced freshwater fish and crayfish in the VWWS directly addresses a previously identified key threat, *i.e.* impact of pest animals - introduced fish and crayfish species, in the Site Investment Guide (WRM, 2007). A long-term control program since 2003 in the Vasse River has resulted in stabilisation of the Goldfish population (Morgan and Beatty, 2005, 2007; Morgan et al., 2008; Beatty and Morgan, 2009). A further reduction (or eradication) in Goldfish biomass will help mitigate their impact such as re-suspending nutrients in the sediment that has been demonstrated to help fuel algal blooms.

While an intensive control program of Goldfish may be successful in this VWWS, additional information on the species is also required to refine these control efforts. The use of passive acoustic telemetry can yield valuable information on the movements of animals within their environment. The current study will therefore identify key seasonal habitats that act as refugia or aggregation sites for invasive Goldfish. We propose to do this by using tagged individuals to determine movement patterns (diurnally and seasonally), habitat associations, and to locate Goldfish aggregations. It is hypothesised that this will enhance the efficiency of the eradication program considerably, as well as allowing a robust management plan to be produced to combat this species.

Along with addressing the threat of Goldfish in the VWWS, it is also paramount that a broader survey be conducted to understand both the native fish communities (such as identifying areas of high conservation value), to detect any unknown introduced species, and develop and prioritise impact mitigation strategies for current and future introductions. In undertaking the control program via a risk management approach, the project will be consistent with the key principles of the Australian Pest Animal Strategy and ensure resources are used in a cost-effective manner.

This study is the first comprehensive survey of the freshwater fishes and crayfishes of the rivers of the VWWS and it aimed to both control existing introduced populations, and identify and prioritise any new incursions detected. It also aimed to engage key stakeholders and the broader local community on its activities to highlight the serious ecological and social impacts of introduced species in order to prevent new introductions. Finally, it aimed to develop a series of management recommendations to mitigate the impact of existing introduced species, and importantly also to prevent and detect any future introductions.

Materials and Methods

Sampling locations and dates

A total of 17 sites were chosen for seasonal sampling including five each in the Ludlow, Abba, and Sabina rivers, and two in the upper reaches of the Vasse River (Fig. 1). At each site, a 50 m long stretch of river was selected for repeated sampling over the duration of the project. Within this area, three stakes spaced 15 m apart from each other (at the 10, 25 and 40 m mark) were driven into the bank to mark the locations of fixed sampling points for the measurement of various environmental and habitat features (see below). Six seasonal sampling trips were conducted in total, commencing in March 2012 and concluding in June 2013.

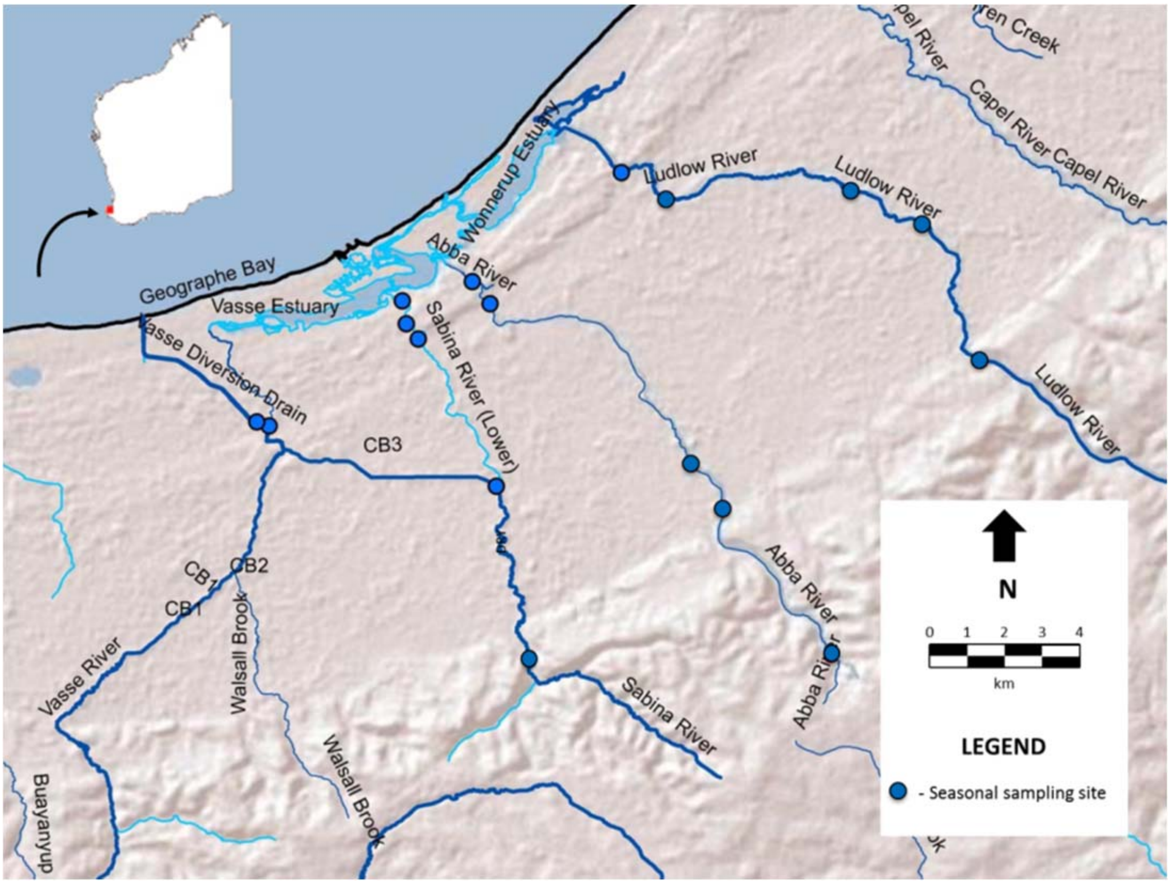

Fig. 1: Map of the Vasse-Wonnerup catchment showing locations of the 17 seasonal riverine sampling sites monitored during the present study.

Habitat and environmental variables

A handheld YSI Professional Plus multiparameter meter was used to measure water quality within each sampling site on each sampling occasion. The mean of three replicate measurements was calculated for water temperature, dissolved oxygen (% saturation and mg/L), conductivity (mS/cm), total dissolved solids (g/L), salinity (ppt), pH, and ORP (mV).

A suite of environmental and habitat features were measured at the three fixed locations at each sampling site. These included percentage cover of various river substrate types (estimated by randomly placing a 1 x 1 m quadrat on the substrate at three locations at each cross section), bank angle (left and right bank), erosion index (left and right bank), percentage riparian vegetation cover (understorey, midstorey and overstorey in a 5 m radius area centred on the fixed locations on both the left and right bank), Pen-Scott foreshore condition index (see Table 1 caption for explanation of grading system used), and stream width (see also Table 1 and Appendix 1). When surface water was present at a fixed sampling location, a cross-sectional profile of the stream was obtained using a handheld flow probe (Global Water FP111) to measure depth (cm) and flow ($cm.sec^{-1}$) at *ca* 10 points along each cross-section.

Digital photographs were taken at fixed locations at each site on each sampling occasion and compiled to track seasonal changes in riparian vegetation cover and surface water availability over the duration of the project (seasonal extremes are presented in Appendix 2).

Spatial and temporal distribution patterns

At each site on each sampling occasion, mean density estimates for fish and crayfish species were determined by conducting three replicate passes with a backpack mounted electrofisher (Smith-Root 12-A). All fish specimens captured were measured to the nearest 1 mm total length (TL), and crayfish specimens were measured to the nearest 1 mm orbital-carapace length (OCL) prior to their release at the point of capture, except introduced species (see *Introduced species removal* below).

Movement patterns of freshwater fishes

Movement patterns were determined at the seasonal sampling sites located uppermost and lowermost in the Sabina, Abba, and Ludlow rivers (Fig. 1) using fyke nets, but only when flow was detectable at the site.

Fyke nets consisted of two 5 x 0.8 m wings, a 1.2 x 0.8 m opening, and 5 m long pocket with two funnels all constructed from 2 mm woven mesh. One net was set facing upstream to capture downstream movements and another net set facing downstream to capture upstream movements. Fyke nets were set for three consecutive nights and checked once each 24 hour period (usually in the morning). Specimens captured in fyke nets were processed in the same manner as described.

Introduced species removal

In order to reduce the population of invasive Goldfish in the lower Vasse River, targeted removal of individuals took place on five separate occasions during the project in March 2012, May 2012, July 2012, November 2012, and March 2013. The extent of the area fished varied on each occasion depending on the river level (Fig. 2). A boat electrofisher (Smith-Root VVP-15B, Fig. 3) was used to stun Goldfish and a scoop net used to remove fish from the river. All Goldfish were euthanised in an ice slurry on board the electrofishing boat, and transported to the laboratory where the TL (to the nearest 1 mm) and wet weight (to the nearest g) of each fish was measured. All other introduced species captured during the study were euthanised in an ice slurry, stored in 100% ethanol, and later counted.

Fin damage by Eastern Gambusia

In order to determine the degree of fin damage that the prolific Eastern Gambusia was having, we used a fin-nipping damage score adopted from Gill et al. (1999), however, set a maximum damage score of 10 (caudal fin completely missing) and a minimum of zero (completely intact).

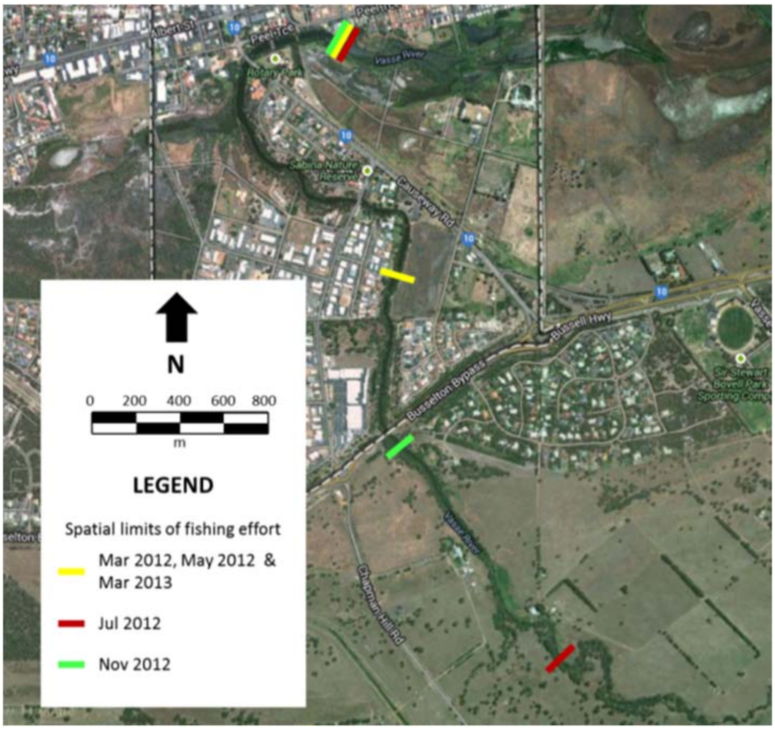

Fig. 2: Map showing the extent of boat electrofishing for Goldfish control in the lower Vasse River during the study.

Fig. 3: Electrofishing boat used in Goldfish control activities (left); a freshly caught Goldfish specimen, approximately 280 mm TL (right).

Acoustic tracking of Goldfish

Eight VR2W 69 kHz (VEMCO) acoustic receivers were deployed at sites throughout the lower and middle reaches of the Vasse River system (Figs 4, 5), a stretch of river known from previous studies (*e.g.* Morgan and Beatty 2007; Beatty and Morgan, 2009) to house a robust, self-sustaining population of Goldfish. Receivers were cable-tied to 25 mm diameter nylon rope and either tied to existing infrastructure (*e.g.* under bridges) or suspended below a 200 mm diameter solid styrene float anchored to the riverbed with a 4.5 kg galvanised sand anchor (Fig. 6). At one site (*i.e.* New River wetland) the receiver was cable tied directly to a 1.5 m long galvanised metal stake driven into the substrate (Fig. 6). V7-4L acoustic transmitters were used in this project following the advice of staff at the biotelemetry equipment manufacturer VEMCO, as they offered the longest transmission range of all transmitters appropriate for the size of the fish to be tagged (*i.e.* adult Goldfish >180 mm).

Acoustic tags were programmed to transmit a signal at a random interval of between 80 and 160 sec, and the estimated tag life was 388 days. The VR2W receivers record these transmissions whenever the tags are within detection range, which can vary depending on factors such as depth, flow, and substrate type (see Whitty et al., 2009). Range testing was performed for three of the eight acoustic receivers (*i.e.* butter factory, town bridge, and New River wetland, see Figs. 2,5) using a V7-4L range test tag that transmits an acoustic signal every 12 seconds. A handheld GPS unit (Garmin eTrex 30) was used to position the range test tag at progressive distance intervals of 25 m from the acoustic receiver up to a maximum distance of 250 m, and the percentage of detections by the receiver at each distance was recorded.

Fig. 4: Aerial photograph of the lower Vasse River showing locations and ID numbers of seven of the eight VR2W acoustic receivers deployed in December 2012. The receiver not shown here (*i.e.* #8 – Below Diversion Drain) was located a further ~3 km upstream of receiver #7.

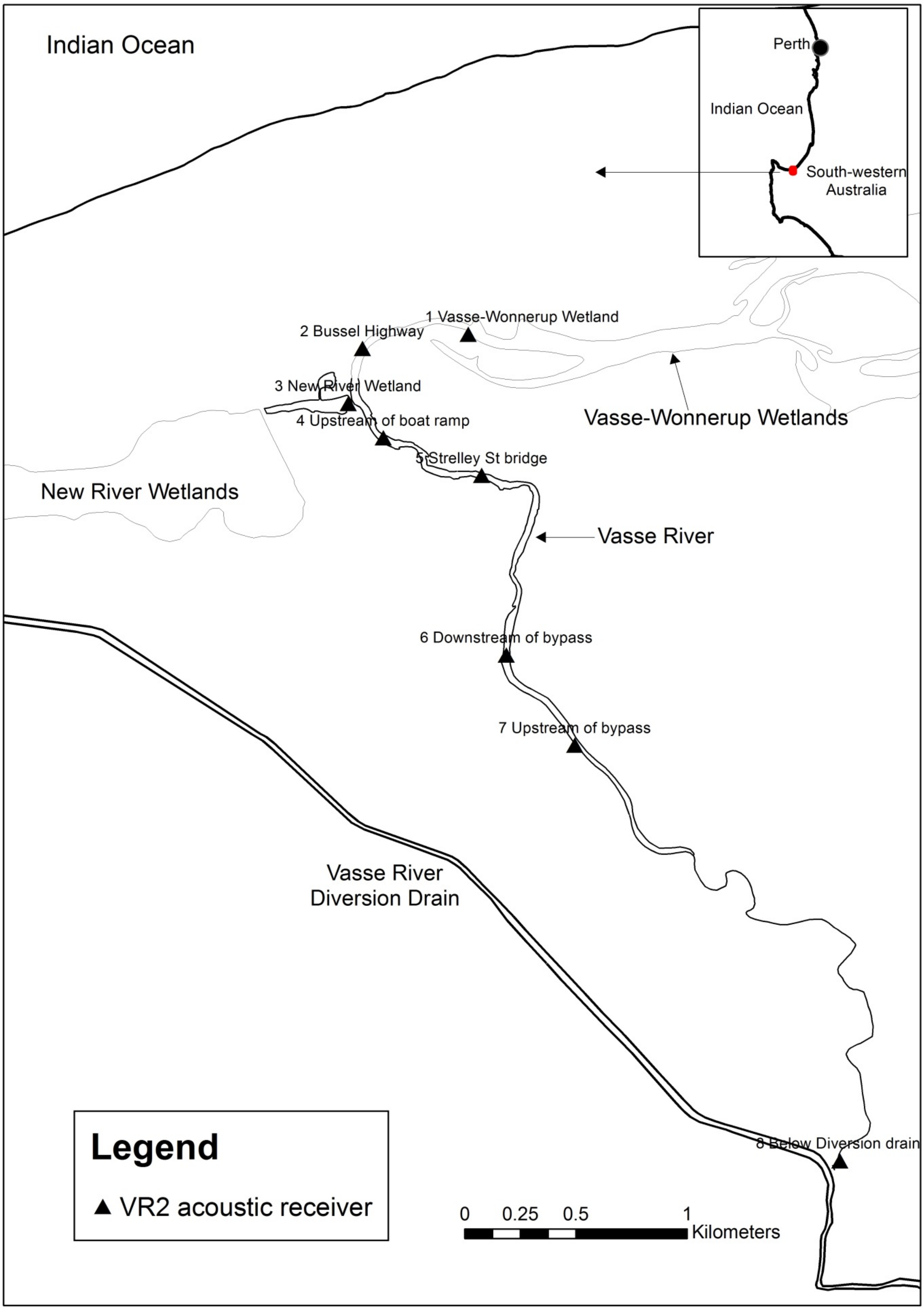

Fig. 5: Map showing the location of all acoustic receivers used (and names) in the tracking of Goldfish in the Lower Vasse River.

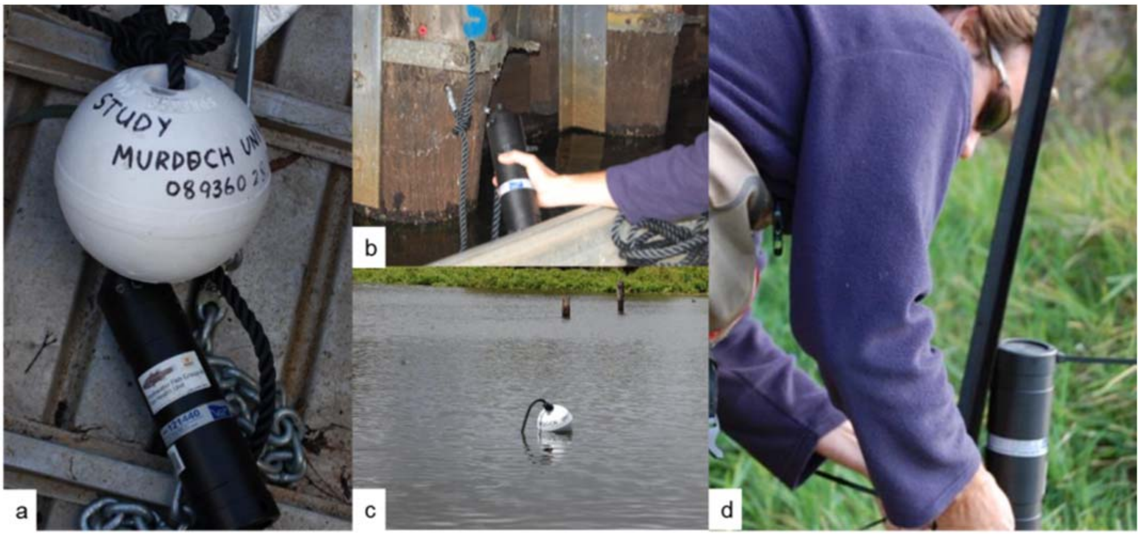

Fig. 6: (a) VR2W acoustic receiver unit attached to styrene float/sand anchor mooring prior to deployment; (b) deployment of receiver attached by rope to bridge infrastructure; (c) styrene float/sand anchor mooring deployment; (d) deployment of receiver attached by cable ties to metal stake (New River Wetland).

Following deployment and range testing of the acoustic receiver array, 21 Goldfish were captured from the Vasse River using a boat electrofisher (Smith-Root VVP 15-B) on 5/12/12 and 6/12/12. Fish were placed in an aerated 110 L holding tank, and moved a short distance (*i.e.* <1 km) to a temporary field laboratory, where each individual fish was anaesthetised by emersion in AQUI-S® solution (dilution rate of 0.125 mL per L of water). Following the loss of equilibrium and all signs of fin movement, an incision of 10 mm was made in the abdominal wall and a V7-4L acoustic tag was placed in the peritoneal cavity (Fig. 7). The incision was then closed with a single suture (4/0) (Fig. 7). All individuals were also implanted with a small (12 mm) Passive Internal Transponder (PIT) tag inserted via injection with a purpose-built applicator (BioMark HPT12 pre-loaded tags). Tagged individuals were then placed in oxygenated holding tanks and monitored throughout a recovery period, which was deemed completed when individuals maintained equilibrium and resumed full fin movement. The fish were then moved to a holding cage in the Vasse River and monitored twice daily for a period of three consecutive days to ensure that no signs of stress, infection (red or swollen areas around the incision), or tag loss were evident. All 21 fish were then liberated into the Vasse River.

Fig. 7: (a) Freshly captured Goldfish specimen; (b) anaesthetised specimen being prepared to have a small incision made in the abdominal wall; (c) V7-4L acoustic transmitter being inserted into the peritoneal cavity; (d) suturing up of the incision; (e) post-operative recovered specimen.

Manual downloading of the receivers took place in January, March, October 2013, and final download occurred in January 2014. Data were compiled using the VUE software package (VEMCO) and analysed and graphically displayed using Excel and Sigmaplot. Distance covered by tagged individuals was calculated by summing the absolute values of the stream distance (in km) between receivers for every consecutive pair of detections for each fish. This yields an estimate of the minimum distance (D_{min}) a fish has covered. The actual distance covered is likely to be much greater as movements outside the detection range of receivers, and small-scale movements within the detection range of receivers cannot be determined from the passive telemetry data.

Results and Discussion

Fishes and decapods in rivers of the Vasse-Wonnerup

Three native freshwater fish species were recorded in the rivers of the VWWS, namely Western Minnow (*Galaxias occidentalis*), Western Pygmy Perch (*Nannoperca vittata*), Nightfish (*Bostockia porosa*) (Fig. 8a-c). Three native estuarine fishes were also captured: Bluespot Goby (*Pseudogobius olorum*), Sea Mullet (*Mugil cephalus*), and Western Hardyhead (*Leptatherina wallacei*) (Fig. 8d-f). Of the estuarine fishes, only the Bluespot Goby was captured regularly, the other two were rare at seasonal sampling sites. Sea Mullet was only encountered during boat electrofishing in the Vasse River. Two introduced fishes were also found in rivers of the Vasse-Wonnerup: Eastern Gambusia (*Gambusia holbrooki*) and Goldfish (*Carassius auratus*), the latter was found only in the Vasse River (Figs 8g-i).

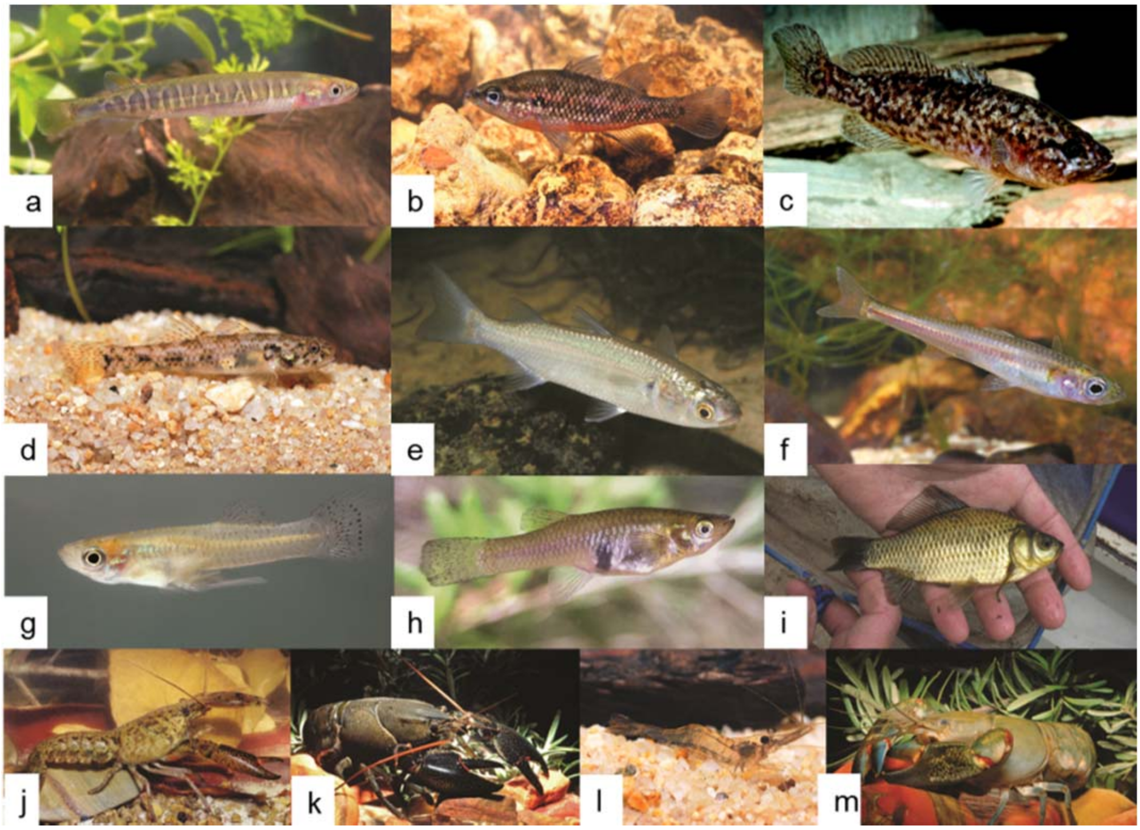

Fig. 8: Fish and decapod crustacean species captured during the study: a) Western Minnow; b) Western Pygmy Perch; c) Nightfish; d) Bluespot Goby; e) Sea Mullet; f) Western Hardyhead; g) Eastern Gambusia male; h) Eastern Gambusia female; i) Goldfish; j) Gilgie; k) Koonac; l) Southwest Glass Shrimp; m) Yabby.

Decapod crustaceans recorded during the study included three native species: Gilgie (*Cherax quinquecarinatus*), Koonac (*C. preissii*), and Southwest Glass Shrimp (*Palaemonetes australis*), and the introduced Yabby (*C. destructor*) (Fig. 8j-m).

Spatial and temporal patterns in distribution

Sabina River (Fig. 9)

Water was present in every season at all of the study sites in the Sabina River, except at the uppermost site (*i.e.* Piggott Road), which only held water in winter and spring. The only species collected from the uppermost site was the Gilgie. Sites further downstream housed between five and seven species, with the highest species richness recorded at Tuart Drive and Bussell Highway. Of the native fishes, both Nightfish and Western Minnow were the most widely distributed (at 4 of 5 sites), whilst Western Pygmy Perch and Bluespot Goby were slightly less widespread (at 3 of 5 sites). Gilgies were found at most sites (4 of 5) and the Southwest Glass Shrimp at just two of the lower sites. The most numerous species in the Sabina catchment was Eastern Gambusia which dominated catches, particularly in the summer samples. The Yabby was also recorded at one site in the middle reaches of the Sabina.

During the dry summer and autumn period, all four native fish species recorded in the system were found in at least one of the lower three sites, all of which were located within 3.3 km of the interface with the Vasse Estuary. In winter and spring, Western Minnows were absent from the lower sites but did occur at the Oates site, located in the middle reaches of the catchment. Nightfish maintained their presence in the lower reaches of the catchment throughout the year and were also present at the Oates site in spring. Western Pygmy Perch were absent at all sites in winter but were present at the Oates site in spring as well. Bluespot Goby was resident in all seasons in the lower catchment but did not penetrate to the middle reaches of the catchment. The seasonal distribution of the two native decapods followed a similar pattern to that of the goby. Introduced Eastern Gambusia occurred at all sites in most seasons, with the exception of the uppermost site.

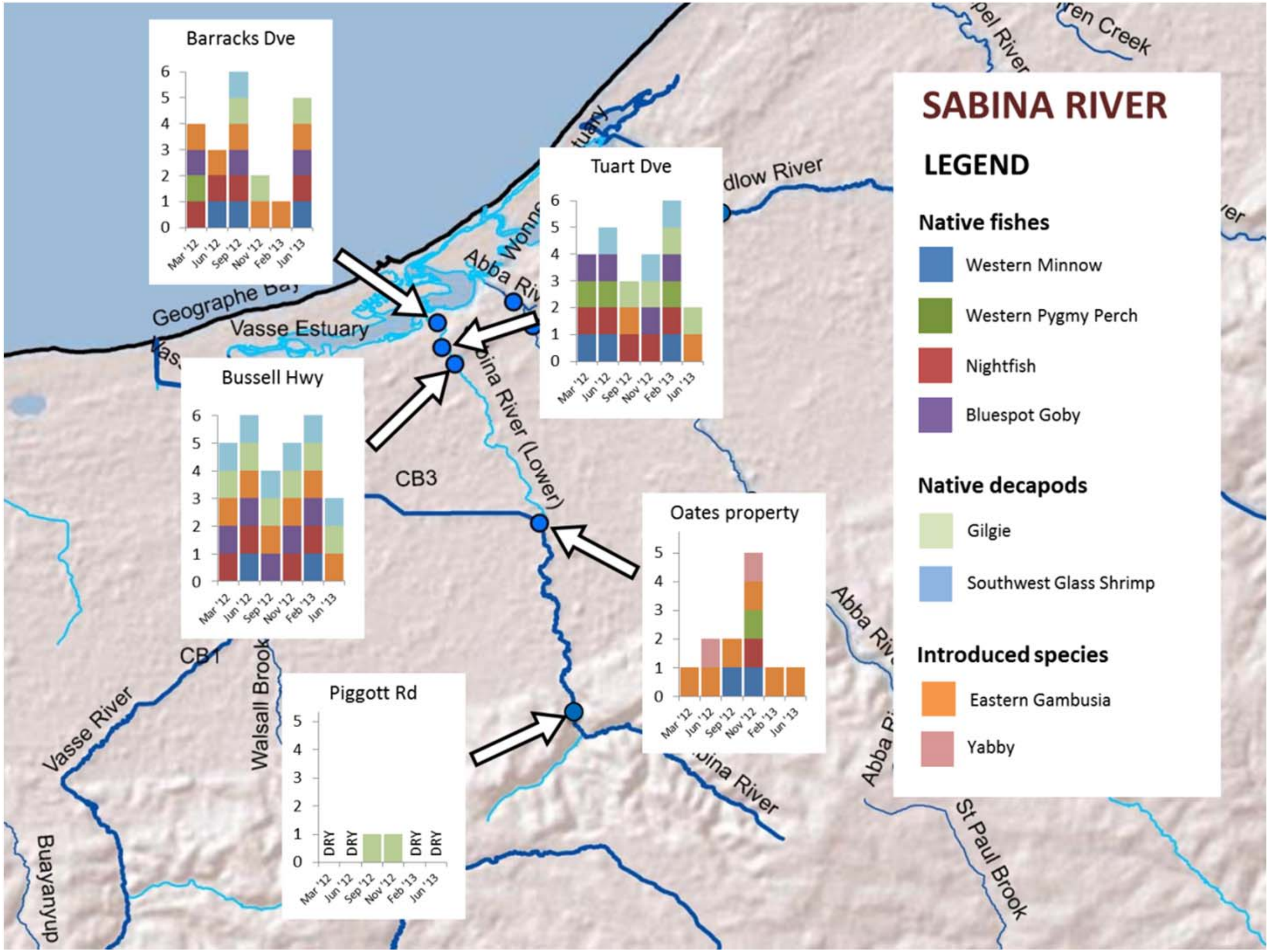

Fig. 9: Results of seasonal sampling at sites in the Sabina River, February 2012 - June 2013.

Abba River (Fig. 11)

The only sampling site that held water year round in the Abba was the Bussell Highway site. All other sites dried periodically during the summer and autumn seasons. The two lowermost sites housed seven different species of fishes and crayfishes, whilst the upper three sites housed between two and three species. Western Minnow (4 of 5 sites) and Nightfish (3 of 5 sites) were the most widely distributed fish species, whilst Western Pygmy Perch, Bluespot Goby and the introduced Eastern Gambusia were all recorded in the two lowermost sites only. Of the decapods, the Gilgie was found at all sites, with the Southwest Glass Shrimp occurring at the two lowermost sites only. The native Koonac was also recorded at one site in the middle reaches of the catchment (Ludlow-Hithergreen).

At the sites where it occurred, Eastern Gambusia was the most abundant species, with densities highest during the summer/autumn period. In terms of native fish abundance, all species were present in roughly comparable numbers; no species was clearly dominant.

The lowermost sites, both located within 3 km of the interface with the Vasse Estuary, acted as a baseflow refuge for the majority of the aquatic macrofaunal diversity in the Abba River. With the onset of flow in winter and spring, the Western Minnow displayed some upstream dispersal to the middle reaches (Williamson Road). Also, Western Minnow and Nightfish were recorded at the uppermost site (Vasse Highway), located in the headwaters about 22 km upstream of the estuary, during the flow period. However, it is suspected that these fish may have moved into this seasonally inundated habitat from refuge pools located on nearby farmland (*e.g.* artificial onstream dams), rather than by migrating from the lower reaches. The appearance of native decapods (Gilgie and Koonac) in the impermanent sites located in the middle and upper catchment was not surprising given the ability of both of these species to persist in seasonally inundated habitats by retreating to moist burrows (Fig. 10).

Of note was the major crayfish death event that occurred at the Bussell Hwy site on the Abba River in early June 2013 (Fig. 10). This was caused by a truck rollover spilling sewerage into the system. At the time of sampling, hundreds of Gilgies were recorded dead and dying on the banks that had walked out likely as a result of extremely low dissolved oxygen levels that would have been expected shortly after the spill occurring.

Fig. 10: (top left): Gilgie burrows in undercut bank at Abba River (Vasse Hwy site March 2012), (other photos) the mass walk-outs of Gilgies in the Abba River (Bussell Hwy) that occurred in early June 2013 in response to sewerage spill.

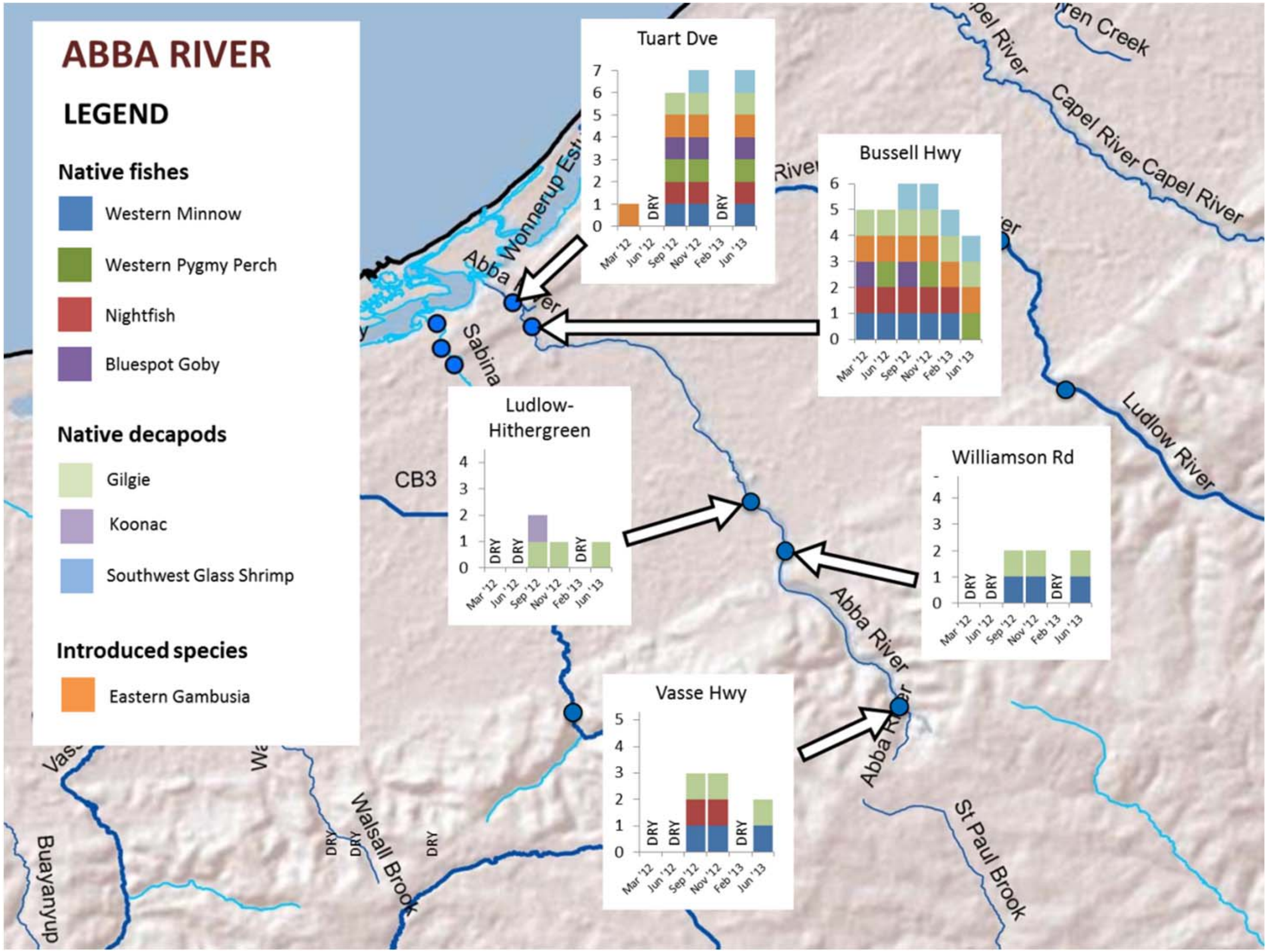

Fig. 11: Results of seasonal sampling at sites in the Abba River, March 2012 - June 2013.

Ludlow River (Fig. 12)

The lowermost site (Tuart Drive) was the only Ludlow sampling site that held water in all seasons. Not surprisingly, it housed the highest diversity of aquatic species (7 species). The other sites in the catchment housed between two and four species. Western Minnow, Western Pygmy Perch, and introduced Eastern Gambusia were the most widespread fish species (3 of 5 sites). The native decapods (Gilgie and Koonac) were also found at 3 of 5 sites. These two decapod species did not overlap in distribution in the Ludlow River except at the Bussell Highway site. Koonacs were distributed in the middle reaches of the catchment (*i.e.* Bussell Highway to Warns Rd), whereas Gilgies were recorded at sites in the lower catchment and at the uppermost site. The two estuarine stragglers (Bluespot Goby and Southwest Glass Shrimp) only occurred at the lowermost site. The introduced Yabby was also recorded at the Bussell Highway site.

As in the other catchments, Eastern Gambusia dominated fish numbers at the sites where it occurred. Of the native species, densities were roughly similar between all species, although Southwest Glass Shrimp were the most abundant species at the Tuart Drive site in the lower reaches.

Three fish species (*i.e.* Western Minnow, Western Pygmy Perch, Eastern Gambusia) dispersed upstream to sites in the middle reaches of the catchment (~15 km upstream of mouth) during the winter/spring flow period. Nightfish was also recorded at the uppermost site (~20 km upstream of mouth) in winter.

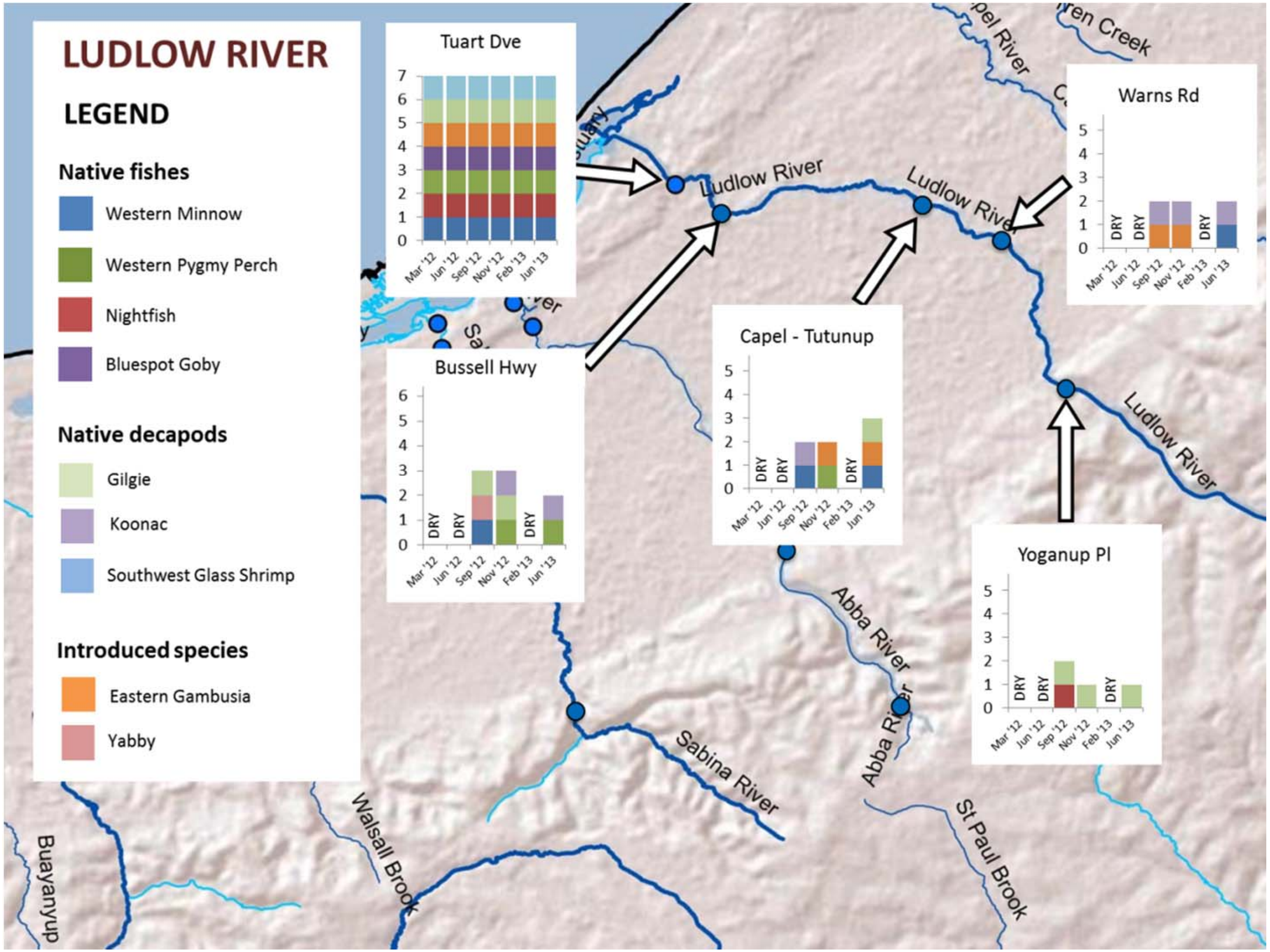

Fig. 12: Results of seasonal sampling at sites in the Ludlow River, March 2012 - June 2013.

Movement patterns of freshwater fishes

Most native freshwater fishes of the south-western Australia display strong seasonal movements within river catchments (Beatty et al., 2014). Typically fish move upstream in winter and spring to breed in seasonally inundated habitats of creeks and wetlands in response to increasing flow rates (Beatty et al., 2014). At the conclusion of the breeding period, a movement downstream in rivers is usually evident where permanent refuge pools are generally more abundant.

Captures in fyke nets during the present study reflected these general movement patterns. At sites located in the lower reaches of catchments, the three common native freshwater species (*i.e.* Western Minnow, Western Pygmy Perch, and Nightfish) were recorded moving predominantly upstream during late winter and spring sampling (Fig. 13a-c). Downstream migrants, although still fewer in number than upstream migrants, showed a proportional increase in November, a time of year when breeding has mostly been completed for these species (Fig. 13 a-c).

Captures of other species, including the introduced Eastern Gambusia, were very low in number and/or highly variable among catchments and seasons (Fig. 13d). Consequently, it was difficult to discern any clear trends in their movement patterns. Likewise, numbers of most species captured in fyke nets at sites located in the upper reaches of the three catchments were extremely low (Fig. 13). The one exception was the Gilgie, which was captured moving in both directions in roughly equal proportions (Fig. 13e). However, this was probably more a function of the propensity for this species to enter fyke nets rather than a reflection of any significant migratory movements. Native decapod crustaceans readily enter fyke nets and other fishing gear types (*e.g.* box traps), presumably for the purpose of seeking out what is likely to be perceived as an opportunity to exploit a newly available shelter or food resource in their habitat (*i.e.* the net itself).

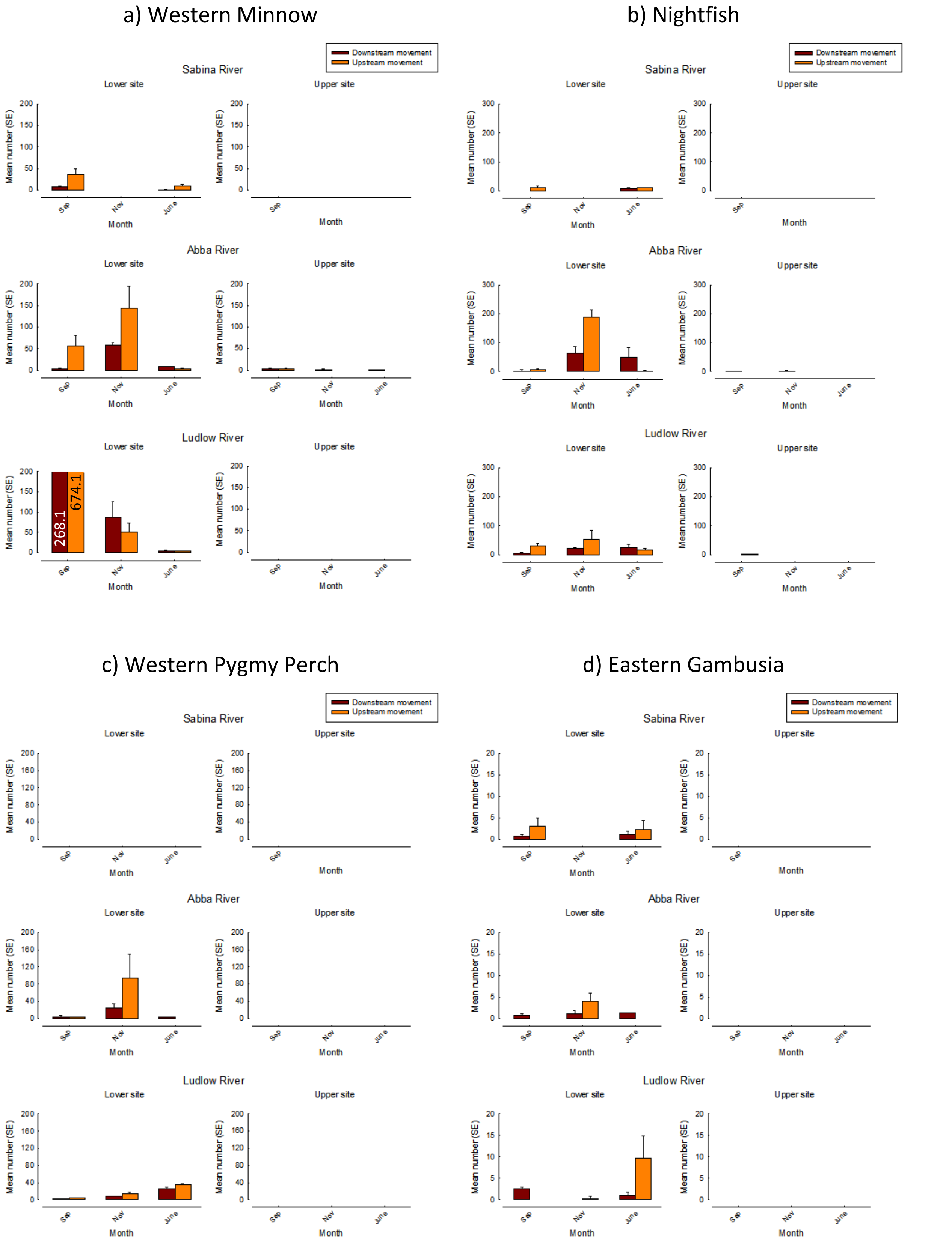

Fig. 13: Upstream and downstream movement of seven aquatic species (a-g) captured in the Sabina, Abba, and Ludlow rivers during three seasons (*i.e.* Sep = spring, Nov = spring/summer, Jun = winter).

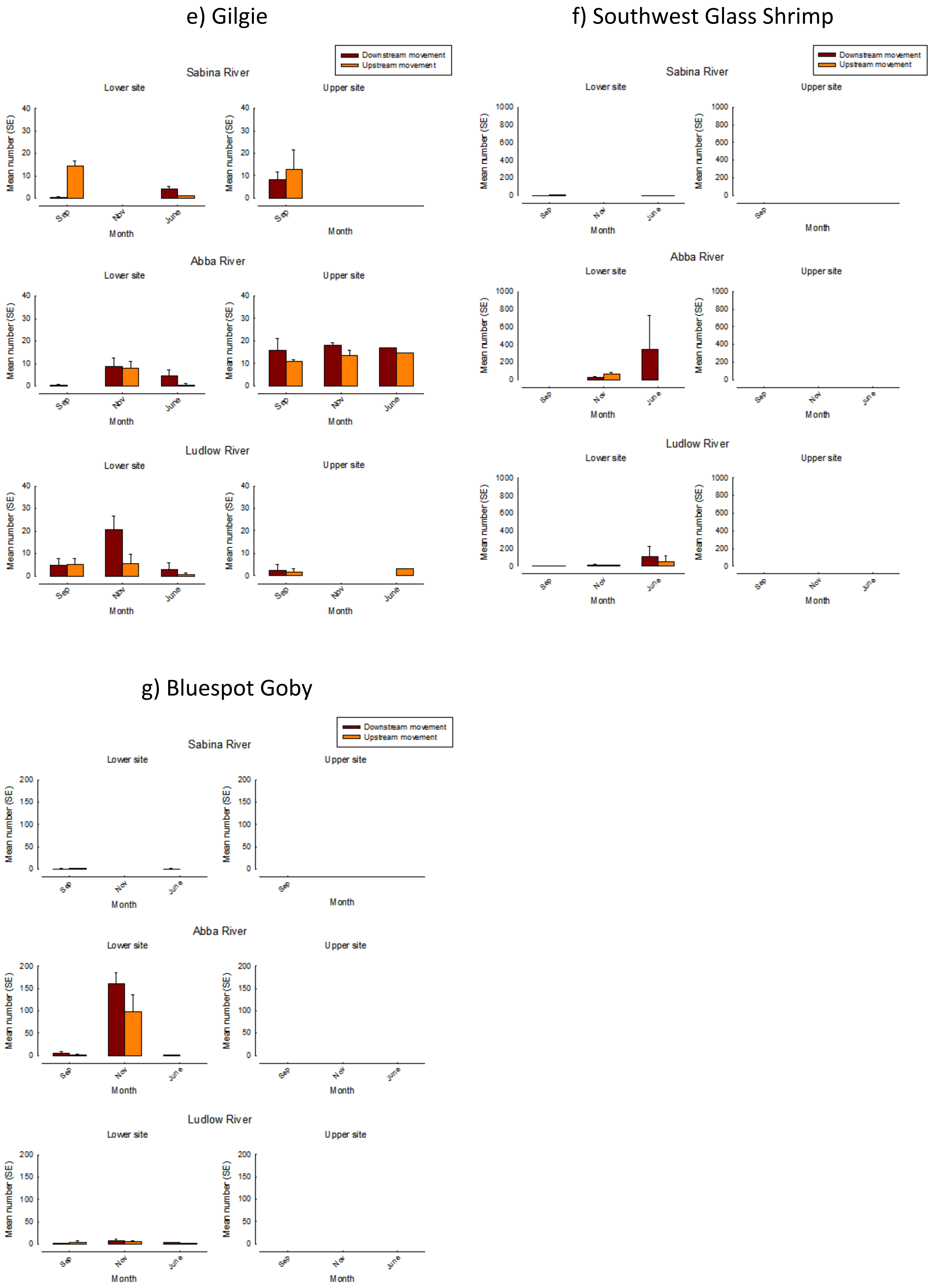

Fig. 13 cont.: Upstream and downstream movement of seven aquatic species (a-g) captured in the Sabina, Abba, and Ludlow rivers during three seasons (*i.e.* Sep = spring, Nov = spring/summer, Jun = winter).

Habitat and environmental variables

The habitat and environmental characteristics of sites were relatively consistent in all three catchments; depending on the distance upstream of the estuary. A number of sites in the lower reaches (*i.e.* within about 3 km of the estuary) were located within or near the boundaries of Ludlow National Park. Most of these sites had a riparian zone that was heavily weed infested or weed dominated (*i.e.* B2/B3 on the Pen-Scott index) (Table 1), with reasonable shading of the streamline (typically ≥ 30%) provided by native tree species including Tuart (*Eucalyptus gomphocephala*), Flooded Gum (*E. rudis*), and Peppermint (*Agonis flexuosa*). Bank erosion at these sites ranged from moderate (~5) to low (~8.5) (Table 1).

The middle reaches of the Vasse-Wonnerup catchments (*i.e.* between Bussell Highway and the foot of the Darling Scarp) comprise land that is mostly developed for agriculture, livestock grazing, and mineral sand mining. Sites in these areas ranged in their level of riparian degradation from heavily weed infested (B2) to eroded (C3). Two sites (*i.e.* Sabina River – Oates property and Vasse River – Chapman Hill Road) consisted of channelised, diversion drains, built to bypass the Vasse-Wonnerup Estuary and mitigate the risk of flooding in Busselton. Stream shading ranged from >50% at sites where some native riparian overstorey remained intact to 0% at the heavily modified diversion drain sites. Bank erosion at these sites ranged from moderately high (~3) to low (~8). Most sites in the middle reaches of the catchments typically only held water in winter and spring with the exception of the two Vasse River sites and the Sabina River – Oates Property site (diversion drain).

Three study sites were located in the upper reaches of the catchments. Two of these were located at slightly higher elevation on the Darling/Whicher Range (*i.e.* Sabina River – Piggott Road and Abba River – Vasse Highway), whilst the third was located at the foot of the Darling Scarp (*i.e.* Ludlow River – Yoganup Place). All three sites exhibited highly seasonal flow (only holding water in winter and spring), and were characterised by near pristine riparian condition (A2) and >60% stream shading provided by native overstorey species including Jarrah (*Eucalyptus marginata*), Marri (*Corymbia calophylla*), Yarri (*E. patens*), and Flooded Gum (*E. rudis*). Bank erosion at these sites was low (~7 and above).

Table 1. Mean values of various environmental/habitat characteristics of seasonal sampling sites. Pen-Scott foreshore condition index was graded as follows: A1 – pristine, A2 – near pristine, A3 – slightly disturbed, B1 – degraded/weed infested, B2 – degraded/heavily weed infested, B3 – degraded/weed dominated, C1 – erosion prone, C2 – soil exposed, C3 – eroded, D1 – eroding ditch, D2 – freely eroding ditch, D3 – weed dominated drain.

		Distance f/mouth (km)	**Erosion Index** 1-10 (1 = 100% eroded, 10 = <5% eroded)		**Bank Angle** 1-3 (1 = <30°, 2 = 30-60°, 3 = 60-90°)		**Pen-Scott**	**Shade** (%)		**Overstorey** (% cover)		**Midstorey** (% cover)		**Understorey** (% cover)	
CATCHMENT	**SITE**		mean	se	mean	se		mean	se	mean	se	mean	se	mean	se
Sabina	Barracks	1.17	5.00	0.40	2.83	0.18	B3	80	6.16	72.5	10.75	6.67	2.71	84.17	12.36
	Tuart	2.06	8.50	0.37	2.83	0.18	B3	10	6.93	17.5	13.1	5	5.48	95	3.74
	Bussell	3.24	6.83	0.52	2.67	0.23	B3	45	7.87	27.5	13.4	20	9.7	91.67	5.42
	Oates	10.23	10.00	0.00	2.00	0.00	D3	10	6.93	0	0	0	0	50.83	14.31
	Piggott	17.61	6.83	1.15	2.33	0.46	A2	80	5.48	42.5	13.32	46.67	11.8	53.33	7.7
Abba	Tuart	1.68	4.67	0.73	2.50	0.37	B2	30	17.89	19.17	14.03	17.5	5.43	45.83	9.94
	Bussell	2.86	8.50	0.37	3.00	0.00	B3	52.5	15.98	53.33	13.9	38.33	14.47	97.5	1.87
	Ludlow-Hithergreen	13.29	6.67	0.54	2.83	0.18	B2	57.5	19.99	53.33	16.83	75.83	71.1	65.83	6.39
	Williamson	15.54	3.17	0.52	2.33	0.37	C1	80	6.16	52.5	11.81	10.83	3.58	59.17	12.92
	Vasse Hwy	23.14	8.00	0.57	2.50	0.37	A2	90	5.10	82.5	4.42	28.33	3.65	24.17	4.98
Ludlow	Tuart	3.22	7.67	0.92	2.33	0.37	B3	37.5	16.96	63.33	8.79	19.17	4.78	63.33	28.05
	Bussell	5.99	7.67	0.46	2.50	0.24	B2	39.17	10.72	62.5	13.17	16.67	5.23	78.33	5.42
	Capel-Tutunup	12.7	2.67	0.23	2.00	0.49	C3	9.17	3.29	13.33	14.61	0	0	89.17	4.34
	Warns	15.65	7.83	0.59	2.50	0.24	B3	41.67	9.87	52.5	7.97	13.33	3.65	79.17	11.26
	Yoganup	22.71	6.67	0.88	3.00	0.00	A2	60.1	10.04	63.33	8.68	21.67	6.73	70.83	4.1
Vasse	Chapman Hill Rd	5.71	4.83	0.34	3.00	0.00	D3	0	0	0	0	1.67	1.83	100	0
	Below div. drain	5.75	7.50	0.84	2.00	0.40	B3	53.33	16.23	51.67	13.17	10	5.1	95.83	2.2

The composition of the river substrate was relatively consistent across all sites with the streambed consisting mainly of sand and silt (Fig. 14). Besides large woody debris (LWD), all other substrate types comprised a relatively small proportion of the streambed at the majority of sites (Fig. 14).

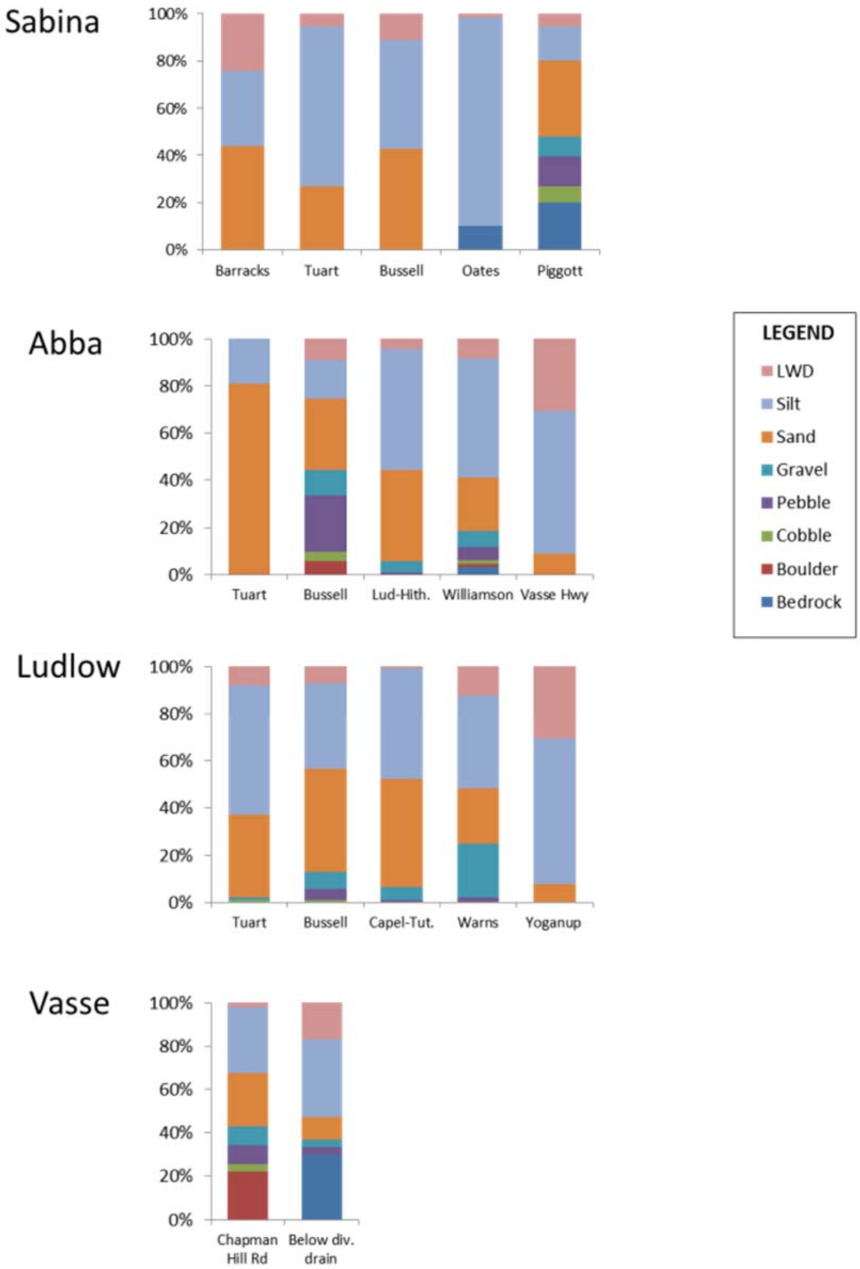

Fig. 14. Cumulative percentage composition of various substrate types at seasonal sampling sites in the Vasse-Wonnerup catchment.

As is typical of most small rivers in south-western Australia, the rivers of the Vasse-Wonnerup catchment displayed a highly seasonal flow regime. In 2012, flow was absent at all except one site during March/May sampling, and was only detected during September sampling at most sites, although there were some sites (mostly in the Abba River) that had detectable flow into November. Flow had ceased at all sites by the following February sampling period, but had resumed at all sites except the upper Sabina River (Piggott Road) in June 2013 following decent autumn rainfall. Salinity levels were intrinsically linked to the prevailing flow regime, with the highest levels occurring during the hottest and driest period in March 2012, and the lowest levels coinciding with a period of strong surface discharge in September 2012 (Fig. 15 b,d). Water temperatures followed a similar pattern to salinity but the timings of the recorded minima and maxima in the various catchments were slightly different. Temperatures peaked during November 2012 and February 2013 sampling and were at their lowest in May 2012 (Fig. 14a). No clear seasonal trends were evident in the dissolved oxygen concentration data (Fig. 15c).

a)

b)

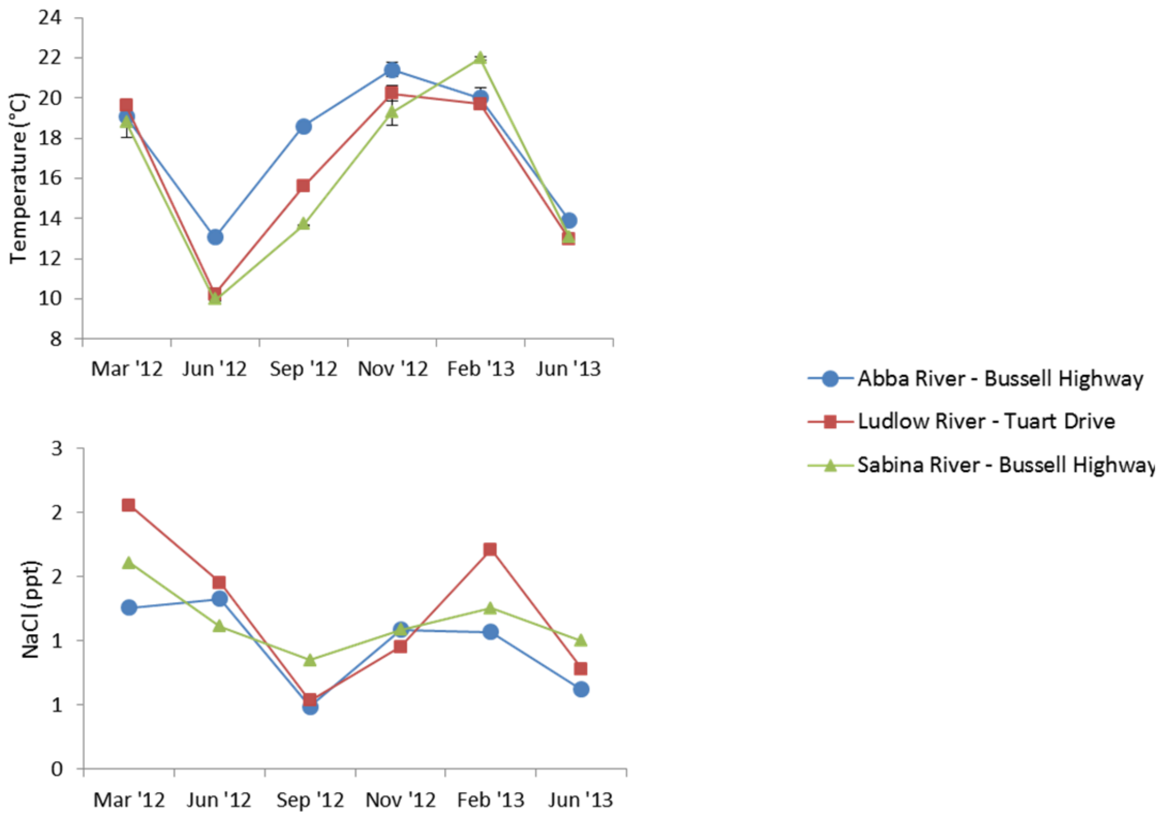

Fig. 15: Seasonal variation in a) mean water temperature, b) mean salinity, c) mean dissolved oxygen, and d) discharge at selected sites in the Sabina, Abba, and Ludlow rivers during the study.

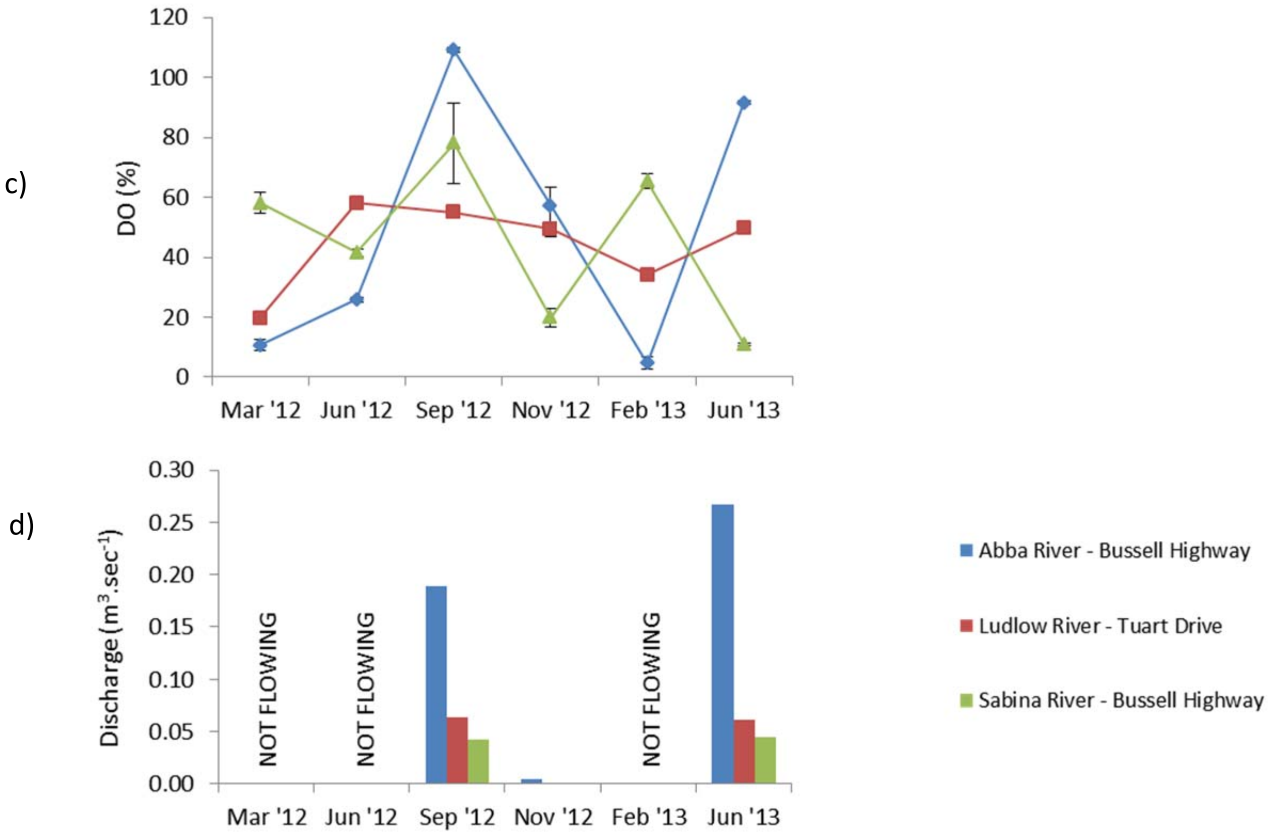

Fig. 15 cont.: Seasonal variation in a) mean water temperature, b) mean salinity, c) mean dissolved oxygen, and d) discharge at selected sites in the Sabina, Abba, and Ludlow rivers during the study.

Introduced species removal

The removal of Goldfish became the focus of our introduced species control efforts during the study when it became apparent from our seasonal sampling data that only two other introduced species occurred in the Vasse-Wonnerup catchment (*i.e.* Eastern Gambusia and Yabby), both of which are notoriously difficult to control effectively. This highlights the adaptive approach the project adopted where constant review of our data with the VWPFSC enabled decisions to be made to adapt our monitoring and control efforts.

Targeted boat electrofishing efforts resulted in the capture and removal of 842 Goldfish from the lower Vasse River. Notably, this amount outnumbers those removed in nine previous removal events combined, stretching back to December 2003. There was a consistent trend in the numbers of Goldfish removed (usually ≤ 100 individuals per removal event) with one notable exception: the first removal event of the current study in March 2012, in which over 550 specimens were captured (Fig.

15). This unprecedented catch was probably caused by the drying of habitat in the adjoining New River Wetland, due to exceptionally hot and dry conditions over the preceding summer, which resulted in a mass downstream migration of Goldfish into the lower Vasse main channel where they were subsequently caught. This event helped improve the success of this project in terms of boosting the numbers of introduced fish removed, but more significantly, it highlighted the importance of the New River Wetland for this population. This information will greatly assist future Goldfish control efforts in this system. Following the exceptionally large catch in March 2012, the numbers removed declined noticeably with each control event throughout the remainder of the year (Fig. 16), reflecting the fact that the Goldfish population had been greatly reduced by the removal program. However, following spring breeding and a flush of new recruits in the population, the number of Goldfish removed increased to over 100 in March 2013 (Fig. 16).

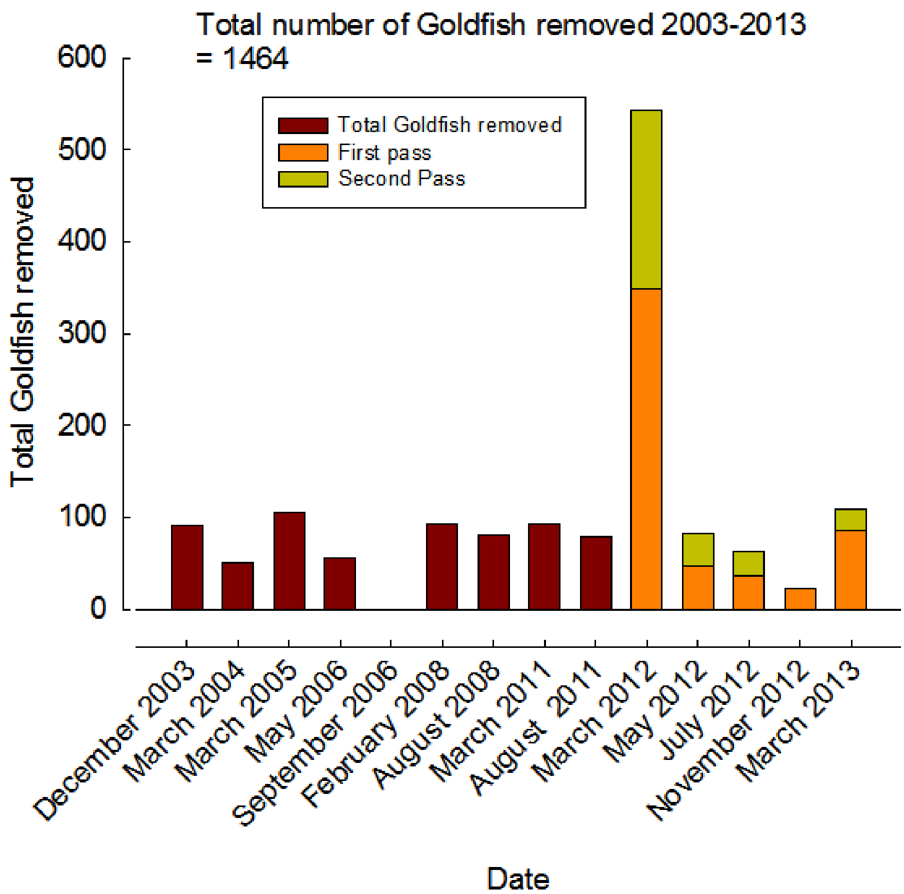

Fig. 16: Numbers of Goldfish removed from the lower Vasse River, 2003-2013. Only the final five removal events were undertaken as part of the current project; however, note that all removal events involved the same methodology and comparable eradication effort.

Of the other introduced aquatic species captured in rivers of the Vasse-Wonnerup, a total of 26,081 Eastern Gambusia and five Yabbies were removed. The low numbers of Yabbies captured during the study reflects a low relative abundance for this species in these rivers. Importantly, no other introduced species were detected as part of the comprehensive seasonal monitoring program in the estuary or rivers of the Vasse-Wonnerup. This project has provided a valuable baseline dataset for long-term monitoring of both the native and introduced fish communities in these rivers. A key recommendation is ongoing community education on the impacts of introduced aquatic species to help mitigate the risk of additional introductions (see Recommendations).

Fin damage by Eastern Gambusia

All native fish received some damage to their fins that typified attack by Eastern Gambusia (Fig. 17). Fin-nipping was most severe on Western Pygmy Perch and peaked at 1.55 (±0.30) in March 2012 falling to a minimum of 0.21 (±0.11) in September 2012 (Figure 2). The life-cyle of the Eastern Gambusia lifecycle results in its densities being greatest in late summer – early autumn and it appeared that the degree of damage to native species was associated with the densities of the introduced species.

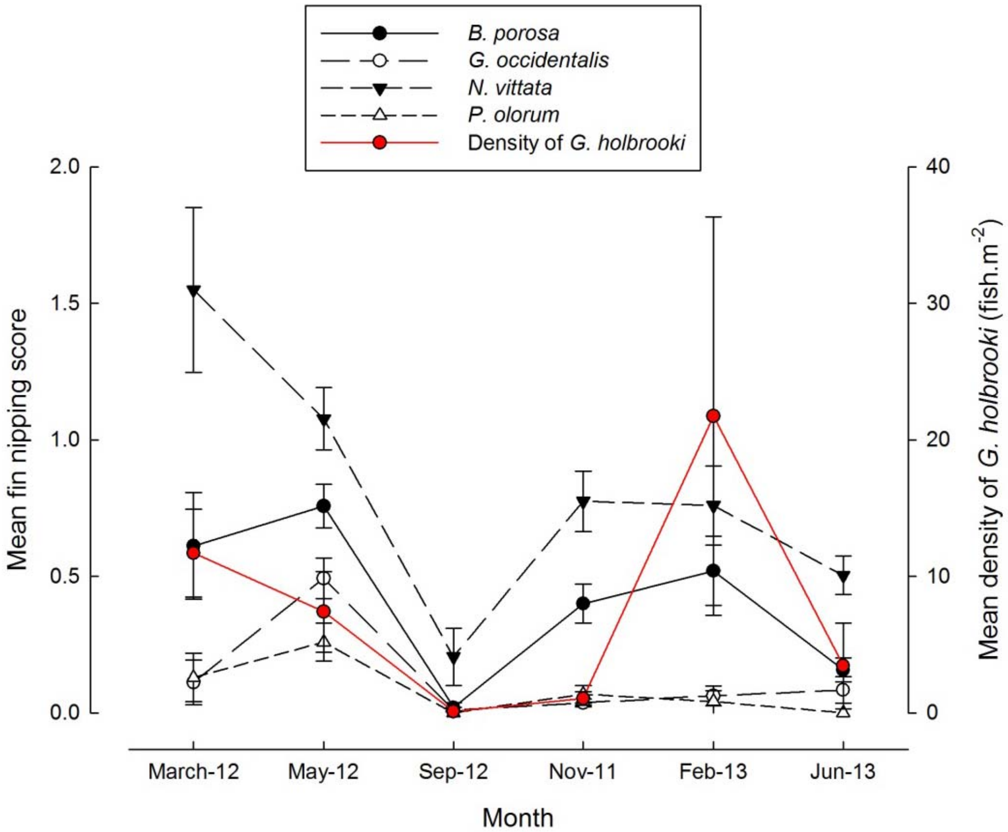

Fig. 17: Combined (across all rivers sampled) mean fin-nipping scores for each native fish captured in each month sampled. N.B. also presented is the mean density of *G. holbrooki* in each sampling month.

Acoustic tracking of Goldfish

Overall number of detections and site fidelity

In total, each fish was detected an average of 27189 (±5573 S.E.) times in the receiver array. Five of the 21 fishes were excluded from the analysis (i.e. 7574, 7575, 7580, 7583 and 7593) as they were not recorded for more than 30 of the 391 day period and 7576 was also excluded because it appeared to have died (recorded at one receiver constantly) (Table 2). The data for subsequent analysis was also trimmed slightly to encompass the period December 15 2012 - December 14 2013 (i.e. 365 days). This was undertaken to allow for more standardised analyses among fishes (i.e. the period ends on the last full day before the first battery died) and to account for any unusual behaviour the fish may have displayed in the 10 day period following tagging.

Examination of the remaining 15 fishes revealed that there were a total of 403728 detections over the 1 year period (Table 2). The fishes spent the majority of their time within the array with several notable exceptions of those with lengthy non-detection periods (Fig. 18). Of these, most displayed "home-ranging" tendencies, where regular detections were made at one or two receivers, interspersed with comparatively less detection at other receivers in the array (Fig. 18). A minority of tagged Goldfish did not display such site fidelity, with large numbers of consecutive detections at any one receiver being the exception for these individuals (e.g. #7576, #7579, #7581, Fig. 18).

No fish were detected at the eighth receiver downstream of the bypass (Figs 18, 19). Highest site fidelity was recorded at the receiver upstream of the boat ramp adjacent to the Busselton Shire (Figs 2, 3) which was situated within the infestation of water lilies that expanded rapidly throughout the one year study to eventually completely prevent boat passage (Fig. 20). Fourteen of the 15 fish (93%) visited that site at least once and on average fish visited this site on 27% of the total days throughout the year (Table 3, Fig. 18). Other key habitats included the New River Wetlands (80% of fish detected at least once and on average each fish detected on 22% of all days), Strelley St (93% of fish detected and average each fish detected on 21% of all days) (Table 3, Fig. 19). The least visited site (other than the receiver below the Diversion Drain) was the Vasse-Wonnerup Wetlands immediately downstream of the Butter Boards; however, this site was still visited by 47% of the fishes at an average rate of ~5% of all days (Table 3, Figs 18, 19).

Two fish (#7585 and #7591) spent considerable time outside of the array likely in the lengthy (~3 rkm) region between receiver 7 (upstream of the Bypass) and the Diversion Drain (that had zero detections). This was assumed as there were long periods (>3 months) between detections at the former receiver during which time they were not detected at the other receivers (Fig. 18). One fish (#7582) spent considerable time at receiver 1 in the Vasse-Wonnerup Wetlands (downstream of the array) and was last detected at that site in mid-September (Figure 18).

Seasonal patterns in movement

There were clear seasonal shifts in habitat usage for most fish. Of particular note was the decline from the summer/autumn period compared to the winter/spring period in the number of fish and detections at the two receivers at Bussell Hwy and downstream of the Bypass (Fig. 18). The catch-per-unit-effort (CPUE) had been recorded as consistently high at the Bussell Hwy site and around the Boat Ramp during previous Goldfish control events. Those results combined with the acoustic telemetry data from this study provide solid evidence to indicate that these sites are likely to be the most important refuge or shelter site for Introduced Goldfish in the Vasse River during the summer/autumn period. Conversely, a considerable increase in the number of fish was detected in the New River Wetland during this high flow period (Fig. 19). The receiver upstream of the boat ramp had relatively consistent residency throughout the study period (Fig. 19).

During the month encompassing the known breeding period of Goldfish (birth day was assigned as September 1st in Morgan and Beatty, 2007) between 15th August and 15th September, there were clear preferences for regions (Table 2, Figs 18, 19). Equal numbers of fish (i.e. nine) were detected at the New River Wetlands and the Boat Ramp during that time (Table 2). The third most number of fish (i.e. 8) were detected at the Strelley St receiver followed by upstream (four fish) and downstream (three fish) of the bypass with the receiver at the upstream end of the Vasse Wonnerup Wetlands detecting one fish (Table 2). This suggests that the New River Wetland and the habitat upstream of the Boat Ramp (likely to be within the infestation of water lilies) are the key spawning sites of the fish as all those tagged were mature individuals.

Table 2: Detection data from the 21 *C. auratus* passive acoustically tracked in the Lower Vasse River between December 2013 and January 2014. N.B. shaded fish were excluded from the overall analysis.

Fish ID	TL	Tag location	Tag date	Days at liberty	# of detections	# receivers detected at	Days detected	Residency index	Dmin (km)
7574	374	DS of Bypass	06-Dec-12	3	384	4	3	100.0	4.6
7575	233	US of Bypass	06-Dec-12	22	1168	5	18	81.8	7.23
7576	277	US of Bypass	06-Dec-12	375	97881	6	344	91.7	33.67
7577	355	Boat ramp	05-Dec-12	391	67324	6	381	97.4	82.55
7578	260	Boat ramp	05-Dec-12	391	16792	4	154	39.4	7.41
7579	280	Boat ramp	05-Dec-12	391	5718	6	186	47.6	129.08
7580	290	DS of Bypass	06-Dec-12	10	1278	5	10	100.0	14.14
7581	294	US of Bypass	06-Dec-12	391	37497	5	308	78.8	116.96
7582	371	US of Bypass	06-Dec-12	282	20439	7	177	62.8	82.8
7583	265	Boat ramp	05-Dec-12	5	497	3	5	100.0	0.62
7584	370	Boat ramp	05-Dec-12	391	35606	6	378	96.7	229.42
7585	290	Boat ramp	05-Dec-12	382	7985	6	96	25.1	8.71
7586	297	US of Bypass	06-Dec-12	390	31363	7	329	84.4	165.34
7587	314	US of Bypass	06-Dec-12	383	19547	6	293	76.5	111.5
7588	265	US of Bypass	06-Dec-12	391	48561	5	377	96.4	93.06
7589	309	US of Bypass	06-Dec-12	390	25008	7	342	87.7	231.32
7591	280	Boat ramp	05-Dec-12	382	40645	6	223	58.4	34.5
7592	357	Boat ramp	05-Dec-12	391	56735	5	290	74.2	99.34
7593	262	US of Bypass	06-Dec-12	17	106	6	8	47.1	9.19
7594	306	US of Bypass	06-Dec-12	391	37476	7	357	91.3	163.23
7595	273	US of Bypass	06-Dec-12	391	18966	5	347	88.7	86.83
min	233			3	106	3	3	25.1	0.62
max	374			391	97881	7	381	100.0	231.32
avg	301.0			293.3	27189.3	5.6	220.3	77.4	81.5
se	9.03			35.62	5573.42	0.23	31.49	4.87	15.88

Table 3: Overall number of fish and the number of detections at each receiver, and the mean percentage of daily visits per fish at each receiver (i.e. site fidelity). Also shown are the same detection data during the month around the known peak breeding period of Goldfish (i.e. 15th August – 15th September) in the Vasse River.

	Entire study			Breeding period		
Receiver ID	**Number of fish detected (% total)**	**Total detections (% total)**	**Mean % of total days visited per fish (± 1S.E.)**	**Number of fish detected (% total)**	**Total detections (% total)**	**Mean detections/fish (± 1 S.E.)**
U/S of Bypass	10 (67)	58150 (14)	18.57 (7.34)	4 (27)	3519 (12)	880 (748)
D/S of Bypass	12 (80)	52186 (13)	18.93 (3.65)	3 (20)	37 (0.1)	12 (1)
Strelley St	14 (93)	19521 (5)	21.02 (6.36)	8 (53)	488 (1.6)	61 (22)
Boat Ramp	14 (93)	104756 (26)	27.03 (7.67)	9 (60)	17298 (58)	1922 (1012)
New River Wetlands	12 (80)	48449 (12)	21.87 (7.73)	9 (60)	7023 (24)	780 (335)
Bussel Hwy	13 (87)	117177 (29)	16.31 (4.60)	0 (0)	-	-
Vasse-Wonnerup Wetlands	7 (47)	3489 (0.86)	4.73 (1.78)	1 (7)	1388 (47)	1388
TOTAL		403728			29753	

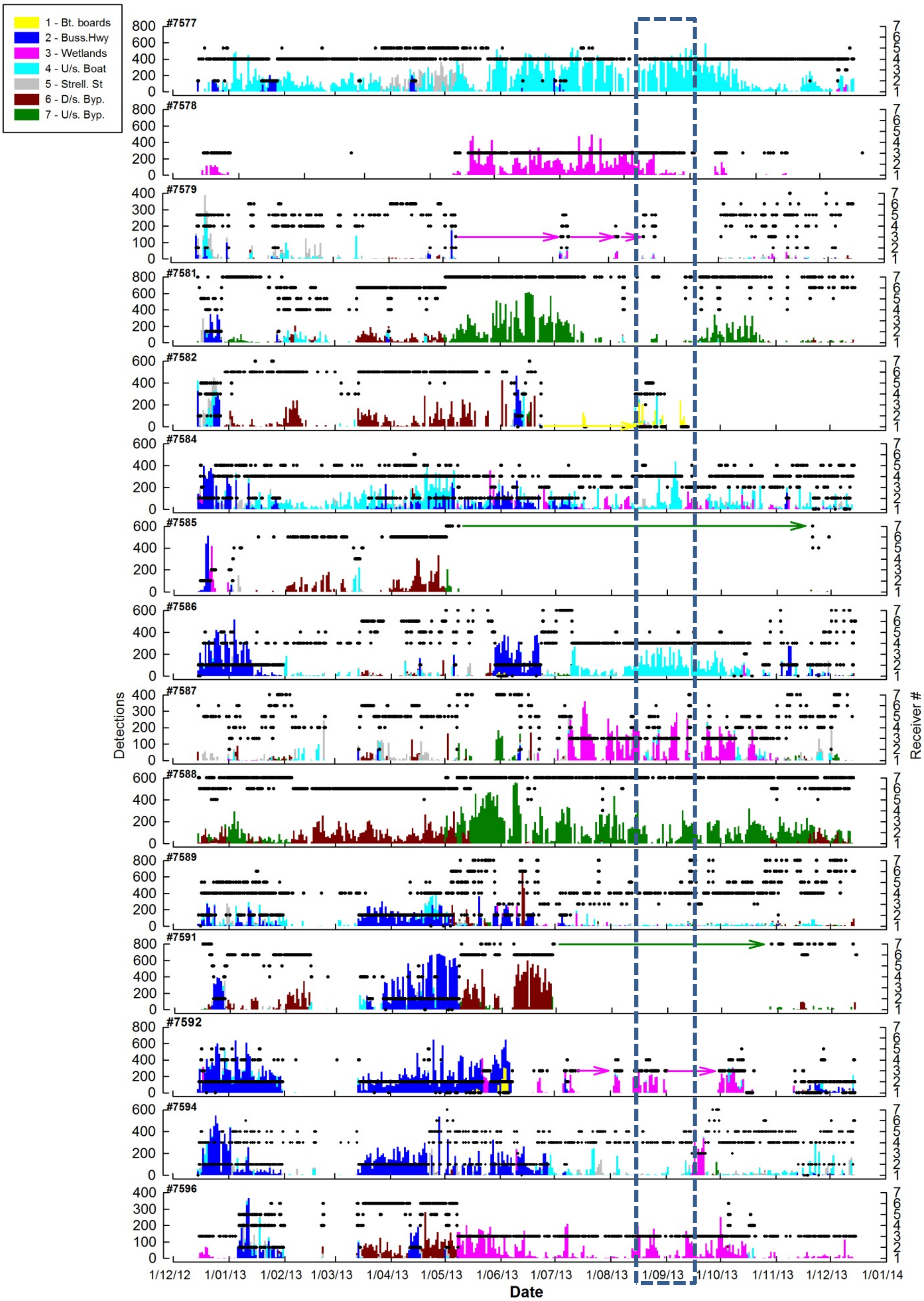

Fig. 18: Detections at seven receivers for 15 Goldfish tracked for a year in the lower Vasse River. N.B. total detections per day for each fish are displayed as colour coded bars (corresponding to each receiver). Dots are actual individual detections corresponding to the receivers (coded 1-7 on the right hand axes). Horizontal coloured arrows indicate a fish left a receiver for an extended period but was again subsequently detected at that receiver (i.e. suggesting it remained in that habitat for that undetected period, see text for details). The box indicates the peak spawning period of Goldfish.

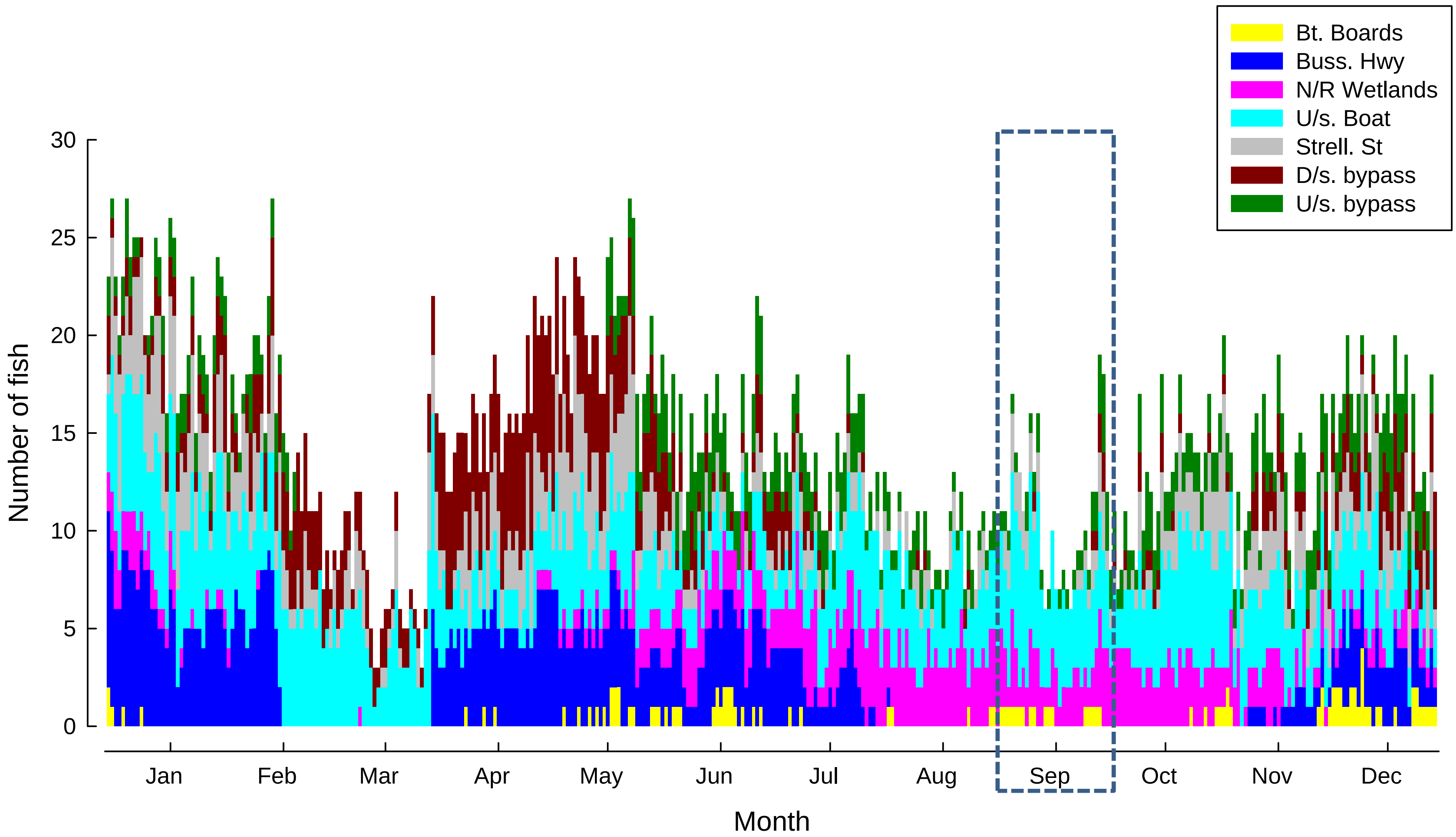

Fig. 19: Number of fish at each of the seven receivers in each day over the one year period in the lower Vasse River. N.B. each receiver is colour coded and note the clear increase in the number of fish detected in the New River Wetland (pink) during the winter and spring, and decrease in the fish detected at the Bussell Hwy Bridge and downstream of the Bypass during spring. N.B. The box indicates the peak spawning period of Goldfish.

Fig. 20: Images of a phenomenal expansion in the water lily infestation in the lower Vasse River during the study period.

Diurnal patterns in movement

There was a clear increase in the overall number of detections between midday and midnight in autumn and winter with this pattern not evident in summer and spring (Fig. 21). In terms of diurnal patterns at individual receivers, a clear reduction in the number of detections occurred at the Bussell Hwy site between mid-morning to dusk in suggesting that fish were using the bridge as an evening refuge (Fig. 22). In autumn, an increase in the number of detections occurred at the site downstream of the Bypass and the Boat Ramp between late morning to early evening. In winter, a similar increase in detections occurred from early morning to early evening at the Boat Ramp, New River Wetlands, and upstream of the Bypass. There was no clear diurnal pattern in number of detections in spring (Fig. 22).

An example of a diurnal movement pattern is presented for fish #7595 that measured 273 mm TL (Fig. 23). For example, during the period 7/1/13 to 14/1/13, fish #7595 was recorded undertaking regular movements back and forth between the Town Bridge and Strelley Street Bridge receivers (Fig. 23); a round trip distance of about 1.8 km. Most of these movements took place during daylight hours, followed by sedentary periods of 12-16 hours where the fish was detected at regular intervals, mostly at the Town Bridge site (Fig. 23).

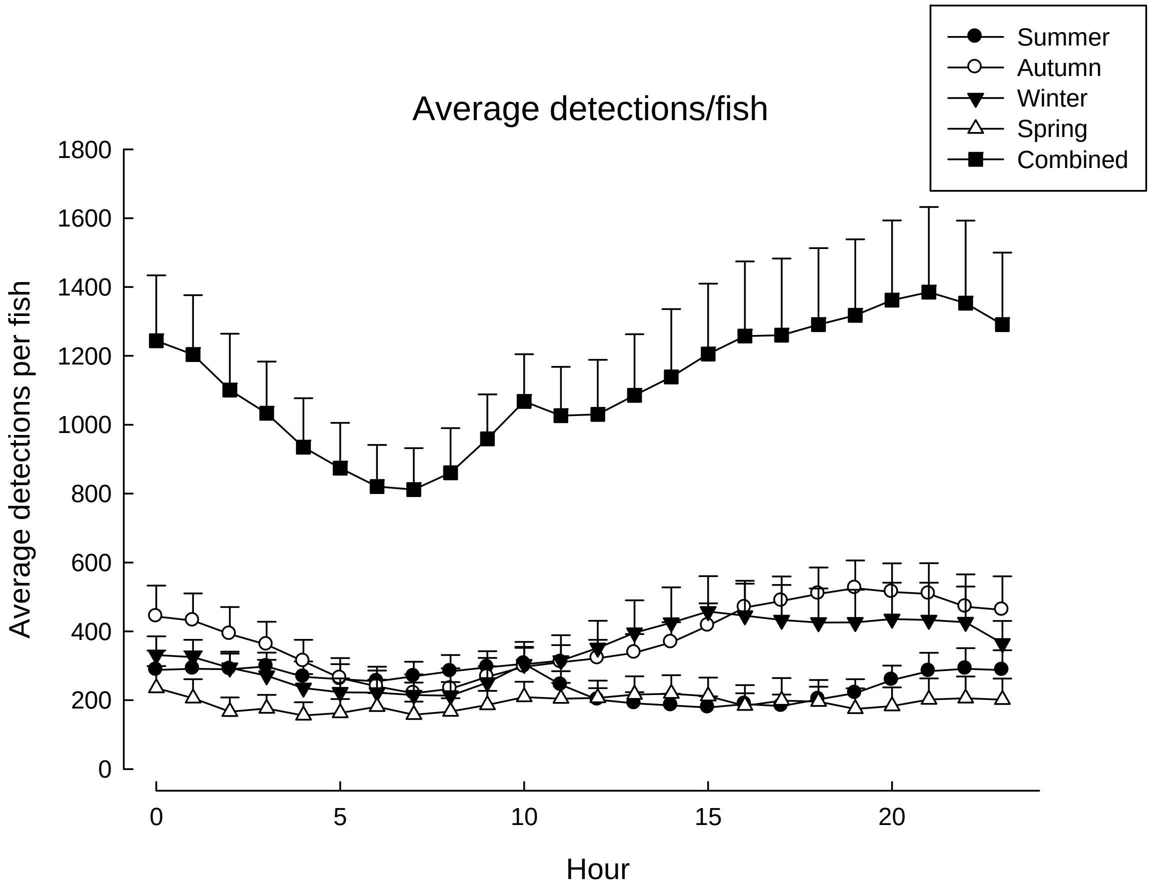

Fig. 21: Average detections over 24 hour period in each season and over all seasons. N.B. the increase in detections between midday through to midnight during the autumn and winter.

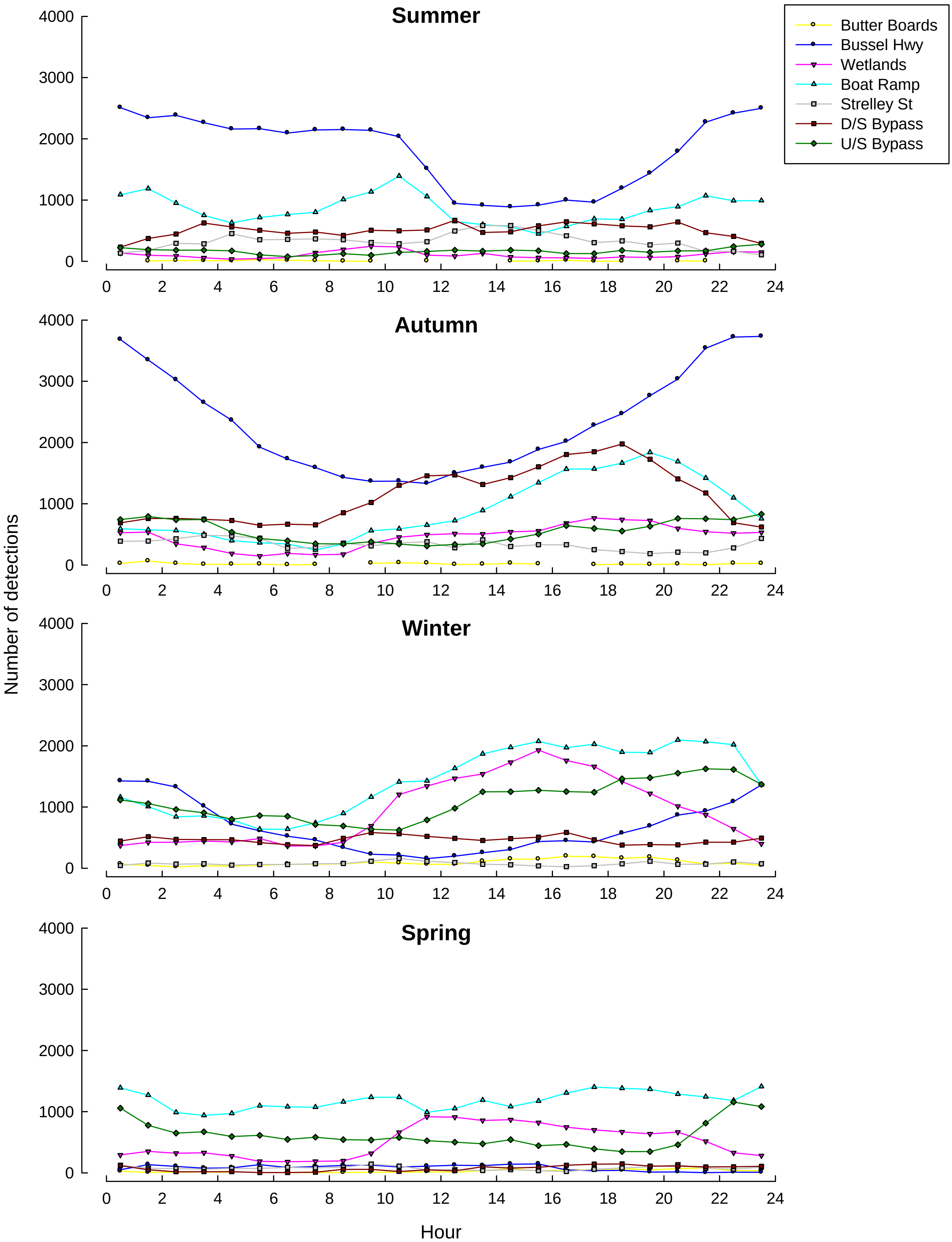

Fig. 22: Total detections over 24 hour period in each season at the seven receivers in the lower Vasse River.

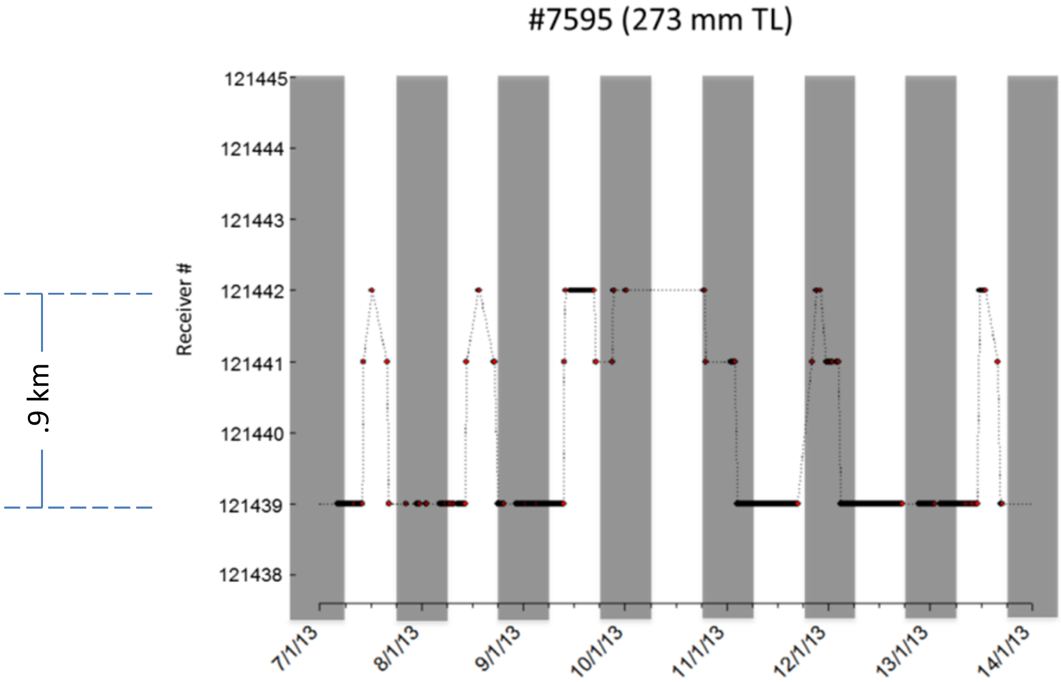

Fig. 23: Detection plot for fish #7595 during a week in January 2013. Dark bars indicate night periods, and white bars daylight periods.

Average Dmin (km) was 81.5 rkm of movement with a maximum of 231.3 rkm recorded for fish 7589 (309 mm TL) (Table 2). A closer look at the data for some of the wider ranging individuals revealed relatively high levels of mobility over short timeframes. For example, fish #7589 (309 mm TL at time of capture) moved back and forth between the receiver located upstream of the Bussell Highway Bypass and the Town Bridge site over a 24 hour period on 16-17 May 2013, covering a stream distance of at least 5.4 km, and repeated the journey, albeit more slowly (~48 hours), two days later (Fig. 17). These data on movement demonstrate that Goldfish in the Vasse River are highly mobile, which, along with the hardy and adaptable biology of the species, adds to the challenge of controlling this introduced population. Moreover, these distances obviously greatly underestimate the actual distance travelled by the fishes over the one year period as are only calculated based on movement between receivers. However, as discussed below, this study has also identified a potentially exciting opportunity for control due to it identifying key breeding habitats.

Prior to this study, we hypothesised that the New River and associated wetlands may play an important role as a potential spawning area for introduced Goldfish in the Vasse River. The extent to which the New River was utilised varied greatly among individual tagged fish, with some being detected there regularly, whilst others considerably less so with 80% of individuals being detected in the system at least once during the study and 60% during the peak breeding period (as revealed by Morgan and Beatty 2007 (Table 3, Fig. 17). The study has therefore confirmed that the wetlands were one of the top two breeding habitats and perhaps also nursery habitat (along with the site upstream of the Boat Ramp). Therefore there exists an opportunity to design a simple funnel barrier that could be installed at the narrow entrance to the New River Wetland that enables one-way passage of Goldfish in but then prevents downstream movement back into the lower Vasse River. As the New River Wetland usually dries substantially, this could then enable targeted removal (such as netting or application of a piscicide such as rotenone) to eliminate a large proportion of the breeding stock. Consideration would need to be made on monitoring the barrier to prevent build-up of debris, and also the possibility of trapping other native fishes (i.e. Sea Mullet) in the Wetland.

We had previously suspected that the Vasse-Wonnerup estuary could be utilised as a pathway of dispersal by Goldfish, particularly during periods of peak flow. Confirming this fear, the current study detected 40% of large goldfish accessing the Vasse-Wonnerup Wetlands at least once (in the Lower Vasse Wetlands). Furthermore, the estuarine sampling component in the current report revealed juvenile Goldfish to be present in the Lower Vasse Wetlands, and the Upper and Lower Vasse Estuary at salinities of ~17 ppt.

Lab trials on the salinity tolerances of Goldfish conducted as part of this project determined that salinity levels in the estuary do indeed fall to within threshold tolerance levels of the species in winter (see also the *Spatial and temporal changes the abundance and distribution of introduced fish* in the Wetland section in the current report). The salinity tolerance of the Vasse River Goldfish revealed the population had an acute tolerance of ~11 ppt, but alarmingly a gradual tolerance of ~21ppt. Therefore, it is clear that this species is seasonally moving into the Vasse-Wonnerup Wetland, and a small proportion of individuals may even be reproducing in the Lower Vasse Wetland. Therefore, it is plausible and possibly inevitable, that Goldfish could spread to other rivers in the Vasse-Wonnerup catchment via the Estuary. Fortunately, our rigorous seasonal monitoring in the rivers revealed that this dispersal avenue has not yet taken place and there remains an opportunity to concentrate control efforts on the Vasse River population.

Summary

- A total of 66,944 individual fishes and decapod crustaceans representing 12 species were captured during riverine sampling. These included three south-western Australian endemic freshwater fishes, three native estuarine fishes, two introduced fishes, three native decapod crustaceans, and one introduced decapod crustacean.
- The project has resulted in the removal of 26,081 introduced Eastern Gambusia and 842 Goldfish from rivers of the Vasse-Wonnerup catchment. The removal of large numbers of mature Goldfish during this project was likely to significantly reduce the rate of recruitment in spring 2013.
- The comprehensive seasonal sampling did not reveal any unexpected pest species incursions.
- The highest aquatic biodiversity sites were in the lower reaches of the river catchments. These sites tend to hold permanent water and act as vital refugia for aquatic species during the dry summer/autumn baseflow period.
- Sites located further upstream in the rivers only held water in winter/spring and housed fewer aquatic species. However, native fishes were found to utilise upstream sites, indicating that seasonal migration is an important life history trait and longitudinal stream connectivity must be considered to effectively manage native freshwater fish populations in the Vasse-Wonnerup.
- Stream habitats were generally degraded throughout the catchments, except in the headwater reaches located on the Darling/Whicher range, which were near pristine. Habitats were most highly degraded in the middle reaches of rivers, in the agricultural and grazing land lying between Bussell Highway and the foot of the scarp.

- The project resulted in a world-first passive acoustic tracking study of an invasive Goldfish population in order to better focus future control efforts.
- In the 12 month duration of the tracking study, 530,913 detections were recorded on the array. Of the 15 Goldfish tracked for the entire year, highest site fidelity (in terms of percentage of days visited) was recorded at the receiver upstream of the Boat Ramp near the Shire (93% of fish, 27% of days), followed by the New River Wetlands (80% of fish, 22% of days). The least amount of detections were recorded in the Ramsar listed Vasse-Wonnerup Wetlands (i.e. the Lower Vasse River Wetlands), however, 47% of fish were still detected there at least once during the study.
- Tagged Goldfish were far from being sedentary, and on average covered a minimum stream distance (Dmin) of 84.28 km over a 10 month period (one fish moved 231.3 km). However, actual distances travelled would have been much greater than these values as Dmin only takes into account straight line movements between receivers. Movements of at least 5.4 km in a 24 hour period were recorded for some individuals, indicating that the species is highly mobile in the Vasse River.
- There were considerable seasonal patterns in habitat use with a distinct movement away from the Bussell Hwy and downstream of the Bypass sites between summer/autumn and winter/spring. Between these same periods, a distinct shift occurred towards the New River Wetlands.
- The key sites utilised during the known period site of Goldfish were the site upstream of the Boat Ramp and the New River Wetland (60% of fish detected) suggesting they were the key spawning habitats. Furthermore, 80% of Goldfish were detected in the New River Wetland at some stage during the study. Several trends in diurnal movement patterns were also detected that also changed seasonally.
- As almost half of tagged Goldfish were detected in the Ramsar listed Lower Vasse River Wetland and the salinity tolerance of Goldfish determined during the study (i.e. 11 ppt acute, and 21 ppt gradual tolerance), it is plausible that the species can use the estuary as a dispersal pathway into other rivers of the Vasse-Wonnerup during spring when diluting flows lower salinity levels within threshold limits. This was also supported by the

findings of the Wetland component of the current study that detected large numbers of juveniles using the Vasse axis of the wetlands.

- However, rigorous seasonal sampling in both estuarine and riverine waters throughout the Vasse-Wonnerup failed to detect the species outside of the lower Vasse River and upper Vasse Estuary, indicating that such dispersal has not yet taken place.
- Information gathered during this study will be critical to future management as it allows clarity and confidence in the allocation of resources for ongoing introduced species prevention and control efforts in the VWWS (please see the **Table of Monitoring and Management Recommendations** at the rear of the report).

Fish faunas of the Vasse-Wonnerup Wetland System

Wonnerup Estuary

Introduction

The Vasse-Wonnerup Wetland System (VWWS) is a shallow, intermittently-open, nutrient-enriched system located near the town of Busselton, Western Australia (Brearley, 2005). The wetland provides habitat for over 37,500 water birds comprising ~90 species, a function that is recognised by its designation in 1990 as a Wetland of International Importance under the Ramsar Convention (Lane et al., 2007). However, although the importance of the VWWS is well recognised, its catchment has undergone substantial anthropogenic modification. For example, much of the catchment has been cleared, primarily for cattle grazing, extensive drainage networks have been constructed, several rivers that used to flow into the system have been diverted to the sea and floodgates have been installed in the exit channels of the estuaries to prevent seawater intrusion (Lane et al., 1997). Furthermore, the large amounts of fertilizer applied to crops, combined with animal waste discharged from pastures into the estuaries, resulted in the VWWS becoming "*the most grossly enriched major wetland system known in Western Australia*" (McAlpine et al., 1989). Moreover, without management intervention, nutrient loads are expected to increase over the next 20 years due to increased urbanisation and more intensive agriculture (Department of Water, 2010). These factors have led to a multiplicity of detrimental effects, including increases in the prevalence of eutrophication, algal blooms, anoxia, fish kills, undesirable odours, mosquito problems and the death of fringing vegetation (Lane et al., 1997; Brearley, 2005).

Fish kills occur regularly in the Vasse-Wonnerup. Reports of such events date back to 1905 and they have occurred as recently as January and February 2012, April 2013 and February 2014, with the causes ranging from anoxia to high temperatures, algal blooms and/or toxic phytoplankton (Lane et al., 1997; Lynch, 2013; Hart, 2014; J.Tweedley per. obs.). However, despite the frequency of these events and the ecological importance of the VWWS, the only quantitative information on the fish fauna of any parts of this system, was that collected from the nearshore waters of the Vasse and Wonnerup estuaries during January 2012 by Tweedley et al., (2012). Such a study is fundamental to understanding the ecological functioning of this system, particularly as fish constitute a substantial component of the diet of at least 24 bird species which utilise this system and are key to the Vasse-Wonnerup maintaining its Ramsar status (Wetland Research and Management 2007). Furthermore, the collection of quantitative data on the fish fauna is essential in developing an understanding of the ecology of the wetland and, if sufficient in scope, would also allow the development of quantitative tools for monitoring the future ecological health of the VWWS (e.g. Harrison and Whitfield 2004; Breine et al., 2007; Hallett et al., 2012a; 2012b). Such empirically-based tools could underpin a rigorous assessment of the effectiveness of management actions, including recent efforts to reduce nutrient input (Department of Water 2010).

In light of the above, the main aim of this study was to conduct a detailed quantitative survey of the characteristics of the fish fauna of the nearshore waters of all regions of the Vasse-Wonnerup seasonally over 18 months between February (summer) 2012 and May (autumn) 2013, including the presence of any introduced species of fish, as two species were found by Tweedley et al., (2012).

Materials and Methods

Sampling regime

The fish fauna of the shallow, nearshore waters of the Vasse-Wonnerup was sampled during the last month of each season between February (summer) 2012 and November (spring) 2013 at each of the seven regions of the VWWS, *i.e.* the Deadwater, Wonnerup Inlet, lower and upper Vasse Estuary, lower and upper Wonnerup Estuary and the Lower Vasse River Wetlands (Fig. 1; Appendix 3). Note that no sampling was conducted in the upper Vasse and Wonnerup estuaries in May 2012 and February 2013 as the sites in those regions were completely dry (see Appendix 3) and thus contained no fish. Sampling of the deeper, offshore waters was also undertaken in August and November 2013 in the Deadwater and Wonnerup Inlet. During each of the above sampling occasions, samples of the fish fauna were collected at four sites representing each of the regions (Fig. 1).

Samples of the fish fauna in nearshore waters were collected using a seine net that was 21.5 m long and consisted of two 10 m long wings (6 m of 9 mm mesh and 4 m of 3 mm mesh) and a 1.5 m long bunt made of 3 mm mesh. The net, which was laid parallel to the shore and then hauled onto the beach, fished to a depth of 1.5 m and swept an area of 116 m^2 (Fig. 2). Two replicate samples were collected at each site, on each sampling occasion.

Upon capture, all fish collected using the seine net were immediately euthanised in an ice slurry (Murdoch University Animal Ethics Permit #RW2471_12). The total number of individuals of each fish species in each sample was then recorded and the total length of each individual measured to the nearest 1 mm, except when a large number of any one species was caught, in which case the lengths of a random subsample of 100 fish were measured.

Three replicate values for salinity, water temperature (°c) and dissolved oxygen concentration (mg L^{-1}) were also measured in the middle of the water column at each site at the time of fish collection using a Yellow Springs Instrument 556 water quality meter (www.ysi.com).

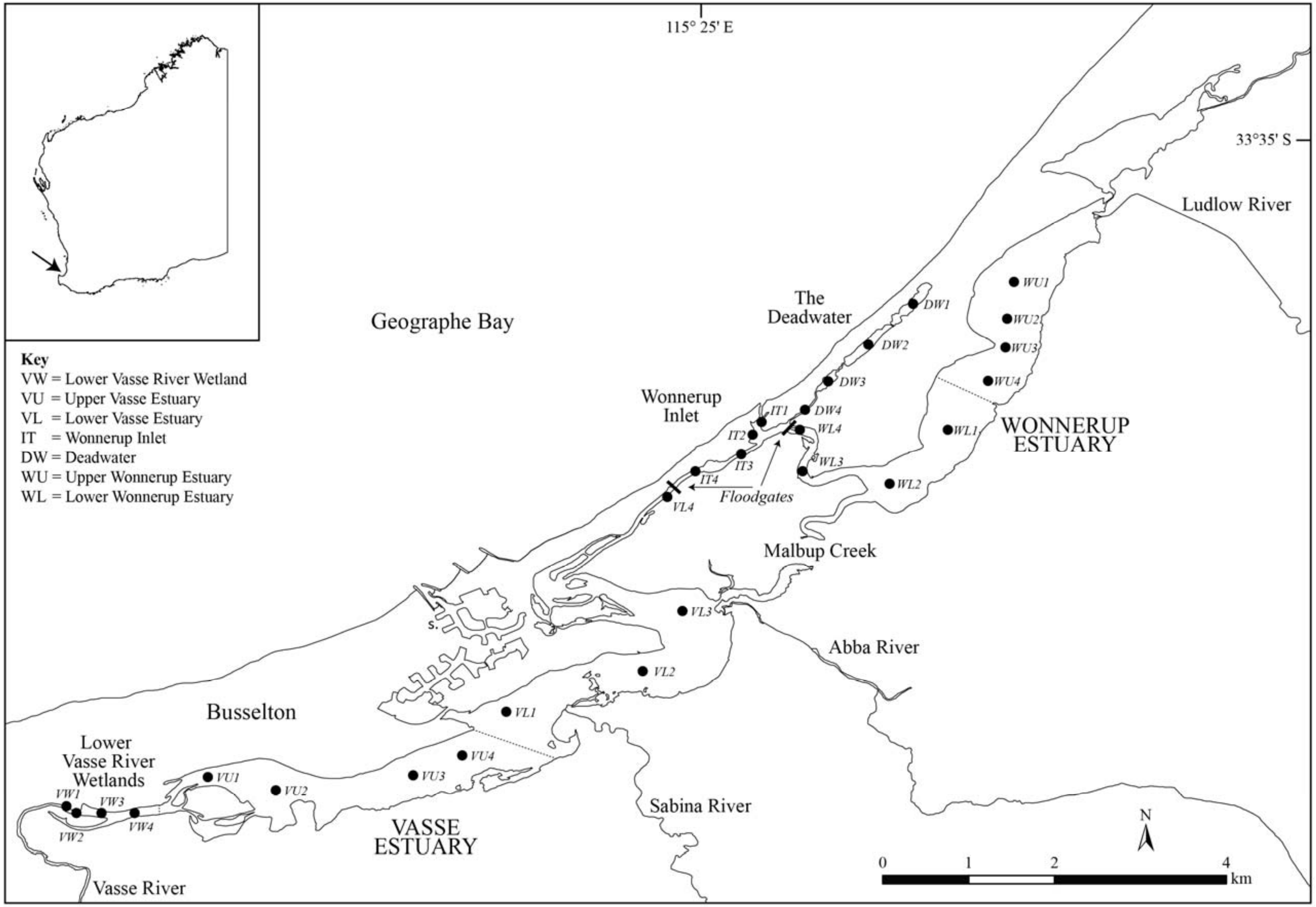

Fig. 1: Map of the Vasse-Wonnerup wetland System, showing the location of the sites sampled with a 21.5 m seine net seasonally between February 2012 and November 2013.

Fig. 2: Photograph showing the sampling of the nearshore waters of the Deadwater using a 21.5m seine net. Photo James Tweedley.

Life cycle category and guild

Each fish species was assigned to a life-cycle guild within a category according to the way in which it uses estuaries (Potter et al., 2014), based on numerous studies of the biology of fish species in south-western Australia as reported in the review of Potter and Hyndes (1999). Definition of the three categories and seven guilds relevant to the current study are as follows. **Marine category**, *i.e.* species that spawn at sea. *Marine straggler* (MS) guild, *i.e.* species that spawn at sea and typically enter estuaries sporadically and in low numbers and are most common in the lower reaches, where salinities typically do not decline far below ~35. *Marine estuarine-opportunist* (MEO) guild, *i.e.* species that spawn at sea and regularly enter estuaries in substantial numbers, particularly as juveniles, but can also use coastal marine waters as alternative nursery areas. **Estuarine category**, *i.e.* species with populations in which the individuals complete their life cycles within the estuary. *Solely estuarine* (E) guild, *i.e.* species typically found only in estuaries. *Estuarine and marine* (E&M) guild, *i.e.* species represented by populations whose individuals complete their life cycle either in estuaries or coastal marine waters. *Estuarine and freshwater* (E&F) guild, *i.e.* species represented by populations whose individuals complete their life cycle either in estuaries or fresh water. **Freshwater category**, *i.e.* species that spawn in freshwater. *Freshwater straggler* (FS) guild, *i.e.* species that are found in low numbers in estuaries and whose distribution is usually limited to the low salinity, upper reaches of estuaries. *Freshwater estuarine-opportunist* (FEO), *i.e.* species found regularly and in moderate numbers in estuaries and whose distribution can extend well beyond the oligohaline sections of these systems. No species belonging to the **diadromous category**, *i.e.* species that migrate between the sea and freshwater, such as the Pouched Lamprey (*Geotria australis*) were recorded in this study.

Statistical analyses

Each of the following statistical analyses were performed using the PRIMER v6 multivariate software package (Clarke and Gorley, 2006) with the PERMANOVA+ add-on module (Anderson et al., 2008). As no samples were collected from the upper Vasse and Wonnerup estuaries in May 2012 or February 2013 due to them being dry the data for those regions in those seasons were excluded from any statistical analyses. However, unlike 'traditional' parametric analyses, the permutational tests employed below remained unaffected by unbalanced statistical designs.

Numbers of species and densities of fishes

The number of species and density (fish 100 m^{-2}) of fishes recorded at each of the four sites sampled in the seven regions of the Vasse-Wonnerup in the eight consecutive seasons between February 2012 and November 2013 (with the exception noted above) were separately input to univariate Analysis of Variance, but using the permutation form in which the statistical tests do not make traditional normality assumptions (Anderson, 2001). This is effected as a special case of multivariate analysis in PRIMER v6 software (Clarke and Gorley 2006) by constructing a Euclidean distance matrix among values of the single variable and entering those into the PERMANOVA routine (Anderson et al., 2008). The resulting tests determined whether the number of species and density of fishes differed significantly between years (2 levels: 2012 and 2013), seasons (4 levels: February [summer], May [autumn], August [winter] and November [spring]) and regions (7 levels: see Fig. 1), and the extent of any interactions among these factors. All factors were considered fixed. In this and in all subsequent PERMANOVA tests the null hypothesis of no significant difference among *a priori* groups was rejected if the significance level (*P*) was < 5%, and the relative influence of each term in the model was quantified using the *F* or, in the case of a multivariate test, *pseudo-F*. Examination of the values for these variables demonstrated that, prior to PERMANOVA, the number of species required a square-root transformation and density a $\log_e(x+1)$ transformation to meet the test assumption of homogeneous dispersions among *a priori* groups (see Anderson, 2001).

Multivariate analyses of spatial changes in species compositions

The numbers of each fish species recorded at each of the four sites in each region in each season in 2012 and 2013, with the exception noted above, were subject to a pre-treatment shown to be effective for fish data of this type (Clarke et al., 2014). This involved a) dispersion weighting to down-weight the effects of those species whose numbers exhibited erratic differences among replicate samples due to schooling (Clarke et al., 2006), followed by b) square-root transformation to down-weight the contributions of species with consistently high values (across replicates within a group) in relation to those with consistently low values. The resultant pre-treated data at each site on each sampling occasion were averaged and used to construct a Bray-Curtis similarity matrix, which was subjected to the same three-way PERMANOVA design as described above, though now with genuinely multivariate data, with the focus being on determining whether there were significant interactions between year, season and region.

Separate two-way crossed Analysis of Similarity tests (ANOSIM, Clarke and Green, 1988; Clarke, 1993) were used to assess the relative magnitudes of overall year, season and region factors (subsuming both main and interaction effects), via the universally-scaled ANOSIM *R* statistic. This was computed, in turn, for each factor (region, year or season) *vs* the other two factors combined, thereby removing the combined effects of those other factors (see Lek et al., 2011). In this and all subsequent ANOSIM tests, the null hypothesis that there were no significant differences in ichthyofaunal composition among levels of a factor was rejected if the significance level (*P*) was < 5%. The extent of any significant differences was determined by the magnitude of the test statistic (*R*), which typically ranges between 0 (*i.e.* no group differences) to 1 (*i.e.* the similarities between samples from different groups are all less than those between samples belonging to the same group).

As the above PERMANOVA tests detected significant interactions between years, seasons and regions, the Bray-Curtis similarity matrix was separated for each level of the relevant temporal factor(s) in order to remove their confounding influence, and the various sub-matrices were then each subjected to one-way Analysis of Similarities (ANOSIM) tests to elucidate, in more detail, the extent to which ichthyofaunal composition differed among regions. The same Bray-Curtis submatrices were then used to construct a distance among centroids matrix, which creates averages in the 'Bray–Curtis space' calculated from the replicate samples (Anderson et al., 2008), and subjected to non-metric Multidimensional Scaling (nMDS) ordination (referred to as centroid nMDS ordination plots) in order to display visually the differences in the fish faunal composition among regions.

When ANOSIM detected a significant difference among regions and the associated *R* statistic was ≥ 0.2, Similarity Percentages (SIMPER, Clarke, 1993) was then used to elucidate which species typified the assemblages in each region and those which contributed most to differences between each pair of regions. Focus was placed on those typifying and distinguishing species that had the highest similarity/standard deviation ratio and dissimilarity/standard deviation ratio, respectively, and those that were the most abundant.

Whilst an *a priori* hypothesis was able to be constructed, stating that the fish fauna in the Vasse-Wonnerup would differ among the seven regions (see above), it was not possible to predict which pairs of regions would be similar (see Tweedley et al., Submitted for a similar problem and approach). Therefore, to identify those regions in which the fish fauna composition did not differ significantly (and thus could represent 'management units'), the pre-treated faunal data was averaged across seasons and years, thus creating a single value for each of the seven regions) and used to construct a Bray-Curtis similarity matrix. This matrix was then subjected to hierarchical agglomerative clustering with group-average linking (CLUSTER) and associated Similarity Profile (SIMPROF) test (Clarke et al., 2008). A SIMPROF test was performed at each node of the dendrogram to ascertain whether the particular group of samples (*i.e.* regions in the Vasse-Wonnerup) being subdivided contained any significant internal structure. This routine thus provided a sound and objective basis for ascertaining the points in the clustering procedure at which further subdivision of the samples was unwarranted. The null hypothesis that there were no significant faunal differences among regions was rejected if the significance level (P) associated with the test statistic (π) was < 5%.

Multivariate analyses of temporal changes in species compositions

The statistical analyses employed to elucidate any temporal changes in fish faunal composition were identical to those described above for assessing the extent of any spatial changes. For these analyses, the same Bray-Curtis similarity matrix constructed from the dispersion weighted and square-root transformed fish data was employed. However, to assess whether the fish composition varied among seasons and years in of the seven regions, this overall Bray-Curtis similarity matrix was separated into submatrices for each region and subjected to the same one-way ANOSIM, centroid nMDS and one-way SIMPER employed above. Finally the pre-treated fish faunal data was averaged across region, used to construct a Bray-Curtis similarity matrix and subjected to the CLUSTER and SIMPROF routines described above, to determine in which seasons and year the average fish fauna across the entire system was similar. Note that to assess whether differences in the fish faunal compositions among the various season and year combinations were driven by changes in the densities of key species rather than the presence of those species, the CLUSTER-SIMPROF procedure was employed again, only this time using a Bray-Curtis similarity matrix constructed from the presence or absence data of each species in each season and year combination.

Results

Water quality

Mean salinity differed markedly among the seven regions in the Vasse-Wonnerup and generally among seasons (Fig. 5a). The seasonal trends in salinity among the different regions fell into one of three groups, (i) those that underwent pronounced seasonal variation, *i.e.* the upper and lower Vasse and Wonnerup estuaries, (ii) those that underwent some seasonal change in salinity, *i.e.* the Deadwater and Wonnerup Inlet and (iii) those where salinity essentially remained the same, *i.e.* the Lower Vasse River Wetlands.

The largest seasonal changes in salinity were recorded in the upper Vasse and Wonnerup estuaries where salinities ranged from ~ 0.77 in the Upper Vasse in August 2013 to 111 in the Upper Wonnerup in February 2013. Generally salinities were highest in February and May (when water levels were lowest; see Appendix 4) and lowest in August and November. However, rainfall in April 2013 caused a pronounced drop in salinity in May of that year (Fig. 5a). A similar pattern in salinity was recorded in the lower Vasse and Wonnerup estuaries, however, by comparison the maximum mean salinity recorded in either of these regions was 46 in the Lower Vasse Estuary in May 2012. In contrast to the Vasse and Wonnerup estuaries, salinities in the Deadwater and Wonnerup Inlet remained much more stable and generally around that of full strength sea water. In August 2013, however, relatively heavy rainfall resulted in salinities in Wonnerup Inlet dropping to 1.5 and those in the Deadwater to 13 (Fig. 5a). Salinities in the Lower Vasse River Wetland were always low and ranged between 0.32 and 0.85, even during the warm, dry summer months.

Mean water temperature in each of the regions followed a consistent trend, generally being highest in February (~25 °C) and November (~22 °C) and lowest in May (~16 °C) and August (~16 °C; Fig. 5b). Mean dissolved oxygen concentrations exhibited no clear trend either among regions or seasons (Fig. 5c). In all cases the mean values for each season/year and region combination were normoxic (i.e. an oxygen level that is above the ≤ 2 mg L-1 definition of hypoxia; Rosenberg, 1980). However, it is noteworthy that hypoxia was detected at individual sites on three occasions (not shown on graph, but discussed later).

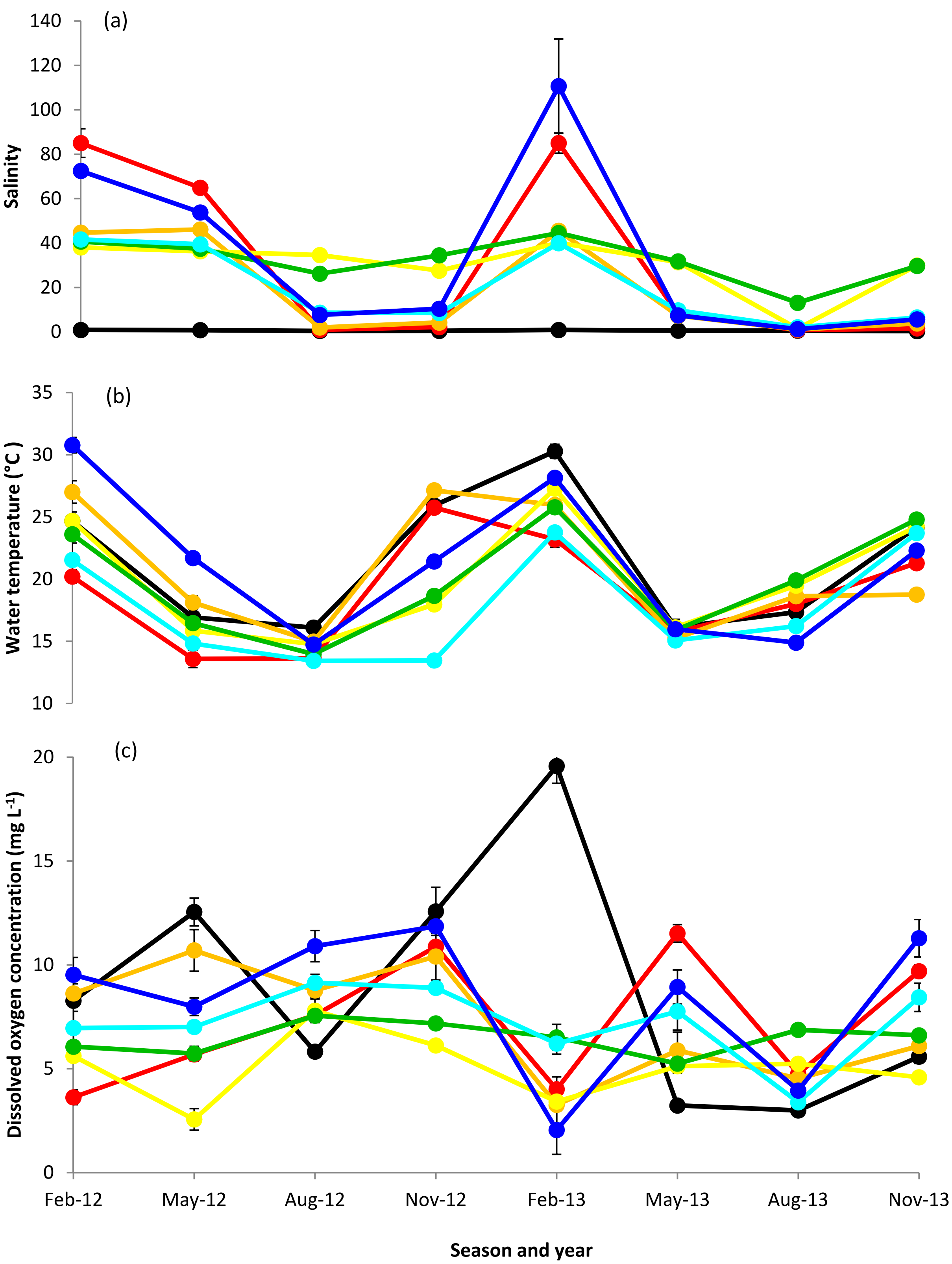

Fig. 5: Mean (a) salinity, (b) water temperature (°C), (c) dissolved oxygen concentration (mg L^{-1}) at each of the seven regions in the Vasse-Wonnerup in each season between February 2012 and November 2013. Error bars represent ±1 standard error. Lower Vasse River Wetlands (●), Upper Vasse Estuary (●), Lower Vasse Estuary (●), Wonnerup Inlet (●), Deadwater (●), Lower Wonnerup Estuary (●) and Upper Wonnerup Estuary (●).

Overall number of species, density and life cycle category among regions

A total of 174,149 fish were recorded in the shallow, nearshore waters of the Vasse-Wonnerup between February 2012 and November 2013. These fish comprised 31 species, representing 18 families. The Atherinidae (silversides), Sillaginidae (whitings) and Gobiidae (gobies) were the most speciose, with the first two families represented by five species and the last by three species. In terms of individuals, the most abundant family were the silversides (~84%), whom together with the gobies accounted for ~94% of all fish recorded.

Of the seven regions sampled throughout the Vasse-Wonnerup, the Deadwater (25) and Wonnerup Inlet (22) were by far the most speciose. Conversely, only five species were recorded in the Upper Wonnerup Estuary (Table 1). However, it should be noted that, like the Upper Vasse Estuary, this region was not sampled in two of the eight seasons as all the water had evaporated (Appendix 3). The remaining regions all contained between 8 and 14 species. Like the number of species, overall mean density of fish was also highest in the Deadwater (~780 and ~600 fish 100 m^{-2}, respectively) and the Lower Vasse River Wetlands (~670 fish 100 m^{-2}). In contrast the lowest mean densities were recorded in the Upper Wonnerup and Vasse estuaries (~19 and ~32 fish 100 m^{-2}, respectively).

Three of the four categories in the estuarine usage functional group were recorded in the nearshore waters of the Vasse-Wonnerup, *i.e.* freshwater, estuarine and marine species. Although marine species were the most speciose, represented by 18 species, compared to estuarine (8) and freshwater (4), fish belonging to this category only comprised ~2% of the total number of fish. In contrast, estuarine species dominated the fish fauna contributing almost 95% to the total number of fish recorded.

Four species, namely the Western Hardyhead, Elongate Hardyhead, Bluespot Goby and Spotted Hardyhead, were the most abundant species, each representing ≥ 5% to the catch and together contributing ~91% to the total number of individuals (Table 1). Particularly abundant where the Western and Elongate hardyheads, which ranked in the top two in all regions except the Lower Wonnerup Estuary and Lower Vasse River Wetlands where they ranked first and third and first and fifth, respectively.

Table 1: Life cycle guilds (LC), mean density (fish 100 m^2; $\bar{X}$), standard error (SE), percentage contribution to the overall catch (%), rank by density (R), mean total length (L^m) and length range (mm; L^R) of each fish species recorded in each of the seven regions in the Vasse-Wonnerup in each season between February 2012 and November 2013. Abundant species (*i.e.* those that contribute ≥ 5% to the catch) are highlighted in grey. In this and subsequent tables, life-cycle guilds are abbreviated as follows: E, solely estuarine; E&M, estuarine & marine; E&F, estuarine and freshwater; MEO, marine estuarine opportunist; MS, marine straggler; FEO, freshwater estuarine-opportunist and FS, freshwater straggler. The total number of species, mean overall density, number of samples and actual and adjusted number of individuals (*i.e.* after the number of individuals in each sample had been adjusted to that in 100 m^{-2}) are given for each region. Species ranked by total abundance. Scientific names are given in Appendix 6.

		Entire estuary						Lower Vasse River Wetlands				Upper Vasse Estuary				Lower Vasse Estuary			
Common name	**LC**	$\bar{X}$	**SE**	**%**	**R**	L^M	L^R	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R
Western Hardyhead	E&F	164.23	53.62	45.51	1	34	14-65	534.56	362.47	79.67	1	17.67	4.02	55.37	1	113.09	49.74	58.01	1
Elongate Hardyhead	E	115.46	22.72	31.99	2	33	14-87	0.85	0.59	0.13	5	8.76	3.59	27.46	2	54.74	28.80	28.08	2
Bluespot Goby	E&F	25.74	4.48	7.13	3	24	8-56	68.75	23.10	10.25	2	1.49	0.60	4.67	4	25.39	13.47	13.02	3
Spotted Hardyhead	E	22.68	8.94	6.29	4	32	18-61									0.16	0.14	0.08	5
Eastern Gambusia	FEO	9.55	7.87	2.65	5	25	9-57	61.83	55.02	9.21	3					0.08	0.08	0.04	8
Southern Longfin Goby	E&M	9.52	2.18	2.64	6	33	12-71									1.02	0.80	0.53	4
Sandy Sprat	MEO	3.82	2.41	1.06	7	39	19-56												
Black Bream	E	3.33	0.74	0.92	8	72	10-242									0.05	0.04	0.03	9
Sea Mullet	MEO	1.96	0.59	0.54	9	47	22-217									0.01	0.01	0.01	14
Goldfish	FS	1.09	0.34	0.30	10	30	10-149	4.16	1.55	0.62	4	3.77	1.76	11.82	3	0.09	0.07	0.05	7
Yelloweye Mullet	MEO	0.60	0.22	0.17	11	83	26-235												
Silver Fish	E&M	0.59	0.22	0.16	12	34	21-68									0.05	0.05	0.03	9
Common Silverbiddy	MEO	0.54	0.39	0.15	13	34	14-82									0.03	0.03	0.01	13
Trumpeter Whiting	MEO	0.43	0.24	0.12	14	58	33-107												
Bridled Goby	E&M	0.36	0.13	0.10	15	32	21-56									0.11	0.11	0.06	6
Yellowtail Grunter	E	0.18	0.05	0.05	16	80	24-236									0.05	0.05	0.03	9
Western Pygmy Perch	FS	0.18	0.12	0.05	16	28	14-35	0.23	0.17	0.03	7	0.02	0.01	0.06	6				
Western Minnow	FS	0.13	0.04	0.04	18	44	23-82	0.58	0.26	0.09	6	0.13	0.06	0.39	5	0.04	0.02	0.02	12
Tarwhine	MEO	0.12	0.06	0.03	19	60	32-116												
King George Whiting	MEO	0.10	0.04	0.03	19	74	25-145												
Western School Whiting	MEO	0.06	0.05	0.02	21	48	27-75												
Yellowfin Whiting	MEO	0.04	0.02	0.01	22	121	67-250												
Australian Anchovy	E&M	0.03	0.03	0.01	22	58	51-63												
Western Striped Grunter	MEO	0.03	0.01	0.01	22	30	15-151												
Southern School Whiting	MS	0.02	0.02	0.01	22	31	24-66												
Prickly Toadfish	MEO	0.02	0.01	0.01	22	39	22-68												
Soldier	MEO	0.02	0.01	0.01	22	26	12-36												
Tailor	MEO	0.02	0.01	0.01	22	67	30-112												
Common Hardyhead	MEO	<0.01	<0.01	<0.01	29	53	48-57												
Smalltooth Flounder	MEO	<0.01	<0.01	<0.01	29	149	149-149												
Old Wife	MS	<0.01	<0.01	<0.01	29	24	24-24					0.02	0.01	0.06	6				
Number of species		31						7				8				14			
Mean overall density		360.89						670.97				31.91				194.94			

Number of samples	**416**	64	48	64
Adjusted number of fish	**150,128**	42,942	1,532	12,476
Actual number of fish	**174,149**	49,813	1,777	14,472

Table 1 continued:

		Wonnerup Inlet				**Deadwater**				**Lower Wonnerup Estuary**				**Upper Wonnerup Estuary**			
Common name	**LC**	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R
Western Hardyhead	E&F	198.87	63.19	33.09	2	172.70	39.90	22.15	2	23.94	7.24	39.18	1	14.78	5.95	77.42	1
Elongate Hardyhead	E	280.71	71.52	46.71	1	389.05	131.24	49.91	1	16.08	5.99	26.32	3	3.30	1.30	17.31	2
Bluespot Goby	E&F	13.15	4.77	2.19	5	38.40	12.80	4.93	5	19.88	6.46	32.54	2	0.86	0.14	4.52	3
Spotted Hardyhead	E	64.16	53.46	10.68	3	83.07	31.46	10.66	3	0.05	0.03	0.09	6				
Eastern Gambusia	FEO									0.19	0.09	0.31	5				
Southern Longfin Goby	E&M	10.09	1.80	1.68	6	50.77	14.09	6.51	4								
Sandy Sprat	MEO	16.88	16.56	2.81	4	7.95	3.15	1.02	8								
Black Bream	E	9.68	3.15	1.61	7	11.92	3.78	1.53	6								
Sea Mullet	MEO	2.88	1.15	0.48	8	9.87	3.82	1.27	7								
Goldfish	FS																
Yelloweye Mullet	MEO	0.89	0.51	0.15	11	3.03	1.41	0.39	10								
Silver Fish	E&M	1.01	0.86	0.17	9	2.77	1.29	0.36	11								
Common Silverbiddy	MEO	0.19	0.09	0.03	13	3.31	2.75	0.43	9								
Trumpeter Whiting	MEO	0.05	0.03	0.01	17	2.76	1.67	0.35	12								
Bridled Goby	E&M	1.02	0.75	0.17	9	1.20	0.53	0.15	13	0.01	0.01	0.02	8				
Yellowtail Grunter	E	0.86	0.33	0.14	12	0.28	0.13	0.04	17								
Western Pygmy Perch	FS									0.90	0.84	1.48	4	0.02	0.01	0.09	5
Western Minnow	FS					<0.01	0.01	<0.01	25	0.04	0.04	0.07	7	0.13	0.05	0.66	4
Tarwhine	MEO	0.09	0.05	0.02	15	0.71	0.40	0.09	14								
King George Whiting	MEO					0.63	0.25	0.08	15								
Western School Whiting	MEO					0.40	0.33	0.05	16								
Yellowfin Whiting	MEO	0.19	0.10	0.03	13	0.04	0.03	0.01	23								
Australian Anchovy	E&M					0.22	0.22	0.03	18								
Western Striped Grunter	MEO					0.13	0.09	0.02	19								
Southern School Whiting	MS	0.03	0.02	<0.01	19	0.11	0.11	0.01	20								
Prickly Toadfish	MEO	0.03	0.02	<0.01	19	0.09	0.04	0.01	21								
Soldier	MEO	0.04	0.03	0.01	17	0.08	0.04	0.01	22								
Tailor	MEO	0.09	0.05	0.02	15	0.03	0.03	<0.01	24								
Common Hardyhead	MEO	0.01	0.01	<0.01	21												
Smalltooth Flounder	MEO	0.01	0.01	<0.01	21												
Old Wife	MS																
Number of species		22				25				8				5			
Mean overall density		600.94				779.55				61.10				19.09			

Number of samples	64	64	64	48
Adjusted number of fish	38,460	49,891	3,910	916
Actual number of fish	44,614	57,874	4,536	1,063

Numbers of species and density

The mean numbers of species differed significantly among years, seasons and regions and the season x region and year x season x region interaction (Table 2a). As indicated by the *F* value, differences among regions explained the majority of the variance, followed by season. The lack of a significant year x season interaction indicates that the pattern of seasonal difference was consistent in both years. In all seasons, the Deadwater contained the greatest number of species, ranging from ~4.3 in autumn to ~7.9 in summer (Fig. 6a). Wonnerup Inl*et al*so contained appreciable numbers of species, while the lowest numbers of species were generally recorded in the upper Vasse and Wonnerup estuaries. The mean number of species exhibited seasonal trends within a region. For example, values of this variable were markedly higher in summer and spring than in autumn and winter in the Deadwater and Wonnerup Inlet. In contrast, in the remaining regions the number of species increased from a minimum in autumn to a maximum in spring before falling in summer (Fig 6a).

Total density (fish 100 m^{-2}) also differed among years, seasons and regions and the season x region and year x season x region interactions (Table 2b). Differences among seasons and regions were the most important and far larger than that for year and for any of the interaction terms. Mean densities were generally greatest in the Deadwater and Wonnerup Inlet and lowest in the upper Vasse and Wonnerup estuaries (Fig. 6b). Within a region densities changed markedly, with the lowest values generally recorded in autumn and winter. However, the season in which the maximum abundance was recorded differed among regions, with the greatest values recorded in the most downstream locations, *i.e.* the Lower Vasse Estuary, Wonnerup Inlet and the Deadwater in summer, while this peak occurred in autumn in the remaining regions (Fig. 6b).

Table 2. Mean squares (MS), *F* values (F*)* and significance levels (*P*) for three-way PERMANOVA tests employing the Euclidean distance matrices constructed from (a) number of species and (b) mean density (fish 100 m^{-2}) in the seven regions of the Vasse-Wonnerup in each season over the two consecutive years between February 2012 and November 2013. *df* = degrees of freedom. Significant differences (< 5%) are highlighted in bold.

		(a) Number of species			(b) Density		
Main Effects	***df***	***MS***	***F***	***P***	**MS**	***F***	***P***
Year	1	**1.01**	**5.87**	**0.2%**	**20.11**	**10.33**	**0.2%**
Season	3	**6.14**	**35.70**	**0.1%**	**83.42**	**42.84**	**0.1%**
Region	6	**10.33**	**60.07**	**0.1%**	**78.37**	**40.25**	**0.1%**
Interactions							
Year × Season	3	0.44	2.55	6.1%	4.25	2.18	10.2%
Year × Region	6	0.37	2.15	5.1%	3.74	1.92	7.1%
Season × Region	18	**0.70**	**4.06**	**0.1%**	**7.61**	**3.91**	**0.1%**
Year × Season × Region	14	**0.45**	**2.64**	**0.3%**	**4.13**	**2.12**	**1.7%**
Residuals	156						

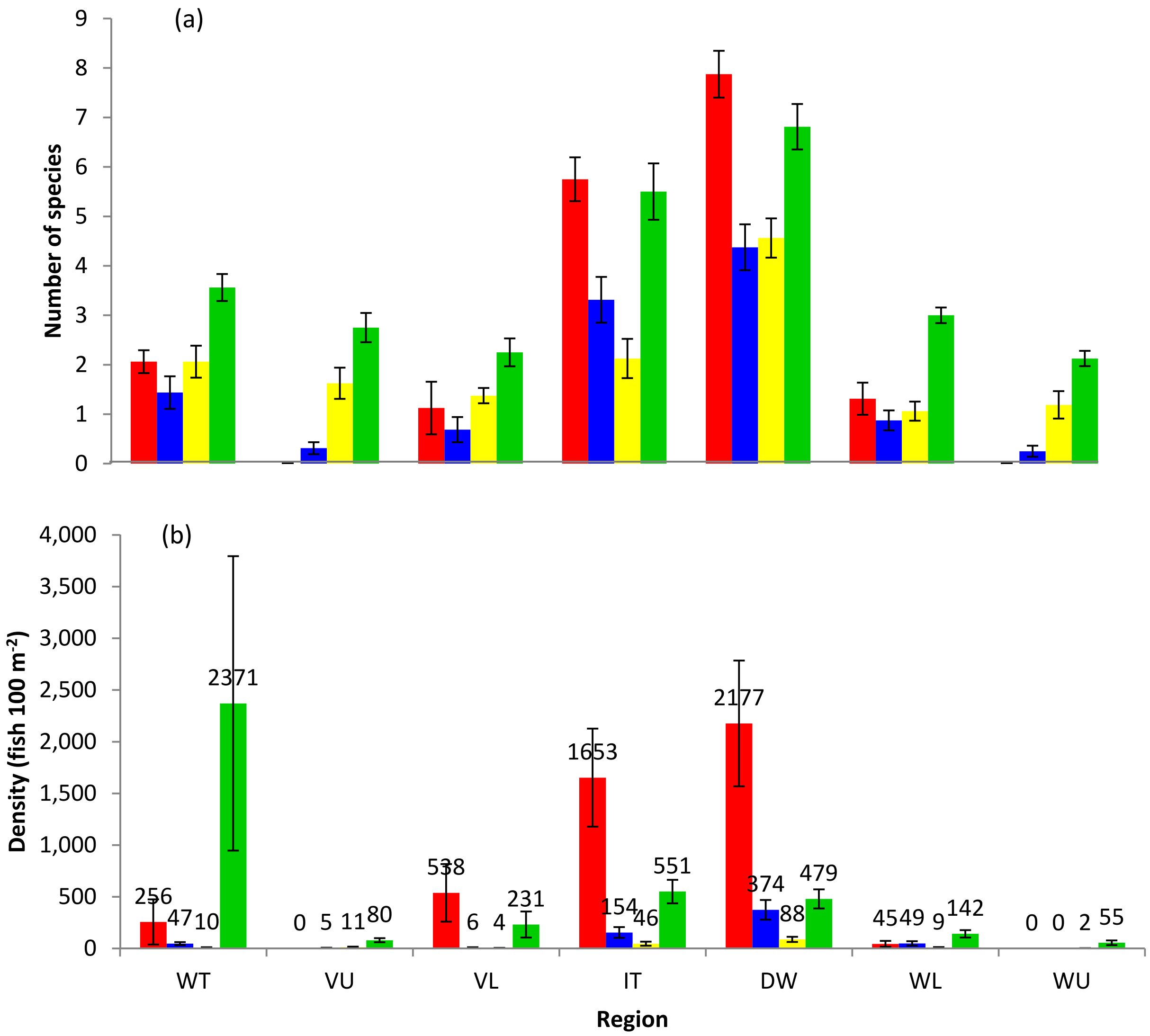

Fig. 6: Mean (a) number of species, (b) density (fish 100 m^{-2}) at each of the seven regions in the Vasse-Wonnerup in each season. Error bars represent ±1 standard error. To aid interpretation of the density plot, the values recorded in each region in each season have been provided above their respective bars. Summer (■), autumn (■), winter (■) and spring (■).

Three-way crossed PERMANOVA identified significant differences in the species composition of the nearshore fish communities among years, seasons, and regions and all interaction terms except the year x region term were significant (all *P* = 0.1%; Table 3). The pseudo-*F* values for region and season were far greater than for any other main effects and interaction terms. Subsuming main effects and interactions, the $\bar{R}$ values for the two-way crossed ANOSIM analyses for each factor *vs* the other two factors combined were greater for season (0.467) and region (0.421) than for year (0.313). On the basis of these results, subsequent analyses were then employed to more thoroughly investigate the differences in fish faunal composition among regions (and later seasons). Note that, these more detailed spatial and temporal analysis were carried out separately for each season and year and each region, respectively.

Table 3. Mean squares (MS), pseudo-*F* values (F*)* and significance levels (*P*) for three-way PERMANOVA tests employing the a Bray-Curtis similarity matrices constructed from the fish abundances recorded using a 21.5m seine net from the seven regions of the Vasse-Wonnerup in each season over the two consecutive years between February 2012 and November 2013. *Df* = degrees of freedom. Significant differences (< 5%) are highlighted in bold.

Main Effects	***df***	***MS***	***Pseudo-F***	***P***
Year	1	**2787**	**6.17**	**0.1%**
Season	3	**8902**	**19.71**	**0.1%**
Region	6	**11992**	**26.55**	**0.1%**
Interactions				
Year × Season	3	**2478**	**5.49**	**0.1%**
Year × Region	6	568	1.26	13.0%
Season × Region	18	**1679**	**3.72**	**0.1%**
Year × Season × Region	14	**1495**	**3.31**	**0.1%**
Residuals	156			

Spatial changes in fish faunal composition

One-way ANOSIM tests, conducted on the data collected in each season and year separately, identified significant differences among regions in each season and year combination (all *P* = 0.1%; Table 4; Fig. 7). The relative extent of the differences in fish composition varied among seasons and years, being higher in February and November than in May and August. The greatest overall difference was recorded during February 2013

Table 4. Global and pairwse *R*-statistic values and significance levels (*P*) for one-way ANOSIM tests for region, employing separate Bray-Curtis similarity matrices constructed from the fish abundances in each season. Insignificant pairwise comparisons are highlighted in grey, while black boxes indicate a comparison where data were not available for the upper Vasse and Wonnerup estuaries due to those areas being dry (see Materials and Methods).

(a) February 2012: Global *R* = 0.449, *P* = 0.1%

	WT	VU	VL	DW	IT	WL
VU	0.458					
VL	0.188	0.271				
DW	0.969	1.000	0.281			
IT	1.000	1.000	0.375	0.094		
WL	0.219	0.417	0.031	0.823	1.000	
WU	0.458	0.000	0.271	1.000	1.000	0.417

(e) February 2013: Global *R* = 0.634, *P* = 0.1%

	WT	VU	VL	DW	IT	WL
VU						
VL	0.458					
DW	1.000		1.000			
IT	0.917		0.917	0.292		
WL	0.458		0.333	1.000	0.875	
WU						

(b) May 2012: Global *R* = 0.589, *P* = 0.1%

	WT	VU	VL	DW	IT	WL
VU						
VL	0.927					
DW	0.917		0.927			
IT	0.646		0.313	0.320		
WL	0.948		0.250	0.969	0.469	
WU						

(f) May 2013: Global *R* = 0.339, *P* = 0.1%

	WT	VU	VL	DW	IT	WL
VU	0.167					
VL	0.000	0.323				
DW	0.531	0.479	0.479			
IT	0.656	0.573	0.625	-0.073		
WL	-0.063	0.479	0.271	0.469	0.635	
WU	0.000	0.458	0.177	0.469	0.625	-0.094

(c) August 2012: Global *R* = 0.359, *P* = 0.1%

	WT	VU	VL	DW	IT	WL
VU	1.000					
VL	1.000	-0.115				
DW	0.917	0.521	0.573			
IT	0.823	0.198	0.118	0.063		
WL	0.792	-0.094	-0.052	0.542	0.208	
WU	0.771	0.094	0.073	0.646	0.240	-0.177

(g) August 2013: Global *R* = 0.244, *P* = 0.1%

	WT	VU	VL	DW	IT	WL
VU	0.052					
VL	0.021	0.063				
DW	0.583	0.510	0.531			
IT	0.375	0.260	0.406	0.219		
WL	0.385	0.115	0.510	0.563	0.281	
WU	-0.094	-0.052	-0.010	0.583	0.260	0.094

(d) November 2012: Global *R* = 0.502, *P* = 0.1%

	WT	VU	VL	DW	IT	WL
VU	0.240					
VL	0.323	-0.052				
DW	0.927	0.865	0.719			
IT	0.896	0.719	0.563	0.000		
WL	0.594	0.240	-0.010	0.990	0.927	
WU	0.646	0.375	0.333	1.000	1.000	0.833

(h) November 2013: Global *R* = 0.566, *P* = 0.1%

	WT	VU	VL	DW	IT	WL
VU	0.885					
VL	0.885	0.396				
DW	1.000	1.000	0.958			
IT	0.938	0.646	0.542	0.188		
WL	0.406	0.604	0.156	1.000	0.646	
WU	1.000	0.656	-0.198	0.938	0.500	0.146

(Global *R* = 0.634), with the fish fauna recorded from the Deadwater and Wonnerup Inlet being particularly distinct from those in the other regions (*i.e.* pairwise *R* > 0.917). This is reflected on the associated centroid nMDS plot, where the points representing those

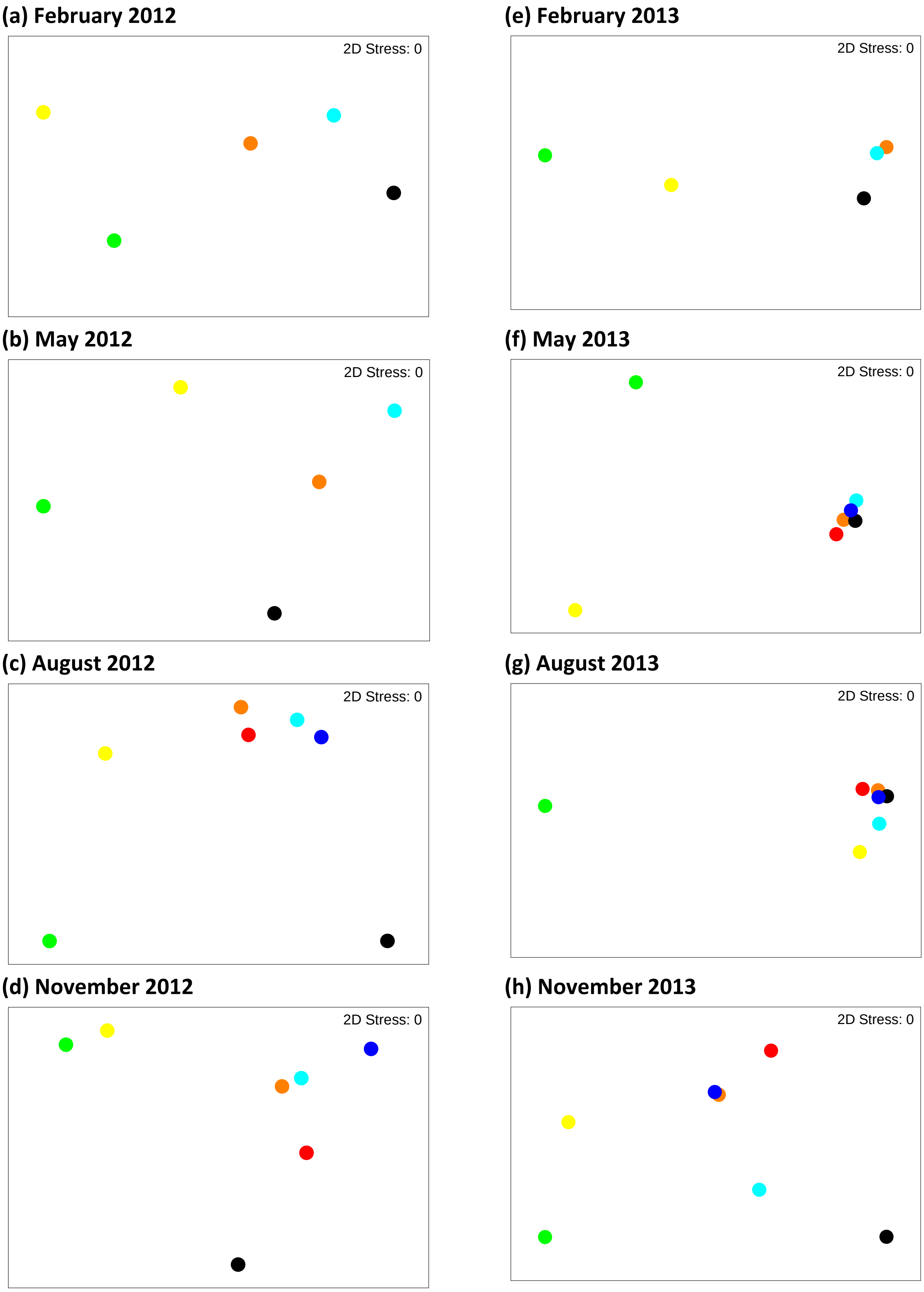

Fig. 7: Centriod nMDS ordinations plots, derived from distance among centroid matrices constructed from Bray-Curtis matrices of the fish composition in each season between February 2012 and November 2013. Lower Vasse River Wetlands (●), Upper Vasse Estuary (●), Lower Vasse Estuary (●), Wonnerup Inlet (●), Deadwater (●), Lower Wonnerup Estuary (●) and Upper Wonnerup Estuary (●).

regions lay on the left side of the plot and far away from those representing the other three regions, *i.e.* the lower Vasse and Wonnerup estuaries and the Lower Vasse River Wetlands (Fig. 7e). In the case of the Deadwater and Wonnerup Inlet, the fish fauna was typified by the consistently large densities of Elongate Hardyhead, Southern Longfin Goby and Black Bream, with these species being more abundant in these regions and any of the three other regions (Table 5a). The next biggest differences, in this season, were in pairwise comparisons involving the Lower Vasse River Wetlands, where the presence of Goldfish distinguished this region from the others as this species was only recorded in this region. In February 2012 a similar trend was recorded, with 9 of the 13 significant pairwise comparisons involving either the Deadwater or Wonnerup Inlet (Table 4a). As in 2013, these regions were distinguished by consistently greater densities of Elongate Hardyhead, Southern Longfin Goby and Black Bream (Table 5a).

In the remaining seasons and years, the largest differences involved comparisons between the Deadwater and other regions, while the same was true for Wonnerup Inlet in all but August 2013 and the Lower Vasse River Wetlands in May and August 2013 (Table 4). This is shown on the associated centroid nMDS plots, where the points representing those regions lay well separated from the main cluster of points representing the upper and lower Vasse and Wonnerup estuaries (Fig. 7). In contrast, these latter points were rarely different in all season and year combinations (Table 4). The presence of substantial densities of the Southern Longfin Goby and to a lesser extent, Black Bream and the Spotted Hardyhead distinguished the Deadwater and the Wonnerup Inlet from the other regions. The fauna in these regions differed significantly in only May 2012 and August 2013, due to greater densities of the Southern Longfin Goby and Sea Mullet in the Deadwater in the former sampling occasion and Yellowtail Grunter and Roach being recorded in Wonnerup Inlet in the latter (Table 5b, h).

In the case of the Lower Vasse River Wetland, this region was typified by consistently greater densities of the Western Minnow and Bluespot Goby in August 2012 and 2013 (Table 5c, g) and of Goldfish, the Bluespot Goby and the Western Hardyhead in November 2012 and 2013 (Table 5d, h) than the other regions. The remaining regions, namely the upper and lower Vasse and Wonnerup estuaries, were generally depauperate, harbouring

Table 5: Species that consistently typified (provided along the diagonal in light grey) and distinguished (provided in the sub-diagonal in white) the fish assemblages in each region of the Vasse-Wonnerup between February 2012 and November 2013 as detected by one-way SIMPER. The region in which each species was most abundant is given in superscript for each pairwise comparison. Insignificant pairwise comparisons, as detected by ANOSIM (Table 4) are highlighted in dark grey, while black boxes indicate a comparison were data were not available for the upper Vasse and Wonnerup estuaries due to those areas being dry (see Materials and Methods). Lower Vasse River Wetlands (WT), Upper Vasse Estuary (VU), Lower Vasse Estuary (VL), Wonnerup Inlet (IT), Deadwater (DW), Lower Wonnerup Estuary (WL) and Upper Wonnerup Estuary (WU). Full species and scientific names are given in Appendix 6.

(a) February 2012

	WT	VU	VL	DW	IT	WL	WU
WT	Eastern Gambusia						
VU	Eastern Gambusia (WT) Bluespot Goby (WT)						
VL			W. Hardyhead Elongate Hardyhead Bluespot Goby				
DW	Elongate Hardyhead (DW) S. Longfin Goby (DW) Black Bream (DW) Eastern Gambusia (WT)	Bluespot Goby (DW) Elongate Hardyhead (DW) S. Longfin Goby (DW)		Elongate Hardyhead Bluespot Goby S. Longfin Goby W. Hardyhead			
IT	Elongate Hardyhead (IT) W. Hardyhead (IT) S. Longfin Goby (IT) Eastern Gambusia (WT)	Elongate Hardyhead (IT) W. Hardyhead (IT) Black Bream (IT) S. Longfin Goby (IT)	Elongate Hardyhead (IT) W. Hardyhead (IT) Black Bream (IT) S. Longfin Goby (IT)		Elongate Hardyhead W. Hardyhead S. Longfin Goby		
WL		Bluespot Goby (WL) Bridled Goby (WL) W. Hardyhead (WL)		S. Longfin Goby (DW) Elongate Hardyhead (DW) Black Bream (DW) W. Hardyhead (DW)	Elongate Hardyhead (IT) W. Hardyhead (IT) Black Bream (IT) Bluespot Goby (WL)	Bluespot Goby W. Hardyhead	
WU	Eastern Gambusia (WT) Bluespot Goby (WT)			Bluespot Goby (DW) Elongate Hardyhead (DW) S. Longfin Goby (DW) W. Hardyhead (DW)	Elongate Hardyhead (IT) W. Hardyhead (IT) Black Bream (IT) S. Longfin Goby (IT)	Bluespot Goby (WL) W. Hardyhead (WL)	

(b) May 2012

	WT	VU	VL	DW	IT	WL	WU
WT	W. Hardyhead Bluespot Goby						
VU							
VL	Bluespot Goby (WT) W. Hardyhead (WT) Elongate Hardyhead(VL)		Elongate Hardyhead				
DW	S. Longfin Goby (DW) Black Bream (DW) Silver Fish (DW) Elongate Hardyhead (DW)		W. Hardyhead (DW) S. Longfin Goby (DW) Black Bream (DW) Silver Fish (DW)	W. Hardyhead S. Longfin Goby Black Bream Silver Fish			
IT	Bluespot Goby (WT) Black Bream (IT) W. Hardyhead (WT) Yellowtail Grunter (IT)			W. Hardyhead (DW) S. Longfin Goby (DW) Yelloweye Mullet (DW) Yellowtail Grunter (IT)	Black Bream Yellowtail Grunter Silver Fish Common Silverbiddy		
WL	Bluespot Goby (WT) Elongate Hardyhead (WL) W. Hardyhead (WT)			W. Hardyhead (DW) S. Longfin Goby (DW) Black Bream (DW) Silver Fish (DW)	Elongate Hardyhead (WL) Black Bream (IT) Yellowtail Grunter (IT)	Elongate Hardyhead	
WU							

(c) August 2012

	WT	VU	VL	DW	IT	WL	WU
WT	Western Minnow Bluespot Goby						
VU	Western Minnow (WT) Bluespot Goby (WT) Eastern Gambusia (WT)	W. Hardyhead					
VL	Western Minnow (WT) Bluespot Goby (WT) Eastern Gambusia (WT)		W. Hardyhead				
DW	Western Minnow (WT) S. Longfin Goby (DW) Bluespot Goby (WT) Prickly Toadfish (DW)	S. Longfin Goby (DW) Prickly Toadfish (DW) Sea Mullet (DW) Elongate Hardyhead (DW)	S. Longfin Goby (DW) Prickly Toadfish (DW) Sea Mullet (DW) Elongate Hardyhead (DW)	S. Longfin Goby Sea Mullet Elongate Hardyhead W. Hardyhead			
IT	Western Minnow (WT) Bluespot Goby (WT) S. Longfin Goby (IT) W. Hardyhead (IT)				S. Longfin Goby W. Hardyhead		
WL	Western Minnow (WT) Bluespot Goby (WT) Eastern Gambusia (WT)			S. Longfin Goby (DW) Prickly Toadfish (DW) Sea Mullet (DW) Elongate Hardyhead (DW)		W. Hardyhead Bluespot Goby	
WU	Western Minnow (WU) Bluespot Goby (WT) Eastern Gambusia (WT)			S. Longfin Goby (DW) Prickly Toadfish (DW) Western Minnow (WU) Sea Mullet (DW)			Western Minnow

(d) November 2012

	WT	VU	VL	DW	IT	WL	WU
WT	Goldfish Bluespot Goby W. Hardyhead						
VU		W. Hardyhead Goldfish Elongate Hardyhead Bluespot Goby					
VL			Bluespot Goby Elongate Hardyhead W. Hardyhead				
DW	Elongate Hardyhead (DW) Goldfish (WT) Bluespot Goby (WT) S. Longfin Goby (DW)	Elongate Hardyhead (DW) Goldfish (VU) S. Longfin Goby (DW) Yelloweye Mullet (DW)	Elongate Hardyhead (DW) S. Longfin Goby (DW) Yelloweye Mullet (DW) Black Bream (DW)	Elongate Hardyhead Bluespot Goby S. Longfin Goby Black Bream			
IT	Goldfish (WT) Elongate Hardyhead (IT) Bluespot Goby (WT) S. Longfin Goby (IT)	Elongate Hardyhead (IT) Bluespot Goby (IT) Goldfish (VU) Yelloweye Mullet (IT)	Elongate Hardyhead (IT) S. Longfin Goby (IT) Yelloweye Mullet (IT) Black Bream (IT)		Elongate Hardyhead Bluespot Goby Yelloweye Mullet		
WL	Goldfish (WT) Bluespot Goby (WT) W. Hardyhead (WT) Elongate Hardyhead (WL)			Elongate Hardyhead (DW) Bridled Goby (DW) S. Longfin Goby (DW) Yelloweye Mullet (DW)	Elongate Hardyhead (IT) Bridled Goby (IT) S. Longfin Goby (IT) Yelloweye Mullet (IT)	Bluespot Goby Elongate Hardyhead W. Hardyhead	
WU	Goldfish (WT) W. Hardyhead (WT) Bluespot Goby (WT) W. Pygmy Perch (WT)	Goldfish (VU) Elongate Hardyhead (VU) W. Hardyhead (VU)	Bluespot Goby (VL) Elongate Hardyhead (VL) W. Hardyhead (VL) Goldfish (VL)	Elongate Hardyhead (DW) Bluespot Goby (DW) S. Longfin Goby (DW) Yelloweye Mullet (DW)	Elongate Hardyhead (IT) Bluespot Goby (IT) Yelloweye Mullet (IT)	Bluespot Goby (WL) W. Hardyhead (WL) Elongate Hardyhead (WL)	Bluespot Goby Elongate Hardyhead

(e) February 2013

	WT	VU	VL	DW	IT	WL	WU
WT	Eastern Gambusia Bluespot Goby						
VU							
VL	Bluespot Goby (WT) Eastern Gambusia (WT) Goldfish (WT)						
DW	Elongate Hardyhead (DW) S. Longfin Goby (DW) Black Bream (DW) Goldfish (WT)		Elongate Hardyhead (DW) S. Longfin Goby (DW) Spotted Hardyhead (DW) Black Bream (DW)	Elongate Hardyhead S. Longfin Goby Spotted Hardyhead Black Bream			
IT	Elongate Hardyhead (IT) Yellowtail Grunter (IT) Black Bream (IT) Goldfish (WT)		Elongate Hardyhead (IT) Yellowtail Grunter (IT) Black Bream (IT) Sea Mullet (IT)		Elongate Hardyhead Yellowtail Grunter Sea Mullet Black Bream		
WL	Eastern Gambusia (WT) Bluespot Goby (WT) Goldfish (WT) W. Hardyhead (WT)		W. Hardyhead (WL) Bluespot Goby (WL)	Elongate Hardyhead (DW) S. Longfin Goby (DW) Spotted Hardyhead (DW) Black Bream (DW)	Elongate Hardyhead (IT) Yellowtail Grunter (IT) Black Bream (IT) Sea Mullet (IT)	Bluespot Goby W. Hardyhead	
WU							

(f) May 2013

	WT	VU	VL	DW	IT	WL	WU
WT							
VU		W. Hardyhead					
VL			Spotted Hardyhead				
DW	Elongate Hardyhead (DW) Spotted Hardyhead (DW)	Elongate Hardyhead (DW) Spotted Hardyhead (DW)	Elongate Hardyhead (DW) Spotted Hardyhead (DW)	Elongate Hardyhead Spotted Hardyhead			
IT	Elongate Hardyhead (IT) Spotted Hardyhead (IT) W. Hardyhead (IT)	Elongate Hardyhead (IT) Spotted Hardyhead (IT) W. Hardyhead (IT)	Elongate Hardyhead (IT) Spotted Hardyhead (IT) W. Hardyhead (IT)		Elongate Hardyhead Spotted Hardyhead		
WL		Bluespot Goby (WL) W. Hardyhead (VU)		Elongate Hardyhead (DW) Spotted Hardyhead (DW) Bluespot Goby (WL)	Elongate Hardyhead (IT) Spotted Hardyhead (IT) W. Hardyhead (IT) Bluespot Goby (WL)	Bluespot Goby	
WU		Bluespot Goby (WU) W. Hardyhead (WU)		Elongate Hardyhead (DW) Spotted Hardyhead (DW)	Elongate Hardyhead (IT) Spotted Hardyhead (IT) W. Hardyhead (IT) Bluespot Goby (WU)		Bluespot Goby

(g) August 2013

	WT	VU	VL	DW	IT	WL	WU
WT	Bluespot Goby Western Minnow						
VU		Western Minnow W. Hardyhead Elongate Hardyhead					
VL			Western Minnow W. Hardyhead				
DW	S. Longfin Goby (DW) Sea Mullet (DW) Bluespot Goby (WT) W. Hardyhead (DW)	S. Longfin Goby (DW) Sea Mullet (DW) Western Minnow (VU) W. Hardyhead (DW)	S. Longfin Goby (DW) Sea Mullet (DW) W. Hardyhead (DW) Western Minnow (VL)	S. Longfin Goby Sea Mullet W. Hardyhead			
IT	Western Minnow (WT) Bluespot Goby (WT) S. Longfin Goby (IT) W. Hardyhead (IT)		Western Minnow (VL) S. Longfin Goby (IT) W. Hardyhead (IT)	Sea Mullet (DW) S. Longfin Goby (DW)	S. Longfin Goby W. Hardyhead		
WL	Western Minnow (WT) Bluespot Goby (WT)			S. Longfin Goby (DW) Sea Mullet (DW) W. Hardyhead (DW)	S. Longfin Goby (IT) W. Hardyhead (IT)		
WU				S. Longfin Goby (DW) Sea Mullet (DW) W. Hardyhead (DW) Bluespot Goby (WU)	S. Longfin Goby (IT) Western Minnow (WU) W. Hardyhead (IT)		Bluespot Goby

(h) November 2013

	WT	VU	VL	DW	IT	WL	WU
WT	Bluespot Goby W. Hardyhead Goldfish						
VU	Bluespot Goby (WT) Western Minnow (WT) W. Hardyhead (WT)	Goldfish W. Hardyhead					
VL	Bluespot Goby (WT) W. Hardyhead (WT) Goldfish (WT)	Goldfish (VU)	W. Hardyhead Bluespot Goby				
DW	Bluespot Goby (WT) Tarwhine (DW) S. Longfin Goby (DW) Sandy Sprat (DW)	Tarwhine (DW) S. Longfin Goby (DW) Sandy Sprat (DW) King George Whiting (DW)	Tarwhine (DW) S. Longfin Goby (DW) Sandy Sprat (DW) King George Whiting (DW)	S. Longfin Goby Sandy Sprat W. Hardyhead King George Whiting			
IT	Bluespot Goby (WT) S. Longfin Goby (IT) Goldfish (WT)	S. Longfin Goby (IT) W. Hardyhead (IT) Goldfish (VU) Sandy Sprat (IT)	S. Longfin Goby (IT) W. Hardyhead (IT) Sandy Sprat (IT)		S. Longfin Goby W. Hardyhead		
WL	Bluespot Goby (WT) Western Minnow (WT) W. Hardyhead (WT) Goldfish (WT)	Bluespot Goby (WL) Goldfish (VU) W. Pygmy Perch (WL)		S. Longfin Goby (IT) Bluespot Goby (WL) W. Hardyhead (IT)	S. Longfin Goby (IT) Bluespot Goby (WL) W. Hardyhead (IT)	W. Hardyhead Bluespot Goby Elongate Hardyhead	
WU	Bluespot Goby (WT) W. Hardyhead (WT)	Goldfish (VU) Elongate Hardyhead		Tarwhine (DW) S. Longfin Goby (DW)	S. Longfin Goby (IT) W. Hardyhead (IT)		W. Hardyhead Elongate

	Western Minnow (WT) Goldfish (WT)	(WU) Bluespot Goby (WU)		0Sandy Sprat (DW) King George Whiting (DW)	Sandy Sprat (IT)		Hardyhead Bluespot Goby

consistently low densities of the Elongate and Western hardyheads and Bluespot goby in all seasons and years, and also the Western Minnow in August 2012 and 2013. Note that the grouping of these regions was slightly less tight during November (Fig. 7d, h), due to the substantial presence of Goldfish in the Upper Vasse Estuary, which helped distinguish the fauna in this region from those at the others (Table 5d, h).

CLUSTER-SIMPROF demonstrated that, on the basis of their fish faunal compositions, the seven regions formed four statistically discrete groups, (I) Lower Vasse River Wetland, (II) Lower Vasse and Wonnerup estuaries, (III) Upper Vasse and Wonnerup estuaries and (IV) the Deadwater and Wonnerup Inlet (Fig. 8). Thus, there is no significant difference in fish faunal compositions between any regions within a group, but the regions within a group are statistically distinct from all regions in other groups.

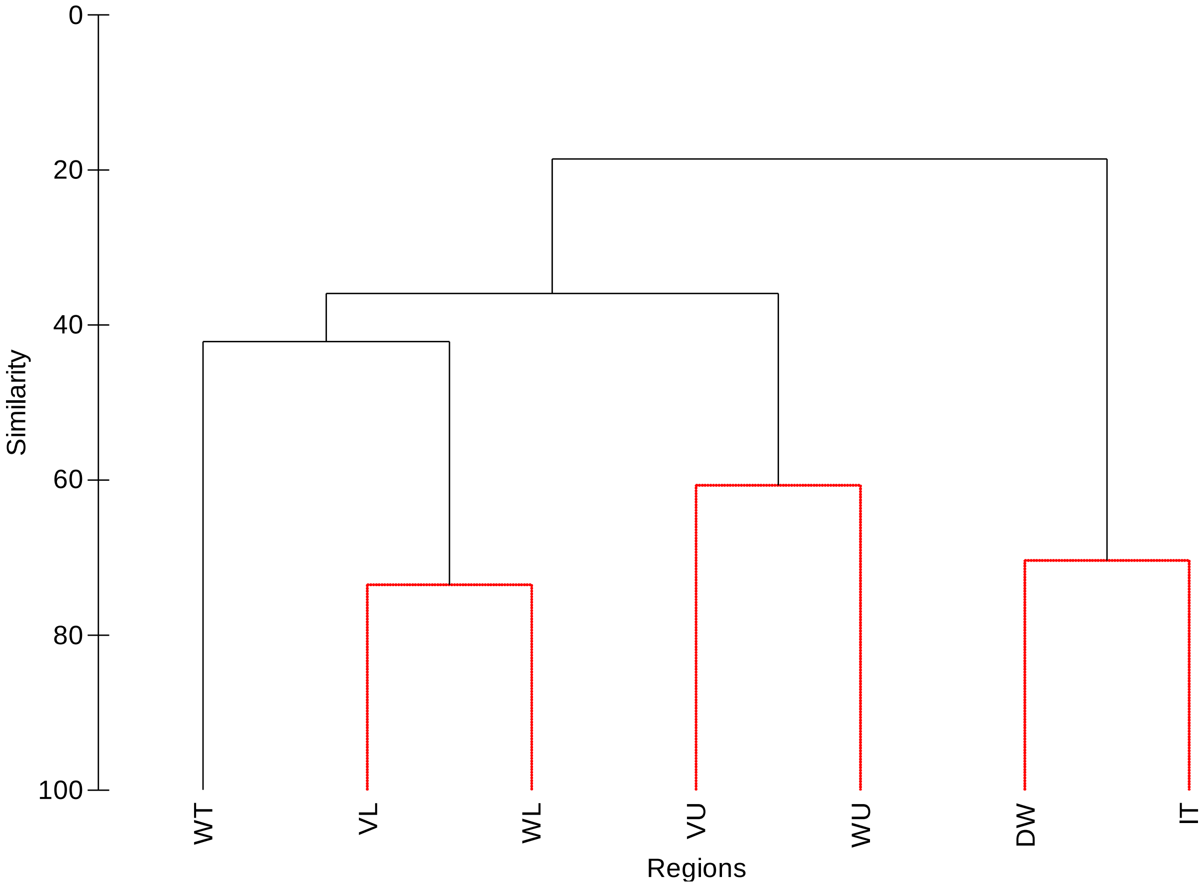

Fig. 8: Dendrogram derived by subjecting, to the CLUSTER and SIMPROF utilizing Bray-Curtis similarities, the average density of each fish species across replicate samples collected from each region on each sampling occasion. The groups in which the species compositions are significantly different from those in all other groups are denoted by thick black lines, whereas connection via a red line indicated those samples no not differ significantly. Lower Vasse River Wetlands (WT), Upper Vasse Estuary (VU), Lower

Vasse Estuary (VL), Wonnerup Inlet (IT), Deadwater (DW), Lower Wonnerup Estuary (WL) and Upper Wonnerup Estuary (WU).

Overall number of species, density and life cycle category among seasons and years

The total number of species recorded across all seven regions of the Vasse-Wonnerup differed markedly among the eight season and year combinations. The highest species richness was recorded in November and February 2012 with 21 and 20 fish species collected, respectively (Table 6). The other seasons in this year exhibited a lower total number of species, *i.e.* 15 in May and 12 in August. A similar trend was reported in 2013, when a lower number of species were recorded in May (13) than the other seasons (17).

Mean overall density underwent a similar pattern to the number of species, being greatest in February and November and lowest during May and particularly, August (Table 6). In fact, densities in this last season in 2012 and 2013 were only 2 and 4%, respectively, of those recorded in February of those same years.

The three most abundant species overall, namely the Western Hardyhead, Elongate Hardyhead and Bluespot Goby, were present in all season and year combinations, representing between 61 and 97% of the total number of individuals (Table 6). Other notably abundant species included the Spotted Hardyhead in February and May 2013 and the Southern Longfin Goby in May and August 2012.

Estuarine species dominated the fish fauna in all seasons and years combinations, representing between ~83 and 98% of the total numbers of fish recorded (Table 6). Relatively large numbers of marine species were recorded in August and November 2013, *i.e.* 15 and 10%, respectively, but generally such species represented < 5% of the total numbers of individuals. Freshwater species, with the exception of February 2012 when large numbers of Eastern Gambusia were recorded in the Lower Vasse River Wetlands, never contributed more than 2.5% to the total fish fauna. The contribution made by this category was substantially greater in August and November than in February and May. In terms of the number of species, estuarine spawning species were the most specious in all seasons except November, when more marine species were recorded (Table 6).

Table 6: Life cycle guilds (LC), mean density (fish 100 m^2; $\bar{X}$), standard error (SE), percentage contribution to the overall catch (%), rank by density (R), mean total length (L^m) and length range (mm; L^R) of each fish species recorded across the entire Vasse-Wonnerup in each season between February 2012 and November 2013. Abundant species (*i.e.* those that contribute ≥ 5% to the catch) are highlighted in grey. The total number of species, mean overall density, number of samples and actual and adjusted number of individuals (*i.e.* after the number of individuals in each sample had been adjusted to that in 100 m^{-2}) are given for each season. Species ranked by total abundance.

		Entire Estuary				February 2012				May 2012				August 2012				November 2012			
Common name	**LC**	$\bar{X}$	**SE**	**%**	**R**	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R
Western Hardyhead	E&F	**164.23**	**57.71**	**45.51**	**1**	325.99	92.65	43.98	1	102.82	36.20	64.16	1	19.38	6.98	66.90	1	537.87	405.85	66.36	1
Elongate Hardyhead	E	**115.46**	**24.43**	**31.99**	**2**	248.68	81.29	33.55	2	25.97	9.30	16.20	2	1.62	0.63	5.58	3	182.67	43.85	22.54	2
Bluespot Goby	E&F	**25.74**	**4.82**	**7.13**	**3**	54.19	19.82	7.31	4	4.29	1.94	2.68	6	1.31	0.58	4.52	4	66.72	20.03	8.23	3
Spotted Hardyhead	E	**22.68**	**9.63**	**6.29**	**4**	0.85	0.83	0.11	9									3.53	2.13	0.43	5
Eastern Gambusia	FEO	**9.55**	**8.48**	**2.65**	**5**	67.06	62.92	9.05	3	0.17	0.11	0.11	11	0.40	0.20	1.38	7	1.42	1.03	0.17	10
Southern Longfin Goby	E&M	**9.52**	**2.35**	**2.64**	**6**	26.28	14.46	3.55	5	11.72	4.52	7.32	3	4.20	1.55	14.51	2	2.36	0.85	0.29	7
Sandy Sprat	MEO	**3.82**	**2.60**	**1.06**	**7**									0.68	0.54	2.34	5	0.06	0.06	0.01	15
Black Bream	E	**3.33**	**0.79**	**0.92**	**8**	10.68	4.48	1.44	6	4.72	1.96	2.95	5	0.09	0.06	0.32	10	2.14	1.13	0.26	8
Sea Mullet	MEO	**1.96**	**0.63**	**0.54**	**9**	0.31	0.19	0.04	13	8.17	5.59	5.10	4	0.57	0.31	1.97	6	2.51	1.36	0.31	6
Goldfish	FS	**1.09**	**0.37**	**0.30**	**10**	0.02	0.02	0.00	19	0.02	0.02	0.01	13					7.05	2.56	0.87	4
Yelloweye Mullet	MEO	**0.60**	**0.24**	**0.17**	**11**	0.60	0.36	0.08	11	0.80	0.46	0.50	7	0.06	0.05	0.21	10	0.31	0.12	0.04	12
Silver Fish	E&M	**0.59**	**0.24**	**0.16**	**12**	1.46	1.09	0.20	8	0.75	0.37	0.47	8	0.29	0.19	1.01	8	0.12	0.09	0.02	14
Common Silverbiddy	MEO	**0.54**	**0.43**	**0.15**	**13**	3.20	3.14	0.43	7	0.45	0.21	0.28	9								
Trumpeter Whiting	MEO	**0.43**	**0.26**	**0.12**	**14**													1.39	0.72	0.17	10
Bridled Goby	E&M	**0.36**	**0.14**	**0.10**	**15**	0.71	0.37	0.10	10									1.96	0.98	0.24	9
Yellowtail Grunter	E	**0.18**	**0.06**	**0.05**	**16**	0.42	0.22	0.06	12	0.19	0.14	0.12	10								
Western Pygmy Perch	FS	**0.18**	**0.13**	**0.05**	**16**													0.26	0.19	0.03	12
Western Minnow	FS	**0.13**	**0.04**	**0.04**	**18**									0.31	0.12	1.06	8				
Tarwhine	MEO	**0.12**	**0.06**	**0.03**	**19**													0.08	0.06	0.01	15
King George Whiting	MEO	**0.10**	**1.00**	**0.03**	**19**	0.03	0.02	0.00	19												
Western School Whiting	MEO	**0.06**	**0.05**	**0.02**	**21**					0.13	0.13	0.08	11								
Yellowfin Whiting	MEO	**0.04**	**0.02**	**0.01**	**22**	0.22	0.12	0.03	14	0.02	0.02	0.01	13								
Australian Anchovy	E&M	**0.03**	**0.03**	**0.01**	**22**	0.25	0.25	0.03	14												
Western Striped Grunter	MEO	**0.03**	**0.01**	**0.01**	**22**	0.06	0.06	0.01	17									0.05	0.03	0.01	15
Southern School Whiting	MS	**0.02**	**0.02**	**0.01**	**22**	0.15	0.12	0.02	16												
Prickly Toadfish	MEO	**0.02**	**0.01**	**0.01**	**22**													0.03	0.02	0.00	19
Soldier	MEO	**0.02**	**0.01**	**0.01**	**22**									0.06	0.04	0.21	10	0.05	0.03	0.01	15
Tailor	MEO	**0.02**	**0.01**	**0.01**	**22**	0.08	0.06	0.01	17									0.02	0.02	0.00	19
Common Hardyhead	MEO	**<0.01**	**<0.01**	**<0.01**	**29**																
Smalltooth Flounder	MEO	**<0.01**	**<0.01**	**<0.01**	**29**													0.02	0.02	0.00	19
Old Wife	MS	**<0.01**	**<0.01**	**<0.01**	**29**					0.02	0.02	0.01	13								
Species richness			**31**				20				15				12				21		

Mean overall density	**360.89**	741.21	160.26	28.97	810.58
Number of samples	**416**	56	40	56	56
Adjusted number of fish	**150,128**	41,508	6,410	1,622	45,392
Actual number of fish	**174,149**	48,149	7,436	1,882	52,655

Table 6 continued

		February 2013				May 2013				August 2013				November 2013			
Common name	**LC**	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R	$\bar{X}$	SE	%	R
Western Hardyhead	E&F	35.80	17.75	4.31	3	10.31	6.02	15.35	3	9.74	3.24	50.20	1	217.69	88.91	71.09	1
Elongate Hardyhead	E	504.09	205.23	60.74	1	39.67	16.28	59.04	1	0.75	0.53	3.89	5	5.70	1.84	1.86	5
Bluespot Goby	E&F	26.62	10.40	3.21	4	1.29	0.60	1.92	4	1.37	0.49	7.06	4	44.29	18.93	14.46	2
Spotted Hardyhead	E	209.59	95.95	25.25	2	14.38	4.81	21.40	2	0.05	0.03	0.24	13				
Eastern Gambusia	FEO	2.52	1.24	0.30	9	0.11	0.11	0.16	7	0.06	0.04	0.32	12				
Southern Longfin Goby	E&M	26.03	11.38	3.14	5	0.69	0.42	1.03	5	3.93	2.00	20.22	2	6.30	1.92	2.06	4
Sandy Sprat	MEO													27.63	19.11	9.02	3
Black Bream	E	11.51	4.25	1.39	6	0.05	0.03	0.07	7	0.20	0.11	1.03	8				
Sea Mullet	MEO	2.59	1.18	0.31	8					2.31	1.62	11.90	3	1.22	0.85	0.40	6
Goldfish	FS	0.84	0.69	0.10	13									0.42	0.16	0.14	10
Yelloweye Mullet	MEO	2.28	2.16	0.28	11	0.62	0.34	0.92	6	0.05	0.03	0.24	13	0.65	0.57	0.21	8
Silver Fish	E&M	2.76	1.93	0.33	7	0.02	0.02	0.02	9								
Common Silverbiddy	MEO	0.71	0.42	0.09	14												
Trumpeter Whiting	MEO	2.54	2.50	0.31	9	0.02	0.02	0.02	9								
Bridled Goby	E&M													0.02	0.02	0.01	14
Yellowtail Grunter	E	1.01	0.46	0.12	12					0.08	0.05	0.40	10	0.02	0.02	0.01	14
Western Pygmy Perch	FS									0.02	0.02	0.08	16	1.05	0.95	0.34	7
Western Minnow	FS					0.02	0.02	0.02	9	0.26	0.08	1.35	7	0.40	0.28	0.13	11
Tarwhine	MEO	0.26	0.18	0.03	16					0.02	0.02	0.08	16	0.65	0.44	0.21	8
King George Whiting	MEO	0.71	0.39	0.09	14					0.08	0.08	0.40	10	0.11	0.06	0.04	12
Western School Whiting	MEO									0.37	0.37	1.90	6				
Yellowfin Whiting	MEO													0.03	0.02	0.01	14
Australian Anchovy	E&M																
Western Striped Grunter	MEO									0.09	0.08	0.48	9				
Southern School Whiting	MS																
Prickly Toadfish	MEO									0.05	0.03	0.24	13	0.06	0.04	0.02	13
Soldier	MEO					0.02	0.02	0.02	9					0.02	0.02	0.01	14
Tailor	MEO	0.06	0.05	0.01	17												
Common Hardyhead	MEO					0.02	0.02	0.02	9								
Smalltooth Flounder	MEO																
Old Wife	MS																
Species richness		17				13				17				17			

Mean overall density	829.94	67.20	19.41	306.22
Number of samples	40	56	56	56
Adjusted number of fish	33,197	3,763	1,087	17,148
Actual number of fish	38,509	4,365	1,261	19,892

Temporal changes in fish faunal composition

Temporal changes in fish faunal composition were detected in all seven regions in the Vasse-Wonnerup, however, the extents of those differences ranged substantially from 0.681 in the Deadwater down to 0.204 in the Lower Vasse Estuary (Table 7). Although the Deadwater and Wonnerup Inlet experienced the greatest temporal changes in fish fauna composition, these changes were due mainly to shift in the abundance of hardyhead and goby species. These species were most numerous in February and to a lesser extent November. Some species were abundant in more seasons (and years) than others, with Black Bream being more numerous during February and May 2012 and February 2013 and with greater densities of Tarwhine and Sandy Sprat being recorded in November 2013 (Table 8d and e).

The composition of the fish fauna in the Lower Vasse River Wetlands changed dramatically in November of both years, with the point representing these sampling occasions widely separated from the others on the nMDS plot (Fig. 9a). This distinction was due to far larger densities of the Western Hardyhead and the Goldfish being recorded (Table 8a). A similar pattern was detected in the Upper Vasse Estuary, which is directly downstream, with November being the most 'unique' season (Table 7b; Fig 9b). In these regions, however, it was the greater densities of Goldfish and the Elongate Hardyhead that were responsible for the marked shift in fish faunal composition (Table 8b).

The two regions in the Wonnerup Estuary exhibited three groups of seasons and years (Figs 8f and g). In the Lower Wonnerup Estuary, there were (i) November 2012 and 2013, which contained the highest densities of the Bluespot Goby, Western Hardyhead and Eastern Hardyhead, (ii) February 2012, characterised by moderate densities of the first two species and (iii) the remaining seasons and years that were fairly depauperate (Table 8f). The Upper Wonnerup Estuary in February 2012 and May 2013 (*i.e.* the summer and autumn seasons in which water was present in this region) yielded very low densities of fish, while the Western Minnow characterised the fish fauna in this region in the August of both years and the Bluespot Goby and Elongate Hardyhead did the same in both Novembers (Table 8g).

The smallest temporal differences were detected in the Lower Vasse Estuary, where only pairwise comparisons involving three season and year combinations were significant (Table 7c). This was due to samples collected in November 2012 containing large densities of the Western Hardyhead, Elongate Hardyhead and Bluespot Goby and those in August 2012 and 2013 containing relatively high numbers of the Western Hardyhead and Western Minnow, respectively (Table 8c).

Table 7: Global and pairwse *R*-statistic values and significance levels (*P*) for one-way ANOSIM tests for region, employing separate Bray-Curtis similarity matrices constructed from the fish abundances in each region. Insignificant pairwise comparisons are highlighted in grey, while black boxes indicate a comparison were data were not available for the upper Vasse and Wonnerup estuaries due to those areas being dry (see materials and methods).

(a) Lower Vasse River Wetland: Global *R* = 0.512, *P* = 0.1%

	Feb-12	May-12	Aug-12	Nov-12	Feb-13	May-13	Aug-13
May-12	0.500						
Aug-12	0.635	0.948					
Nov-12	0.708	0.500	0.760				
Feb-13	0.000	0.344	0.604	0.438			
May-13	0.281	0.885	0.958	0.792	0.177		
Aug-13	0.323	0.677	0.573	0.740	0.104	-0.010	
Nov-13	0.833	0.646	0.979	0.438	0.823	1.000	0.969

(b) Upper Vasse: Global *R* = 0.332, *P* = 0.1%

	Feb-12	May-12	Aug-12	Nov-12	Feb-13	May-13	Aug-13
May-12							
Aug-12	0.229						
Nov-12	0.542		0.490				
Feb-13							
May-13	0.354		-0.073	0.479			
Aug-13	0.417		0.167	0.240		0.219	
Nov-13	0.792		0.677	0.198		0.625	0.510

(c) Lower Vasse: Global *R* = 0.204, *P* = 0.1%

	Feb-12	May-12	Aug-12	Nov-12	Feb-13	May-13	Aug-13
May-12	0.042						
Aug-12	0.104	0.000					
Nov-12	-0.104	0.385	0.427				
Feb-13	0.271	0.042	0.417	0.375			
May-13	0.135	0.000	0.219	0.375	0.083		
Aug-13	0.177	0.354	0.302	0.458	0.500	0.542	
Nov-13	0.000	0.125	0.115	0.156	0.271	0.240	0.385

Table 7 continued.

(d) Wonnerup Inlet: Global *R* = 0.596, *P* = 0.1%

	Feb-12	May-12	Aug-12	Nov-12	Feb-13	May-13	Aug-13
May-12	0.802						
Aug-12	0.917	0.260					
Nov-12	0.740	0.896	1.000				
Feb-13	0.396	0.375	0.813	0.365			
May-13	0.771	0.469	0.583	0.688	0.427		
Aug-13	0.979	0.188	-0.104	1.000	0.729	0.635	
Nov-13	0.615	0.615	0.198	0.875	0.854	0.698	0.490

(e) Deadwater: Global *R* = 0.681, *P* = 0.1%

	Feb-12	May-12	Aug-12	Nov-12	Feb-13	May-13	Aug-13
May-12	0.448						
Aug-12	0.760	0.417					
Nov-12	0.167	0.875	0.969				
Feb-13	0.229	1.000	1.000	0.885			
May-13	0.677	0.698	0.479	0.833	0.958		
Aug-13	0.844	0.490	0.042	0.854	0.969	0.490	
Nov-13	0.698	0.865	0.625	1.000	1.000	0.854	0.479

(f) Lower Wonnerup: Global *R* = 0.400, *P* = 0.1%

	Feb-12	May-12	Aug-12	Nov-12	Feb-13	May-13	Aug-13
May-12	0.552						
Aug-12	0.344	0.333					
Nov-12	0.208	0.906	0.854				
Feb-13	0.406	0.448	-0.210	0.979			
May-13	0.385	0.542	0.167	0.917	0.135		
Aug-13	0.375	0.510	-0.031	0.917	0.031	0.010	
Nov-13	-0.031	0.625	0.542	0.167	0.667	0.646	0.635

(g) Upper Wonnerup: Global *R* = 0.403, *P* = 0.1%

	Feb-12	May-12	Aug-12	Nov-12	Feb-13	May-13	Aug-13
May-12							
Aug-12	0.630						
Nov-12	1.000		0.796				
Feb-13							
May-13	0.313		0.500	0.875			
Aug-13	0.500		-0.037	0.510		0.063	
Nov-13	0.583		0.556	0.385		0.542	0.417

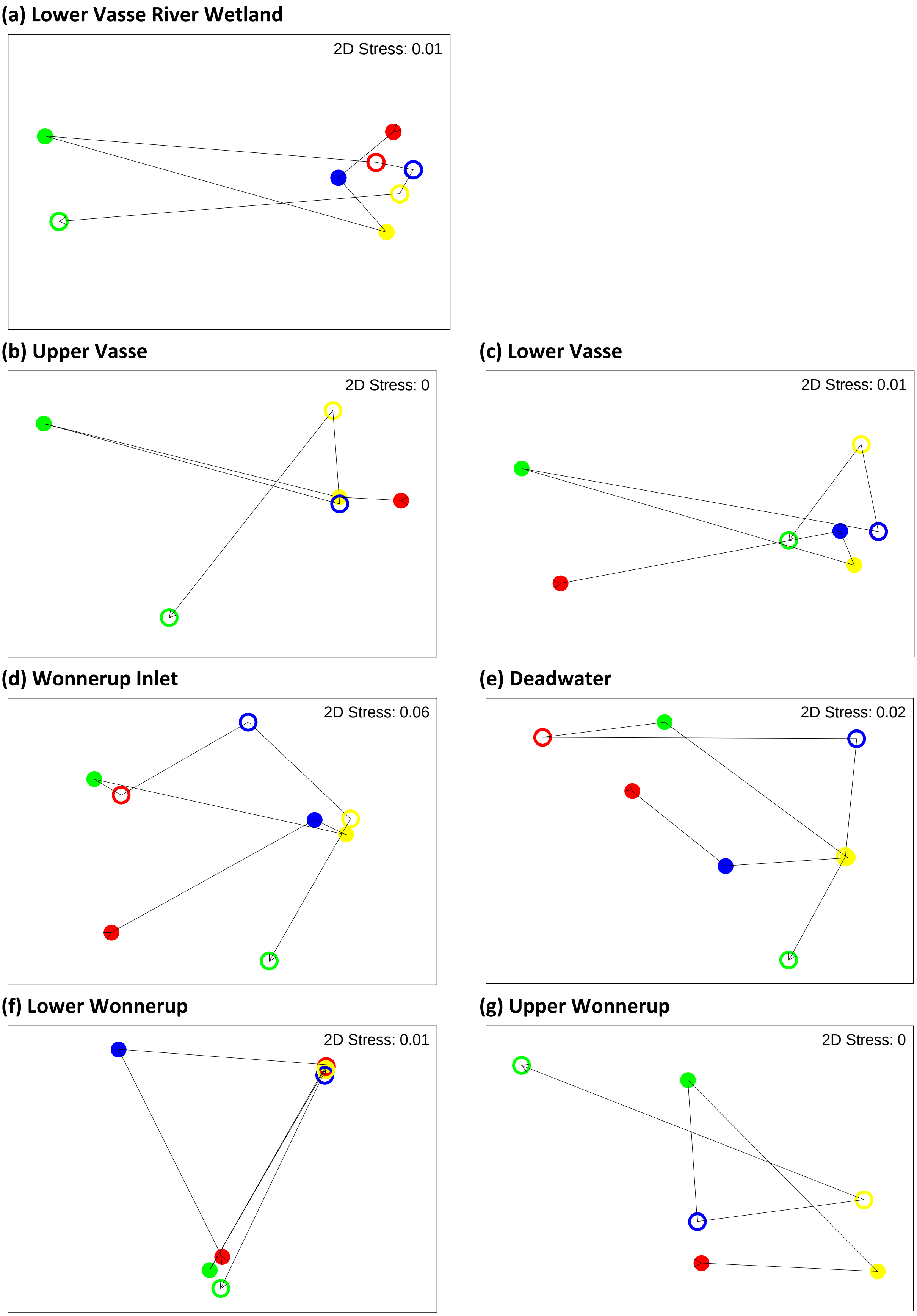

Fig. 9: Centriod nMDS ordinations plots, derived from distance among centroid matrices constructed from Bray-Curtis matrices of the fish composition in each region of the Vasse-Wonnerup. February 2012(●), May 2012 (●), August 2012 (●), November 2012(●), February 2013 (○), May 2013 (○), August 2013 (○) and November (○). The lines represent a trajectory through time.

Table 8: Species that consistently typified (provided along the diagonal in light grey) and distinguished (provided in the sub-diagonal in white) the fish assemblages in each region of the Vasse-Wonnerup between February 2013 and November 2013 as detected by one-way SIMPER. The region in which each species was most abundant is given in superscript for each pairwise comparison. Insignificant pairwise comparisons, as detected by ANOSIM (Table 4) are highlighted in dark grey, while black boxes indicate a comparison were data were not available for the upper Vasse and Wonnerup estuaries due to those areas being dry (see materials and methods). Full species and scientific names are given in Appendix 6.

(a) Lower Vasse River Wetlands

	Feb 2012	May 2012	Aug 2012	Nov 2012	Feb 2013	May 2013	Aug 2013	Nov 2013
Feb 2012	Eastern Gambusia							
May 2012	E. Gambusia (F12) Bluespot Goby (M12) W. Hardyhead (M12)	W. Hardyhead Bluespot Goby						
Aug 2012	W. Minnow (A12) E. Gambusia (F12) Bluespot Goby (A12)	W. Minnow (A12) W. Hardyhead (M12)	Western Minnow Bluespot Goby					
Nov 2012	Goldfish (N12) W. Hardyhead (N12) Bluespot Goby (N12) E. Gambusia (F12)	Goldfish (N12) Bluespot Goby (N12) W. Hardyhead (N12)	Goldfish (N12) W. Hardyhead (N12) Bluespot Goby (N12) W. Minnow (A12)	Goldfish Bluespot Goby W. Hardyhead				
Feb 2013		W. Hardyhead (M12) Goldfish (F12) E. Gambusia (F12)	W. Minnow (A12) Goldfish (F12) E. Gambusia (F12)	Goldfish (N12) W. Hardyhead (N12) Bluespot Goby (N12) E. Gambusia (F12)	Eastern Gambusia Bluespot Goby			
May 2013	E. Gambusia (F12)	Bluespot Goby (M12) W. Hardyhead (M12)	W. Minnow (A12) Bluespot Goby (A12)	Goldfish (N12) W. Hardyhead (N12) Bluespot Goby (N12) W. Pygmy Perch (N12)				
Aug 2013	E. Gambusia (F12) W. Minnow (A13)	W. Hardyhead (M12) Bluespot Goby (M12) W. Minnow (A13)	W. Minnow (A12) Bluespot Goby (A12)	Goldfish (N12) W. Hardyhead (N12) Bluespot Goby (N12) W. Minnow (A13)			Bluespot Goby Western Minnow	
Nov 2013	Bluespot Goby (N13) E. Gambusia (F12) W. Hardyhead (N13) W. Minnow (N13)	Bluespot Goby (N13) W. Minnow (N13) W. Hardyhead (N13) Goldfish (N13)	Bluespot Goby (N13) W. Hardyhead (N13) Goldfish (N13)		Bluespot Goby (N13) W. Hardyhead (N13) W. Minnow (N13)	Bluespot Goby (N13) W. Hardyhead (N13) W. Minnow (N13)	Bluespot Goby (N13) W. Hardyhead (N13) W. Minnow (N13)	Bluespot Goby W. Hardyhead Goldfish

(b) Upper Vasse Estuary

	Feb 2012	May 2012	Aug 2012	Nov 2012	Feb 2013	May 2013	Aug 2013	Nov 2013
Feb 2012								
May 2012								
Aug 2012			Western Hardyhead					
Nov 2012	Goldfish (N12) W. Hardyhead (N12) Bluespot Goby (N12) E. Hardyhead (N12)		Goldfish (N12) E. Hardyhead (N12) Bluespot Goby (N12) W. Hardyhead (N12)	Western Hardyhead Goldfish Elongate Hardyhead Bluespot Goby				
Feb 2013								
May 2013				Goldfish (N12) E. Hardyhead (N12) Bluespot Goby (N12)		Western Hardyhead		
Aug 2013	Western Minnow (A13) W. Hardyhead (A13) E. Hardyhead (A13)						Western Minnow Western Hardyhead Elongate Hardyhead	
Nov 2013	Goldfish (N13) W. Hardyhead (N13)		Goldfish (N13) W. Hardyhead (N13)	Goldfish (N12) E. Hardyhead (N12) Bluespot Goby (N12)		Goldfish (N13) W. Hardyhead (N13)	Goldfish (N13) W. Minnow (A13) W. Hardyhead (N13)	Goldfish Western Hardyhead

(c) Lower Vasse Estuary

	Feb 2012	May 2012	Aug 2012	Nov 2012	Feb 2013	May 2013	Aug 2013	Nov 2013
Feb 2012								
May 2012		Elongate Hardyhead						
Aug 2012			Western Hardyhead					
Nov 2012		Bluespot Goby (N12) E. Hardyhead (N12) W. Hardyhead (N12)	Bluespot Goby (N12) E. Hardyhead (N12) W. Hardyhead (N12)	Bluespot Goby Elongate Hardyhead Western Hardyhead				
Feb 2013			W. Hardyhead (A12)	Bluespot Goby (N12) E. Hardyhead (N12) W. Hardyhead (N12)				
May 2013			W. Hardyhead (A12)	Bluespot Goby (N12) E. Hardyhead (N12) W. Hardyhead (12)		Spotted Hardyhead		
Aug 2013				Bluespot Goby (N12) E. Hardyhead (N12) W. Minnow (A13)	W. Minnow (A13)	W. Minnow (A13)	Western Minnow Elongate Hardyhead	
Nov 2013								W. Hardyhead Bluespot Goby

(d) Wonnerup Inlet

	Feb 2012	May 2012	Aug 2012	Nov 2012	Feb 2013	May 2013	Aug 2013	Nov 2013
Feb 2012	Elongate Hardyhead W. Hardyhead S. Longfin Goby Black Bream							
May 2012	E. Hardyhead (F12) W. Hardyhead (F12) Black Bream (F12)	Black Bream Yellowtail Grunter						
Aug 2012	E. Hardyhead (F12) W. Hardyhead (F12) Black Bream (F12)		S. Longfin Goby W. Hardyhead					
Nov 2012	E. Hardyhead (F12) W. Hardyhead (F12) Bluespot Goby (N12) Black Bream (F12)	E. Hardyhead (N12) Bluespot Goby (N12) Bridled Goby (N12) Black Bream (M12)	E. Hardyhead (N12) Bluespot Goby (N12) Yelloweye Mullet (N12)	Elongate Hardyhead S. Longfin Goby Yelloweye Mullet				
Feb 2013	E. Hardyhead (F12) W. Hardyhead (F12) Black Bream (F12) Yellowtail Grunter (F13)	E. Hardyhead (F13) Yellowtail Grunter (F13) Black Bream (F13)	E. Hardyhead (F13) Yellowtail Grunter (F13) Black Bream (F13)		Elongate Hardyhead Yellowtail Grunter Sea Mullet			
May 2013	E. Hardyhead (F12) W. Hardyhead (F12) Black Bream (F12) S. Longfin Goby (F12)	E. Hardyhead (M13) Black Bream (M12) Spotted Hardyhead (M13)	E. Hardyhead (M13) Spotted Hardyhead (M13) S. Longfin Goby (A12)	Bluespot Goby (N12) E. Hardyhead (N12) S. Longfin Goby (N12) Yelloweye Mullet (N12)	Yellowtail Grunter (F13) E. Hardyhead (F13) S. Hardyhead (F13) Sea Mullet (F13)	Elongate Hardyhead Spotted Hardyhead Black Bream		
Aug 2013	E. Hardyhead (F12) W. Hardyhead (F12) Black Bream (F12) S. Longfin Goby (F12)			E. Hardyhead (N12) Bluespot Goby (N12) Yelloweye Mullet (N12)	E. Hardyhead (F13) Black Bream (F13) Sea Mullet (F13)	E. Hardyhead (M13) S. Hardyhead (M13) S. Longfin Goby (A13) W. Hardyhead (M13)	S. Longfin Goby Sea Mullet W. Hardyhead	
Nov 2013	E. Hardyhead (F12) Black Bream (F12) Yellowfin Whiting (F12)	S. Longfin Goby (N13) W. Hardyhead (N13) Black Bream (M12)		E. Hardyhead (N12) Bluespot Goby (N12) W. Hardyhead (N13)	E. Hardyhead (F13) W. Hardyhead (N13) Sea Mullet (F13)	S. Longfin Goby (N13) W. Hardyhead (M13) S. Hardyhead (M13)	S. Longfin Goby (N13) W. Hardyhead (N13) Sandy Sprat (N13)	S. Longfin Goby W. Hardyhead

(e) Deadwater

	Feb 2012	May 2012	Aug 2012	Nov 2012	Feb 2013	May 2013	Aug 2013	Nov 2013
Feb 2012	Elongate Hardyhead Bluespot Goby S. Longfin Goby Western Hardyhead							
May 2012	Bluespot Goby (F12) E. Hardyhead (F12) S. Longfin Goby (F12) Black Bream (F12)	Western Hardyhead S. Longfin Goby Black Bream Silver Fish						
Aug 2012	Bluespot Goby (F12) E. Hardyhead (F12) S. Longfin Goby (F12)	Sea Mullet (M12) W. Hardyhead (M12) S. Longfin Goby)M12) Black Bream (M12)	S. Longfin Goby Sea Mullet Elongate Hardyhead Western Hardyhead					
Nov 2012		E. Hardyhead (N12) Bluespot Goby (N12) W. Hardyhead (M12) S. Longfin Goby (M12)	E. Hardyhead (N12) Bluespot Goby (N12) Trumpeter Whiting (N12) Black Bream (N12)	Elongate Hardyhead Bluespot Goby S. Longfin Goby Black Bream				
Feb 2013		E. Hardyhead (F13) S. Hardyhead (F13) Bluespot Goby (F13) Black Bream (F13)	W. Hardyhead (F13) S. Hardyhead (F13) Black Bream (F13) Yellowtail Grunter (F13)	E. Hardyhead (F13) S. Hardyhead (F13) S. Longfin Goby (F13) Yellowtail Grunter (F13)	Elongate Hardyhead S. Longfin Goby Spotted Hardyhead Black Bream			
May 2013	S. Longfin Goby (F12) E. Hardyhead (F12) Black Bream (F12)	W. Hardyhead (M12) S. Longfin Goby (M12) Black Bream (M12) Silver Fish (M12)	E. Hardyhead (M13) S. Longfin Goby (A12) Sea Mullet (A12)	E. Hardyhead (N12) Bluespot Goby (N12) Bridled Goby (N12) Black Bream (N12)	E. Hardyhead (F13) S. Longfin Goby (F13) Black Bream (F13) S. Hardyhead (F13)	Elongate Hardyhead Spotted Hardyhead		
Aug 2013	Bluespot Goby (F12) E. Hardyhead (F12) S. Longfin Goby (F12) W. Hardyhead (F12)	Sea Mullet (M12) W. Hardyhead (M12) S. Longfin Goby (M12) Silver Fish (M12)		E. Hardyhead (N12) Bluespot Goby (N12) Bridled Goby (N12) Black Bream (N12)	E. Hardyhead (F13) S. Hardyhead (F13) Black Bream (F13) Bluespot Goby (F13)	E. Hardyhead (M13) Sea Mullet (A13) S. Longfin Goby (A13) W. Hardyhead (A13)	S. Longfin Goby Sea Mullet Western Hardyhead	
Nov 2013	Bluespot Goby (F12) E. Hardyhead (F12) S. Longfin Goby (F12) Sandy Sprat (N13)	Tarwhine (N13) Black Bream (M12) Sandy Sprat (N13) Silver Fish (M12)	Tarwhine (N13) Sandy Sprat (N13) K. George Whiting (N13) W. Hardyhead (N13)	E. Hardyhead (N12) Bluespot Goby (N12) Tarwhine (N13) K. George Whiting (N13)	E. Hardyhead (F13) S. Hardyhead (F13) Black Bream (F13) S. Longfin Goby (F13)	Tarwhine (N13) Sandy Sprat (N13) W. Hardyhead (N13) K. George Whiting (N13)	Tarwhine (N13) Sandy Sprat (N13) Sea Mullet (A13) W. Hardyhead (N13)	S. Longfin Goby Sandy Sprat W. Hardyhead K. George Whiting

(f) Lower Wonnerup Estuary

	Feb 2012	May 2012	Aug 2012	Nov 2012	Feb 2013	May 2013	Aug 2013	Nov 2013
Feb 2012	Bluespot Goby Western Hardyhead							
May 2012	Bluespot Goby (F12) E. Hardyhead (M12)	Elongate Hardyhead						
Aug 2012			Western Hardyhead Bluespot Goby					
Nov 2012		Bluespot Goby (N12) E. Hardyhead (M12) W. Hardyhead (N12)	Bluespot Goby (N12) E. Hardyhead (N12) W. Hardyhead (N12)	Bluespot Goby Elongate Hardyhead Western Hardyhead				
Feb 2013	Bluespot Goby (F12) W. Hardyhead (F12)			Bluespot Goby (N12) E. Hardyhead (N12) W. Hardyhead (N12)	Bluespot Goby Western Hardyhead			
May 2013		E. Hardyhead (M12) Bluespot Goby (M13)		Bluespot Goby (N12) E. Hardyhead (N12) W. Hardyhead (N12)		Bluespot Goby		
Aug 2013		E. Hardyhead (M12)		Bluespot Goby (N12) E. Hardyhead (N12) W. Hardyhead (N12)			Bluespot Goby Western Hardyhead Spotted Hardyhead	
Nov 2013		Bluespot Goby (N13) E. Hardyhead (M12) W. Hardyhead (N13) W. Pygmy Perch (N13)	Bluespot Goby (N13) W. Hardyhead (N13)		Bluespot Goby (N13) W. Hardyhead (N13) E. Hardyhead (N13)	Bluespot Goby (N13) W. Hardyhead (N13) E. Hardyhead (N13)	Bluespot Goby (N13) W. Hardyhead (N13) E. Hardyhead (N13)	Western Hardyhead Bluespot Goby Elongate Hardyhead

(g) Upper Wonnerup Estuary

	Feb 2012	May 2012	Aug 2012	Nov 2012	Feb 2013	May 2013	Aug 2013	Nov 2013
Feb 2012								
May 2012								
Aug 2012	W. Minnow (A12)		Western Minnow					
Nov 2012	Bluespot Goby (N12) E. Hardyhead (N12)		Bluespot Goby (N12) Western Minnow (A12) E. Hardyhead (N12)	Bluespot Goby Elongate Hardyhead				
Feb 2013								
May 2013			Bluespot Goby (M13) W. Minnow (A12)	Bluespot Goby (N12) E. Hardyhead (N12)		Bluespot Goby		
Aug 2013	Bluespot Goby (A13) W. Minnow (A13)			W. Minnow (A13) Bluespot Goby (N12) E. Hardyhead (N12)			Bluespot Goby Western Minnow	
Nov 2013	W. Hardyhead (N13) E. Hardyhead (N13) Bluespot Goby (N13)			W. Hardyhead (N13) Bluespot Goby (N12)		W. Hardyhead (N13) E. Hardyhead (N13)	W. Hardyhead (N13) W. Minnow (A13) E. Hardyhead (N13) Bluespot Goby (N13)	Western Hardyhead Elongate Hardyhead Bluespot Goby

CLUSTER-SIMPROF demonstrated that, on the basis of their fish faunal compositions, the eight season and year combinations formed two statistically discrete groups (Fig. 10a). The first group contained samples from February and November 2012 and 2013 and the other those from May and August 2012 and 2013. However, when the same analysis was run using the presence or absence of each species, rather than a dispersion weighted and square rooted density of each fish species, only a single group was identified (Fig. 10b).

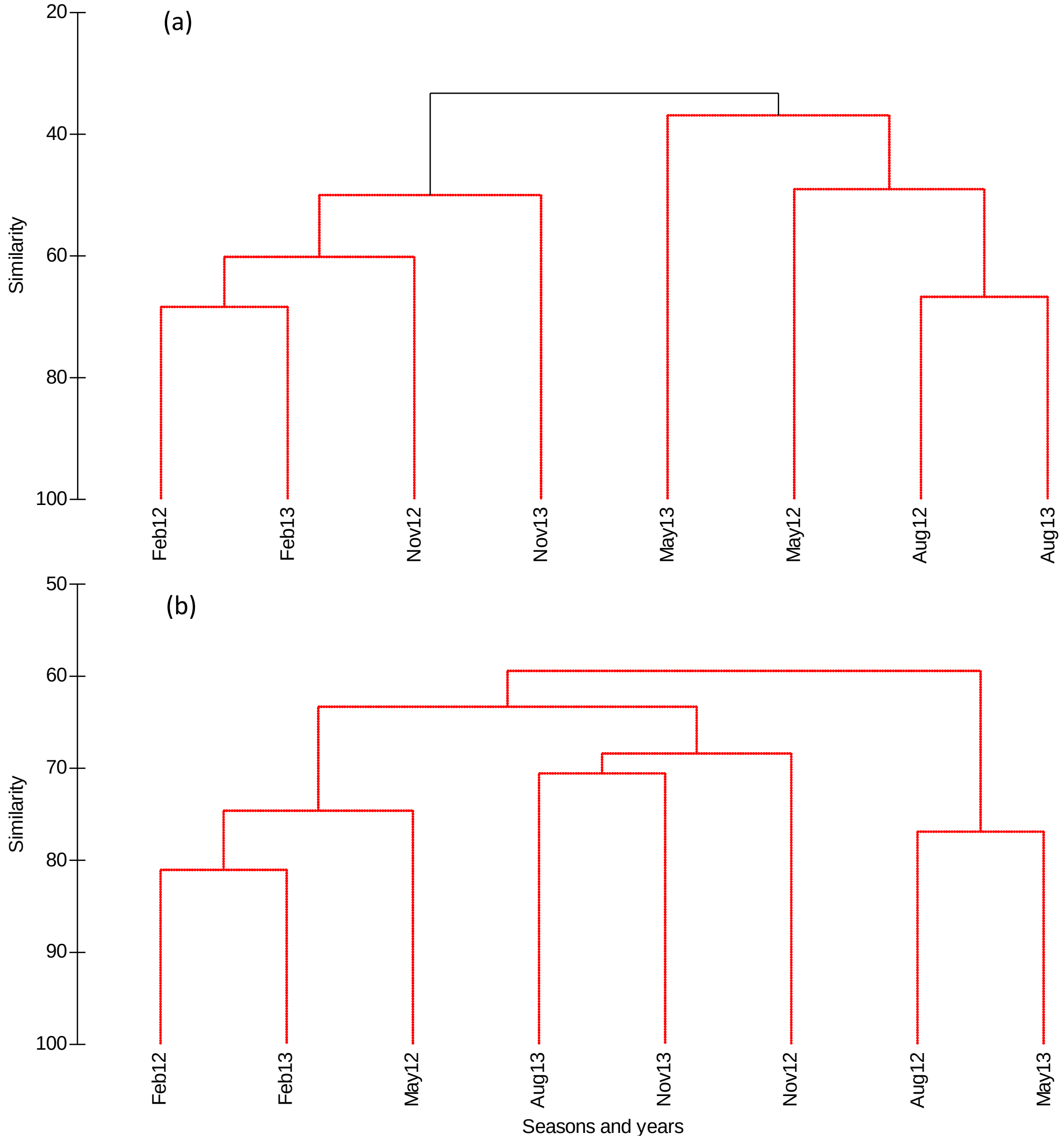

Fig. 10: Dendrogram derived by subjecting, to the CLUSTER and SIMPROF utilizing Bray-Curtis similarities, (a) the average density of each fish species across replicate samples and (b) the presence and absence of all fish species collected throughout all seven regions in the Vasse-Wonnerup on each sampling occasion. The groups in which the species compositions are significantly different from those in all other groups are denoted by thick black lines, whereas connection via a red line indicated those samples no not differ significantly.

Location of introduced fish captures

Two species of introduced species were recorded from the nearshore waters of the Vasse-Wonnerup between February 2012 and November 2013, namely the Goldfish and the Eastern Gambusia (Table 9). Goldfish were only recorded from the Vasse axis of the system, with, at the regional level, their abundance declining downstream from the Lower Vasse River Wetlands (59%), Upper (40%) and Lower (1%) Vasse Estuary (Table 9). Generally, goldfish were most abundant in November, with some fish persisting in the system until February, while only a single individual was recorded in both Mays and none in either August. Eastern Gambusia were predominantly found in the Lower Vasse River Wetlands (>99%; Table 9), with only small numbers recorded in the Lower Vasse Estuary (once) and the Lower Wonnerup Estuary (three times). Abundances peaked in February, particularly in 2012, due over 3,500 individuals being caught in a single sample.

Table 9: The number of (a) Goldfish and (b) Eastern Gambusia captured at each site on each sampling after two replicate deployments of a 21.5m seine net. Note that only regions where each species was recorded are included in the table.

(a)

Region	L. Vasse River Wetland				Upper Vasse Estuary				Lower Vasse Estuary				
Site	1	2	3	4	1	2	3	4	1	2	3	4	Total
February 2012	1												**1**
May 2012				1									**1**
August 2012													**0**
November 2012	38	92	83	8	160	4	3		6				**394**
February 2013	34												**34**
May 2013													**0**
August 2013													**0**
November 2013	5	1	1	3	4	6	3	1					**24**
Total	**78**	**93**	**84**	**12**	**164**	**10**	**6**	**1**	**6**	**0**	**0**	**0**	

(b)

Region	L. Vasse River Wetland				Lower Vasse Estuary				Lower Wonnerup Estuary				
Site	1	2	3	4	1	2	3	4	1	2	3	4	Total
February 2012	128	16	69	3,527				5		5		5	**3,750**
May 2012	4	2	1										**7**
August 2012	1		9	12									**22**
November 2012		22	54	1						2			**79**
February 2013	22	15	56	9									**102**
May 2013				6									**6**
August 2013		2	2										**4**
November 2013													**0**
Total	**155**	**57**	**191**	**3,555**	**0**	**0**	**0**	**5**	**0**	**7**	**0**	**5**	

Discussion

Water quality

Salinity and water temperature

The salinity of estuaries typically increases progressively downstream due to the declining influence of freshwater. However, the seasonal trends exhibited by salinity throughout the Vasse-Wonnerup in 2012-13 demonstrate that, during the warm dry summer months of two successive years, there was an extreme reverse salinity gradient, with salinities increasing from the Deadwater and Wonnerup Inlet to the upper Vasse and Wonnerup estuaries, where they were far greater than sea water, *i.e.* 35, and reached 132 in the Upper Wonnerup Estuary in February 2013. While this gradient was present in February (summer) and May (autumn) 2012 and February 2013, it reversed to a typical salinity gradient during August (winter) 2012 and May 2013 and remained so through to November (spring) or both years. During these cooler, wetter months, salinities in the Vasse and Wonnerup estuaries declined dramatically to values that were always < 10, with the minima being the 0.33 recorded in the Upper Vasse Estuary in August 2012, and progressively increased further downstream.

These salinity patterns are unique for an estuarine environment in south-western Australia. For example, salinities in the Moore River Estuary, which like the Vasse-Wonnerup is also classified as an intermittently-open estuary (Potter and Hyndes, 1999; Brearley 2005), undergo seasonal changes, which relate to the opening of the bar, but always maintain a typical salinity gradient, with values for this variable decreasing with distance upstream (Young et al., 1997). Moreover, even in estuaries which become markedly hypersaline, like the Culham Inlet, where salinity reached 293, a typical salinity gradient was present (Hoeksema et al., 2006). While, a reverse salinity gradient is known to occur in the permanently-open Leschenault estuary during summer, this is due to the fact that the tributary rivers discharge into the lower reaches of the estuary and thus directly opposite the entrance channel (Semeniuk et al., 2000; Veale et al., in press).

The concomitant dramatic increases in salinity during summer and autumn in the Vasse and Wonnerup estuaries are largely due to their shallow depth, coupled with high levels of solar radiation and thus evaporation and the limited precipitation that occurs at this time of year (Bureau of Meteorology, 2014), which results in a decrease in water levels (see photos in Appendix 3 and maps in Lane et al., 2011) and the concentration of salt. Note that this dramatic increase in salinity does not occur in the Lower Vasse River Wetlands or the Deadwater and Wonnerup Inlet. In the case of the first region, this was due to Ford Road acting as a barrier preventing saline water being circulated into the Lower Vasse River Wetland. Thus, salinities in this region were always very low, *i.e.* 0.31-1.18. In regard to the Deadwater and Wonnerup Inlet, salinities reached a maximum of 48 which, although classified as hypersaline, these were substantially lower than those recorded in the Vasse and Wonnerup estuaries. This was due to i) the opening of the bar, which, if not breached naturally, is open artificially in December 14th (Jim Lane, pers. comm.; Appendix 5), and ii) the morphology of these regions. It is thus relevant that the Deadwater and Wonnerup Inlet are significantly narrower (~100 m at the widest point) than the Vasse and Wonnerup estuaries (~600-700 m at the widest point; see also Fig. 1) and that depth measurements taken across the entire VWWS by Lane et al. (2011) on 17 occasions between April 1998 and September 2000 indicated that the maximum depth recorded in the Upper Vasse and Wonnerup estuaries was 1 m shallower than that in the Deadwater and Wonnerup Inlet.

The high levels of solar radiation combined with the shallow water also account for the very high water temperatures particularly in the upper Vasse and Wonnerup estuaries and the Lower Vasse River Wetlands during summer. As with salinity, the maximum recorded water temperature of 33.3°C is almost certainly an under-estimate of the true maximum as temperatures were measured only at the time of sampling.

Dissolved oxygen concentration

Of the 572 measurements of dissolved oxygen concentrations recorded during the day throughout the Vasse-Wonnerup in this study, only 21 values fell below the 2 mg l^{-1} threshold for hypoxia (Rosenberg, 1980). The fact that only a small number of values were classified as hypoxic is partially related to the fact that our measurements were collected during the day, when the high levels of macro and microalgal growth in the system would produce oxygen (Appendix 3). It is thus relevant that the dissolved oxygen concentration was above saturation in 32% of the water quality measurements collected. However, Lane et al. (1997) showed that oxygen concentrations undergo a distinct diel pattern, being highest during the day and deceasing to a minimum just before dawn.

Of the 21 hypoxic dissolved oxygen concentrations recorded, 14 of these values occurred in one of three season and region combinations. The occasion with the lowest value, *i.e.* 0.02 (and two others of 0.23 and 0.49 mg L^{-1}), occurred in the Lower Vasse Estuary several weeks before the large fish kill in April 2013 in an area where the sediment had become thixotropic (see photo in Appendix 3). It is thus relevant that no fish were caught at that site at that time, although sampling efficiency of the seine net was compromised by the gelatinous nature of the sediment.

Very low levels of oxygen 0.17 mg L^{-1} were recorded near the mouth of Wonnerup Inlet in May 2012, during a time when the bar was closed (Appendix 5) and large amounts of seagrass wrack, likely comprised of *Posidonia sinuosa* and *Amphibolis antarctica* (Oldman et al., 2010), had accumulated and were decaying (see Appendix 5 for a photograph). Although the sediment was suitable for a seine net, no fish were collected at this site. They were found, however, at the next site ~ 300 m away, suggesting that fish had moved away from this localised area of hypoxia. Note also that no dead fish were spotted floating or wash up on the banks, although they would likely either sink or be consumed by birds very quickly.

The other occasion where hypoxic conditions were recorded was in the Lower Vasse River Wetlands in February 2012 (*i.e.* 0.93-1.75 mg L^{-1}). Although, to the best of our knowledge, a fish kill did not occur, this may be due to the fact that the two most abundant species in this region at this time were the Eastern Gambusia and the Bluespot Goby. The introduced Eastern Gambusia is known to prefer degraded environments near urban areas (Lloyd et al., 1986) and are able to slow their metabolism in oxygen poor waters (Cech et al., 1980; 1985). They are also able survive in waters with ~1 mg L^{-1} (Odum and Caldwell, 1955; Cherry et al., 1976) and even down to 0.28 mg L^{-1} if they are able to gulp air from the surface (Odum and Caldwell, 1955; Pyke, 2005). Likewise, the Bluespot Goby is able to use atmospheric oxygen via aquatic surface respiration in response to hypoxia (Gee and Gee, 1991).

Number of species and density

Over the course of this two year study, 174,149 fish belonging to 31 species were recorded from the nearshore waters of the Vasse-Wonnerup. While, it is difficult to compare the numbers of species across studies, as the number of species is directly related to the number of samples, it is notable that the number of species here (from 416 samples) is similar to the 27 species (from 268 samples) recorded from the Moore River Estuary, which like the Vasse-Wonnerup, is also classified as an intermittently-open estuary (Young et al., 1997). Furthermore, the average mean density was also similar being recorded as 366 and 388 fish 100 m^{-2}, for the Moore River Estuary and the Vasse-Wonnerup, respectively. The silversides and gobies were amongst the most speciose families and together accounted for ~94% of all fish recorded. The domination of the nearshore waters by individuals of these families is typical of the nearshore waters of estuaries in south-western Australia (e.g. Potter and Hyndes, 1999; Hoeksema et al., 2006; Valesini et al., 2009).

Four species recorded during this study are particularly noteworthy, the first two of which are the Spotted Hardyhead and Common Hardyhead. These species, which ranked 4th and 29th, respectively, are tropical species whose distributions have expanded southwards recently due to the warming of nearshore coastal waters (Veale et al., in press). These species were not recorded in the Leschenault Estuary, ~60 km to the north, in 1994, but were recorded in 2008-2011 (cf. Potter et al., 1997; Veale et al., in press), and this study provides further evidence of a southwards extension of their range. Two introduced freshwater species were also recorded during this study, namely the Eastern Gambusia and the Goldfish (see later for more details).

Both the total and mean number of species were greatest in the Deadwater and Wonnerup Inlet, reflecting the presence of many marine estuarine-opportunist species, such as the five species of whiting, two species of grunter, Tarwhine and Tailor, that were only found in these regions. These species probably remained in the lower reaches and did not penetrate into the Vasse or Wonnerup estuaries as salinities in the Deadwater and Wonnerup Inlet, remained the closest to sea water throughout the entire year as opposed to going markedly hypersaline in summer and autumn and oligohaline in winter and spring. It is thus relevant that Veale et al., (in press), found that the contributions of marine species (which includes the marine estuarine-opportunists) declined as salinities increase along the Leschenault Estuary. In contrast, the lowest mean and total number of species was recorded in the Upper Vasse and Wonnerup estuaries, which reflects the fact that there are only a few species capable of tolerating the extreme changes in salinity that occurs in these regions.

Densities followed a similar pattern to number of species, in that they were generally greatest in the Deadwater and Wonnerup Inlet and lowest in the Upper Vasse and Wonnerup estuaries, which is likely due to changes in salinity. Among regions, the deeper areas of the VWWS, namely the Lower Vasse Estuary and the Deadwater and Wonnerup Inlet, density was greatest in summer. This is during a time when water levels are receding (see Appendix 4; Lane et al., 2011) and may reflect the movement of individuals downstream and away from the shallow, hypersaline upper reaches. In contrast, densities in these latter reaches was greatest in autumn, which is likely to reflect an increase in water level and consequent marked decline in salinity (Appendix 4) and the presence of young Western Hardyheads, that spawn in the preceding months (Prince and Potter, 1983) and which represented between 55-80% of the total number of individuals recorded in these regions.

Spatial changes in fish faunal composition

Fish faunal composition changed markedly among the seven regions in the Vasse-Wonnerup, producing many pairwise comparisons with corresponding *R* values > 0.900 (Table 4), however, the pattern of regional differences (*i.e.* which regions were different from which other regions) changed in every season and year combination. Thus, to simplify this pattern and remove seasonal variability, CLUSTER-SIMPROF was employed to determine, without an *a priori* hypothesis, in which regions the compositions of the fish fauna were different from one another and those where they were not. This analysis, which pooled across regions, identified four statistically different groups or 'management units'; (I) the Lower Vasse River Wetlands, (II) Lower Vasse and Wonnerup estuaries, (III) Upper Vasse and Wonnerup estuaries and (IV) the Deadwater and Wonnerup Inlet. The grouping of the upper and lower estuaries together, rather than the Vasse and Wonnerup estuaries together, demonstrated that environmental differences between regions within an estuary (*i.e.* upper *vs* lower) exhibit a stronger influence on the fish fauna than those between estuaries (*i.e.* Vasse *vs* Wonnerup), a conclusion alluded to by Tweedley et al*.,* (2012) in their snapshot survey.

The fish fauna in the Lower Vasse River Wetlands was dominated by the Western Hardyhead, which contributed 80% to the total number of fish recorded in this region. This silverside is known to exhibit a preference for the upper reaches of estuaries where salinities are lower (Prince et al., 1982). Therefore, its high dominance in the Lower Vasse River Wetlands is likely due to the stable, low salinities there and a lack of competition from other hardyhead species, such as the Elongate Hardyhead, which was abundant in all other regions of the Vasse-Wonnerup, but hardly recorded in that region. Furthermore, although this species generally spawns in estuaries (Prince and Potter, 1983), it has been known to spawn in freshwater lakes (D. Morgan, unpublished data) and is classified as an estuarine & freshwater species (Potter et al., 2014). The same is also true for the Bluespot Goby, which generally prefers the upper reaches of estuaries, was most abundant in the Lower Vasse River Wetlands and is capable of surviving and spawning in freshwater (Gill and Potter, 1993; Gill et al., 1996). Four of the other five species recorded in this region were freshwater species namely, the Eastern Gambusia, Goldfish, Western Minnow and Western Pygmy Perch and their presence is related to the fact that the water in this region always remained fresh, with salinities never exceeding ~1.

The next management unit, the upper Vasse and Wonnerup estuaries, had a similar species complement to the Lower Vasse River Wetlands, except that the densities for all species declined, with the exception of the Elongate Hardyhead which increased. This low mean density is a reflection of the extreme changes in salinity (Fig. 16a) and water level (Fig. 16b), which occur in these regions over the course of a year. For example, no fish were recorded at any site in these regions three of the four summer and autumn seasons. While, in winter and spring, these areas are recolonised by low numbers of the Western Minnow and Western Pygmy Perch from the tributary rivers, the Elongate Hardyhead and the Bluespot Goby from the lower reaches of these estuaries. Substantial numbers of juvenile Goldfish also recruit into the Upper Vasse Estuary (and to a lesser extent into the Lower Vasse Estuary) during November from the Lower Vasse River Wetlands (see later). The timing of the recolonisation is likely to be determined by the pattern of rainfall as, all of these five species can breed in, or in the case of the Elongate Hardyhead, survive in freshwater. Furthermore, the only time any fish were recorded in the Upper Vasse or Wonnerup estuaries during summer or autumn was in May 2013, following unseasonal rains that occurred in late March/ early April, *i.e.* around the time of the large fish kill, which would have raised water levels and lower salinities to enable recolonisation by the Western Hardyhead and Bluespot Goby (Table 5f) from the Lower Vasse River Wetlands and Lower Wonnerup Estuary, respectively.

Fig. 16: The (a) Upper Wonnerup and (b) Upper Vasse estuaries in February 2013, showing shallow puddles of hypersaline waters (salinity = 132) and the dry expanses of benthos exposed, respectively, that are a result of extensive evaporation and a lack of freshwater discharge that occurs during the hot, dry summer period.

Moving downstream, the next 'management unit' identified by CLUSTER-SIMPROF was the lower Vasse and Wonnerup estuaries. Unlike their counterparts in the upper reaches, these regions generally always remain wet, albeit hypersaline (Lane et al., 2011). Thus, it is unsurprising that, these areas yield a greater number of species and density of individuals than the upper estuaries. This is due to the presence of freshwater species during winter and spring, but also several estuarine species, such as the Spotted Hardyhead and the Bridled Goby in the lower Vasse and Wonnerup estuaries, and the Black Bream, Common Silverbiddy and Southern Longfin Goby in the Lower Vasse Estuary. The greater densities in the lower reaches of the estuaries are mainly driven by increased densities of the Western and Elongate hardyheads and the Bluespot Goby, which may reflect the more stable water levels and the fact that these species are all euryhaline, with the last two species able to tolerate salinities of 76 and 136, respectively (Hoeksema et al., 2006).

The furthest downstream and most unique of the 'management units' is that containing the Deadwater and Wonnerup Inlet. These two regions yielded by far the most number of species, *i.e.* 25 and 22, respectively, out of the full complement of 31 and recorded the highest densities of fish in all seasons except spring. This high species richness is due to the fact that the only species that were not recorded in this management unit were the two native (Western Pygmy Perch and Western Minnow) and two introduced freshwater species (Eastern Gambusia and Goldfish) that breed in freshwater and the marine straggler species Old Wife, of which only a single individual was recorded. Furthermore, the Deadwater and Wonnerup Inlet harboured many marine estuarine-opportunist species, which were generally not caught in other regions. This included five species of Whiting and two species of Mullets, Porgies and Grunters. The presence of these species is related to these regions being located close to the ocean and having a relative stable water level and salinity regime, which was always similar to full strength sea water. The presence of so many juveniles belonging to marine estuarine-opportunist species provides a strong indication that many fish recruit into these regions of the Vasse-Wonnerup and use them as a nursery area. Furthermore, as these areas always contain water, they also act as an important refuge area when water levels recede upstream.

Temporal changes in fish faunal composition

Analysis of the extensive data collected during this study indicates that the composition of the fish fauna varies seasonally, with significant changes found to occur in each of the seven regions. These were generally related to far higher densities of fish being recorded in February and November, than in May and August, with the trend being present in both 2012 and 2013. This is reflected in the CLUSTER-SIMPROF dendrogram, which indicated that changes in the density of species among sampling occasions at the whole estuary level were responsible for the seasonal differences rather than a change in the presence or absence of species themselves.

Temporal changes in density were particularly marked in the most abundant four species namely the Western, Elongate and Spotted hardyheads and the Bluespot Goby, which together represented >90 % of all fish recorded (Table 6). Densities of the Western Hardyhead and Bluespot Goby peaked in November of both 2012 and 2013, and substantial numbers were also recorded in February. The peak in November is likely to reflect the presence of 0+ recruits, as the peak spawning period of both of these species is October (Prince and Potter, 1983; Gill et al., 1996). Likewise, in the Swan-Canning Estuary, the Elongate Hardyhead and the Spotted Hardyhead spawn around November-December (Prince and Potter 1983), which explains the peak abundance of these two species occurring in February. However, it is unlikely that the breeding cycle alone is responsible for the dramatic declines in some of these species during May and August. For example, while densities of the Southern Longfin Goby also decline conspicuously from a peak in February, Gill and Potter et al. (1993) found that the abundance of this species did not change seasonally. Furthermore, while these authors also reported that the density of the Bluespot Goby was significantly greater in spring than in winter, due to fish dying after spawning (Gill et al., 1996), the difference was not as marked as was recorded during this study where the density of this species declined from 55 and 40 individuals 100m^{-2} in spring and summer, respectively, to 2.5 and 1 individuals 100m^{-2} in autumn and winter, respectively.

Another factor that may explain the increase in total density in February may be the reduction in the surface area of the VWWS due to the substantial evaporation that occurs at this time of the year together with the lack of freshwater discharge (Lane et al., 2011) and thus the subsequent concentration of fish in the lower reaches. In contrast, when total densities are lower, particularly in August, water levels have increased. The conspicuous decline in density between February and May 2013 may be a result of the substantial fish kill (see later), however, it is worth noting that a similar although less marked decline was seen between these two seasons in the preceding year.

Spatial and temporal changes the abundance and distribution of introduced fish

Of the 13 introduced fish species that have been recorded in south-western Australian freshwater systems (Morgan et al., 2004), two species, namely the Eastern Gambusia and the Goldfish, were known to occur in the lower reaches of the Vasse River (Morgan and Beatty, 2007; Tweedley et al., 2013). While a snapshot survey by Tweedley et al. (2012), confirmed suspicions that these species had spread down into the estuary (Wetland Research and Management, 2007), this survey indicates that no more introduced fish species are present in the Vasse-Wonnerup. Furthermore, a detailed survey of the four tributary rivers undertaken concurrently with the current study confirmed that no additional introduced fish species were present (Tweedley et al., 2013).

The results of the current study demonstrate that Eastern Gambusia were present within three of the regions in the Vasse-Wonnerup, with their abundance varying markedly among them. Of the 4610 individuals, the vast majority 99.5% were found in the Lower Vasse River Wetlands. This is unsurprising as, despite being very tolerant of saline conditions Eastern Gambusia are essentially a freshwater species (Pyke 2005; 2008). It should be noted, however, that two individuals were collected using a sweep net from the Upper Wonnerup Estuary in a salinity of 72, breaking the previous world record of 58.8 (Chervinski, 1983).

While Eastern Gambusia were recorded in the Lower Vasse River Wetlands in seven of the eight seasons (*i.e.* all except November 2013), their densities varied markedly. Over 90% of the individuals were recorded in February, which corresponds to the peak breeding period of this species (Pyke 2005). Furthermore, it is relevant that Chapman and Warbourton (2006) found that flooding depleted densities of Eastern Gambusia, and, that the lowest densities were recorded during August and November following freshwater discharge. Low densities of Eastern Gambusia were recorded in the lower Vasse and Wonnerup estuaries, but only in February 2012, with the exception of two individuals caught in the latter region in November 2012. This suggests that although they possess a salinity tolerance that would enable them to survive in most of the Vasse-Wonnerup (Chervinski 1983; Pyke 2005), they generally remain in both the tributary rivers and the Lower Vasse River Wetlands.

The other species of introduced fish found by Tweedley et al. (2012) in the Vasse-Wonnerup was the Goldfish with a small number recorded in the Vasse Estuary during January 2012 in salinities of ~17 and this species has been thought to inhabit the Vasse River since at least 1996 (Morgan and Beatty 2007). As in Tweedley et al. (2012), Goldfish were found to occur only along the Vasse axis of the VWWS (*i.e.* Lower Vasse Wetlands, and the Upper and Lower Vasse estuary). Along this distribution the abundance of Goldfish declined, with 309 individuals being recorded in the Lower Vasse River Wetlands and 210 and 7 in the Upper and Lower Vasse Estuary, respectively. As most of these recordings were during times when the salinities in these regions were < 5, salinity is unlikely to be a prohibiting factor. As average length of goldfish recorded was 30 mm TL, It is more likely that the decline in abundance downstream reflects an increase in distance from the Lower Vasse River where they were born, which is ~7 km upstream.

The use of the estuary by juvenile goldfish occurred seasonally. Thus, while only 1, 1 and 0 fish were recorded in February and August 2012, respectively, 458 were recorded in November of that year. In 2013, 39 goldfish were recorded in February, none in May and August and 27 in November. These data clearly demonstrate that, following their birth around October (Morgan and Beatty, 2007), a large number of 0+ goldfish move downstream, either through flushing or perhaps swimming to avoid intraspecific completion from larger fish. Given that Tweedley et al., (2012) recorded juvenile goldfish in a salinity of 17 there is a risk that these fish might use the estuary as a 'saltbridge' to gain access to new river systems, such as the Sabina and Abba rivers, and to the Wonnerup Estuary and Ludlow River through Melbup creek during a wet winter, thus expanding its distribution (Schofield et al., 2006). It is relevant that such movements have been recorded in other introduced freshwater fish species elsewhere in the world, such as Zander, *Stizostedion lucioperca* (Brown et al., 2001; 2007). Furthermore, given that the Goldfish in the Lower Vasse River are the fastest growing in the world and are able to breed after their first year of life (Morgan and Beatty, 2007), the movement of several juveniles into a new tributary on a single occasion may be all that is needed to establish a new population.

Management and Monitoring Recommendations

Management

Issue	Findings	Actions
Controlling current introduced species in the Vasse-Wonnerup Wetland System	• Despite the large scale of the sampling regime, no new species of introduced fish or crayfish were recorded in the Vasse-Wonnerup with introductions currently limited to Goldfish (Vasse River and Vasse Diversion Drain), Eastern Gambusia (all rivers, wetland and estuarine habitats), and the eastern Australian Yabby (Vasse River, Vasse Diversion Drain, Sabina and Ludlow rivers). • Eastern Gambusia are widespread in the rivers, wetland and estuarine habitats and 26,081 removed from the rivers and 3,970 from the wetlands during this project. • Damage to the fins of native fish by Eastern Gambusia was considerable and was related to the seasonal patterns in the density of the invasive species. • Yabbies were recorded in very low abundance but remain a threat as they are known to compete with native crayfish for food, and have an invasive life-history including being tolerant of poor water quality and can burrow into sediment to escape drought. • A total of 842 Goldfish were removed from the Vasse River and 472 from the Lower Vasse River Wetland and the Upper Vasse	• There is little chance of controlling or eradicating Eastern Gambusia or Yabbies given their distribution, abundance (in terms of Eastern Gambusia), and invasive life-cycles. • Ongoing public education on the severe ecological damage caused by Eastern Gambusia and the Yabby should be conducted as part of the broader campaign to limited their additional spread (e.g. to artificial water bodies). • Ongoing control of the Goldfish in the Vasse River should occur and a fish trap be designed and trialed at the entrance to the New River Wetland. This could be a funnel design that would allow one-way movement of Goldfish in to the system during winter and subsequently block their movement back into the river during the drying summer months. This would allow

	Estuary, which exceeded the number from all of the previous control attempts combined. • The tracking study revealed that Goldfish were highly mobile with many travelling at least hundreds of km over a one year period. • Two key spawning habitats were revealed with 60% of Goldfish being recorded in the New River Wetland during its peak breeding period. Almost half the tracked fish also utilised the Lower Vasse River Wetland at some time. • Due to its salinity tolerance elucidated during the current project, without continued control and eradication efforts to reduce level of downstream movement to the wetland system, there is a high risk that the species will eventually colonise other rivers.	concentration of the species for control to occur in the near-dry wetland in autumn (using a piscicide or pumping). Consideration would need to be made regarding the monitoring (e.g. clearing debris) and possibly also trapping Sea Mullet in the wetland as it dries.
Preventing future introduced species in the Vasse-Wonnerup Wetland System	• The system was found to house only three of the 15 species of introduced fish and crayfish known from wild systems in south-western Australia. This presents an opportunity to protect this important system from these and future novel introductions. • There has been a 44% increase in the number of introduced freshwater fishes in south-western Australia over the past decade with 80% of new introductions being aquarium species. • Investing in prevention is the most cost-effective way to mitigate the impacts of introduced aquatic species as their control and eradication is usually costly, difficult or impossible.	• Ongoing public education on the high ecological significance of the Vasse-Wonnerup system along with the severe ecological damage that can result from introduced fish and crayfish should be conducted. • Public education should be coordinated across agencies (led by Department of Fisheries) to maximise the efficient use of resources and be targeted at a broader section of the community as possible. • A specific target should be the aquarium

		industry as this is the source of most new introductions in the region.
Protecting habitats for native fishes	• Areas of highest conservation value in the rivers were found to be in the lower reaches where permanent refuges existed. • The Deadwater and Wonnerup Inlet were identified as the key high-conservation regions of the Vasse-Wonnerup Wetland. • Fish kills are a major stressor on the system, particularly the Black Bream population that cannot recruit from outside the system (see Tweedley et al. 2014).	• Ongoing efforts to protect and rehabilitate riparian zones should be made, particularly in the lower reaches of rivers, to project refuge pools and other areas of high conservation value. • Ensuring priority is given to protecting the Deadwater and Wonnerup Inlet which houses the greatest diversity of fishes and act as refuges during times of poor water quality. • Continuing to address excessive nutrient inputs into the rivers of the Vasse-Wonnerup will benefit native fish and crayfish and the ecosystems more generally. • Immediately following any fish kill an independent assessment of its effects (e.g. fish mortality and subsequent ecological effects) should be undertaken (see Tweedley et al. 2014).
Reinstating migratory pathways for native fishes	• Limited seasonal migrations of native fishes were recorded into upstream sections.	• Instream barriers to fish migrations should be assessed and prioritised in the Sabina and Abba Rivers using the process

		developed and implemented for the Ludlow River (as part of a project that was conducted concurrently for the National Climate Change Adaptation Research Facility).

Monitoring

Issue	Need	Action
Preventing future introduced species in the Vasse-Wonnerup Wetland System	• The current study revealed no unexpected introduced species; however, given the recent increase in south-western Australia, it is paramount that any future introductions are detected early to provide greatest change of containment or eradication.	• Coordinated, annual monitoring of key sites in the Wetland and also the lower reaches of the Vasse, Sabina, Abba, and Ludlow Rivers during baseflow (e.g. February) should be conducted to detect any new introductions and also detect Goldfish should they spread into the latter three rivers.
Monitoring the health of the Vasse-Wonnerup Wetland System using fishes as an indicator group	• The Vasse-Wonnerup Wetland and its rivers were shown to have considerable ecological value in terms of fish fauna. • These fauna could be used as part of a broader monitoring program to track the health of the system and to assess	• Annual monitoring of the nearshore fish community in the Wetland and the lower reaches of its four rivers should occur in February in order to monitor the condition

	effectiveness of management actions (e.g. floodgate operation, nutrient reductions).	of the fish fauna in order to both track long-term changes, such as climate change, and also assess the effectiveness or any management actions undertaken to remediate the system.

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Appendices

Appendix 1: Field data sheet (environmental and habitat characteristics)

River: Site Description: Coordinates: S E

Date:

Freshwater Fish Group & Fish Health Unit

Cross-section 1 (downstream point)

SUBSTRATE (% est. in 3 x 1m^2 quadrates)	Bedrock	Boulder (>256mm)	Cobble (64-256mm)	Pebble (16-64mm)	Gravel (2-16mm)	Sand (1.44-0.06mm)	Silt (<0.06 mm)	Large Woody Debris
1								
2								
3								
BANK CONDITION	**Bank Angle** (1 = <30°, 2=30-60°, 3=60-90°)	**Erosion index 1-10** (1 = 100% eroded, 10= <5%)						
Left bank								
Right bank								
HYDROLOGY	Width=							
Depth (cm)								
Flow (cm/sec)								
RIPARIAN VEG 1 = <5%, 10 = 100%	% Channel shaded	% Overstorey	% Mid storey	% Under storey	% Weed cover			
Left bank								
Right bank								

Cross-section 2 (mid point)

SUBSTRATE (% est. in 3 x 1m^2 quadrates)	Bedrock	Boulder (>256mm)	Cobble (64-256mm)	Pebble (16-64mm)	Gravel (2-16mm)	Sand (1.44-0.06mm)	Silt (<0.06 mm)	Large Woody Debris
1								
2								
3								
BANK CONDITION	**Bank Angle** (1 = <30°, 2=30-60°, 3=60-90°)	**Erosion index 1-10** (1 = 100% eroded, 10= <5%)						
Left bank								
Right bank								
HYDROLOGY	Width=							
Depth (cm)								
Flow (cm/sec)								
RIPARIAN VEG 1 = <5%, 10 = 100%	% Channel shaded	% Overstorey	% Mid storey	% Under storey	% Weed cover			
Left bank								
Right bank								

Cross-section 3 (upstream point)

SUBSTRATE (% est. in 3 x 1m^2 quadrates)	Bedrock	Boulder (>256mm)	Cobble (64-256mm)	Pebble (16-64mm)	Gravel (2-16mm)	Sand (1.44-0.06mm)	Silt (<0.06 mm)	Large Woody Debris
1								
2								
3								
BANK CONDITION	**Bank Angle** (1 = <30°, 2=30-60°, 3=60-90°)	**Erosion index 1-10** (1 = 100% eroded, 10= <5%)						
Left bank								
Right bank								
HYDROLOGY	Width=							
Depth (cm)								
Flow (cm/sec)								
RIPARIAN VEG 1 = <5%, 10 = 100%	% Channel shaded	% Overstorey	% Mid storey	% Under storey	% Weed cover			
Left bank								
Right bank								

Water quality (mid-water column)

	1	2	3		1	2	3
Temp (°C) (30cm depth)				O_2 (ppm)			
Conductivity (µS/cm)				O_2 (%)			
TDS (mg/l)				NO3			
NaCl (mg/l)				NH4+			
pH				Water Sample frozen for TP/TN (tick)			
ORP							

Appendix 2: Site photographs

SABINA RIVER	Summer	Winter/spring
Barracks Drive		
Tuart Drive		
Bussell Highway		
Oates property		
Piggott Road		

ABBA RIVER	Summer	Winter/spring
Tuart Drive		
Bussell Highway		
Ludlow-Hithergreen Road		
Williamson Road		
Vasse Highway		

LUDLOW RIVER | **Summer** | **Winter/spring**

Tuart Drive

Bussell Highway

Capel-Tutunup Road

Warns Rd

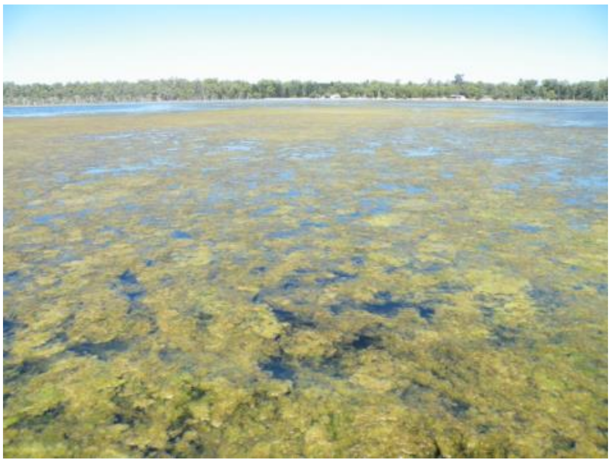
Yoganup Place

VASSE RIVER	**Summer**	**Winter/spring**
Chapman Hill Road		
Below Diversion Drain		

Appendix 3: Photographs highlighting the differences among regions and seasons in the Vasse-Wonnerup (for locations see Fig. 1). Photographs taken by James Tweedley.


The Deadwater in May and November 2013. Note the marked increase in macroalgae in November.


Wonnerup Inlet in May and November 2013. Note the exposed areas of seagrass wrack in May.




Lower Vasse Estuary in February 2012 and 2013. The environment in 2012 is characterised by large macroalgal blooms, while in 2013 low water levels exposed fine anoxic sediments.

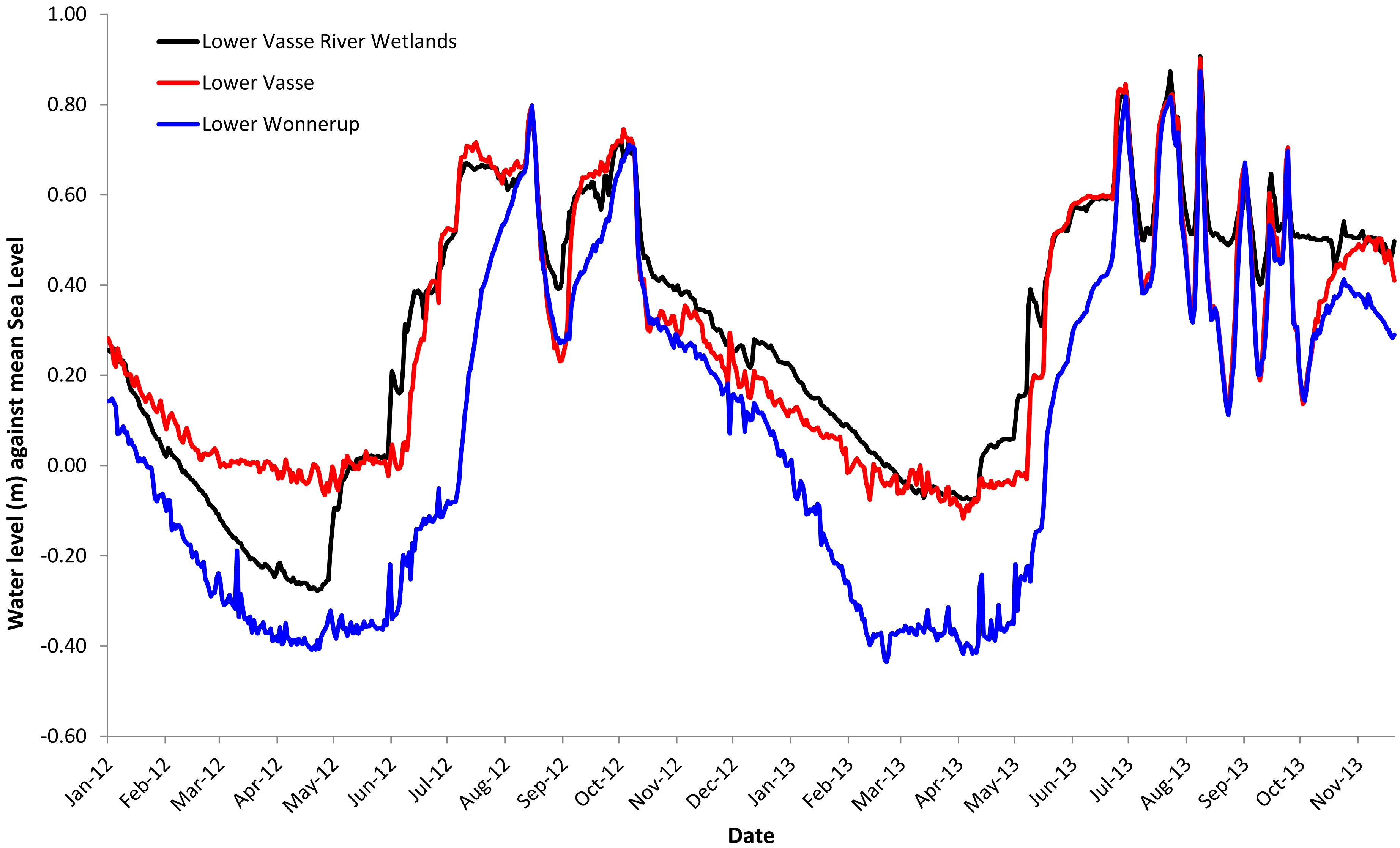
Appendix 3 continued: Photographs highlighting the differences among regions and seasons in the Vasse-Wonnerup (for locations see Fig. 1). Photographs taken by James Tweedley.

Lower Wonnerup Estuary in February and May 2013. Note the presence of serpulid tubeworm mounds in February and the subsequent dramatic increase in water levels by May of that year.

Upper Wonnerup Estuary in November 2012, shallow water with extensive macrophyte beds, however, by February 2013 the macrophytes had been consumed by birds, primarily Black Swans, and the water evaporated.

Lower Vasse River Wetlands in November 2012 and February 2013. Note the decrease in water levels and presence of a phytoplankton bloom in February 2013.

Appendix 4: Daily water levels in comparison to mean sea level recorded by the Department of Parks and Wildlife in the Lower Vasse River Wetlands, Lower Vasse Estuary and Lower Wonnerup Estuary. Data kindly provided by Jim Lane and Yvonne Winchcombe (DPaW).



Lower Vasse River Wetlands
Lower Vasse
Lower Wonnerup
Water level (m) against mean Sea Level
1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
Jan-12
Feb-12
Mar-12
Apr-12
May-12
Jun-12
Jul-12
Aug-12
Sep-12
Oct-12
Nov-12
Dec-12
Jan-13
Feb-13
Mar-13
Apr-13
May-13
Jun-13
Jul-13
Aug-13
Sep-13
Oct-13
Nov-13
Date

Appendix 5: State of the bar at the mouth of Wonnerup Inlet between December 2011 and December 2013. Photographs taken by James Tweedley.

Late December 2011: Deep, straight connection to the ocean as the bar had been dug open two weeks prior. Remained open in February 2012.

Late May 2012: Bar closed to the ocean

Late August 2012: Bar remained closed to ocean

November 2012: A relatively wide but shallow connection established

Appendix 5 continued: State of the bar at the mouth of Wonnerup Inlet between December 2011 and December 2013.

Late February 2013: A very narrow and shallow connection remains

Late May 2013: Bar closed to the ocean, despite being opened following the fish kill in April of that year.


Late August 2013: Deep and wide connection to ocean following heavy rain. Note the erosion on southern bank due to substantial outflow


Late November 2013: Bar closed.

Appendix 6: List of common and scientific names for fish collected from the Vasse-Wonnerup during this study. Species denotes with an * indicate that they have been collected from the system by the authors of this report since the completion of the current study.

Common Name	Species Name	Family	Family Name
Western Australian Salmon	*Arripis truttaceus*	Arripidae	Australasian Salmons
Common Hardyhead	*Atherinomorus vaigiensis*	Atherinidae	Silversides
Elongate Hardyhead	*Atherinosoma elongata*		
Spotted Hardyhead	*Craterocephalus mugiloides*		
Silver Fish	*Leptatherina presbyteroides*		
Western Hardyhead	*Leptatherina wallacei*		
Skipjack Trevally*	*Pseudocaranx wrighti*	Carangidae	Jacks and Trevally
Sandy Sprat	*Hyperlophus vittatus*	Clupeidae	Herrings
Goldfish	*Carassius auratus*	Cyprinidae	Carps
Australian Anchovy	*Engraulis australis*	Engraulidae	Anchovies
Old Wife	*Enoplosus armatus*	Enoplosidae	OldWives
Western Minnow	*Galaxias occidentalis*	Galaxiidae	Galaxiids
Common Silverbiddy	*Gerres subfasciatus*	Gerreidae	Mojarras
Bridled Goby	*Arenigobius bifrenatus*	Gobiidae	Gobies
Southern Longfin Goby	*Favonigobius lateralis*		
Bluespot Goby	*Pseudogobius olorum*		
Southern Garfish*	*Hyporhamphus melanochir*	Hemiramphidae	Halfbeaks
Yelloweye Mullet	*Aldrichetta forsteri*	Mugilidae	Mullets
Sea Mullet	*Mugil cephalus*		
Western Butterfish*	*Pentapodus vitta*	Nemipteridae	Threadfin Breams
Smalltooth Flounder	*Pseudorhombus jenynsii*	Paralichthyidae	Sand Flounders
Nightfish*	*Bostockia porosa*	Percichthyidae	Temperate Perches
Western Pygmy Perch	*Nannoperca vittata*		
Estuary Cobbler	*Cnidoglanis macrocephalus*	Plotosidae	Eeltail Catfish
Eastern Gambusia	*Gambusia holbrooki*	Poeciliidae	Livebearers
Tailor	*Pomatomus saltatrix*	Pomatomidae	Bluefishes
Mulloway*	*Argyrosomus japonicus*	Sciaenidae	Drums
King George Whiting	*Sillaginodes punctata*	Sillaginidae	Whitings
Southern School Whiting	*Sillago bassensis*		
Trumpeter Whiting	*Sillago maculata*		
Yellowfin Whiting	*Sillago schomburgkii*		
Western School Whiting	*Sillago vittata*		
Black Bream	*Acanthopagrus butcheri*	Sparidae	Porgies
Tarwhine	*Rhabdosargus sarba*		
Yellowtail Grunter	*Amniataba caudavittata*	Terapontidae	Grunters
Western Striped Grunter	*Pelates octolineatus*		
Prickly Toadfish	*Contusus brevicaudus*	Tetraodontidae	Puffers
Soldier	*Gymnapistes marmoratus*	Tetrarogidae	Wasp Fish