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Preliminary evidence of spawning phenologies of freshwater fish in a wet-dry tropical river: the importance of both wet and dry seasons

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Lay Summary

This study demonstrated that freshwater fish spawn throughout the year in a range of hydrological conditions in a wet-dry tropical river. Whilst a high number of fish spawned in the wet season, dry season and dry-wet transition periods were also significant. The study has important implications for future research and for managing the impacts of future flow modifications in the wet-dry tropics.

Abstract

Wet-dry tropical rivers are characterised by highly predictable, yet highly variable, seasonal flow regimes. The wet season is often regarded as an important period of ecosystem productivity, dispersal and connectivity, and also for freshwater fish spawning and recruitment. However, few studies have examined fish spawning across hydrological seasons in these rivers. We conducted a pilot study to determine (i) the temporal occurrence (and hence spawning period), and (ii) the suitability of standard sampling methods of young fish in the Daly River, Northern Territory, Australia. Fish spawned throughout the year, with spawning phenologies varying substantially among species. The highest diversity and abundance of young fish occurred during the wet season, although early life stages of a high number of species were also present in the dry season and transition periods. A high number

of species spawned all year round, while others had very discrete spawning periods. Three of the four sampling methods tested were successful in catching early life stages and should be employed in future studies. This study highlights that all hydrological seasons in the wet-dry tropics are important for fish spawning, and has important implications for future research on the drivers of spawning patterns, and for predicting the effects of flow modifications on freshwater fishes of the wet-dry tropics.

Introduction

The early life stages are a critical phase in the life cycle of fishes, as the number of individuals that survive this period determines the strength of that cohort in the population (Houde 1997, Maceina and Pereira 2007; King *et al.* 2013). Recruitment or cohort strength is influenced by a range of environmental and biotic factors, including availability of food, predation and competition, habitat, water quality and inherited genetic factors (Wootton 1990, Maceina and Pereira 2007, Humphries *et al*. subm). Most freshwater fish display seasonal reproductive and recruitment cycles linked to favourable environmental conditions that maximise the success of spawning, fertilization and the rearing of young (Humphries *et al*. subm). In temperate freshwater ecosystems, numerous studies have demonstrated that the seasonality of fish reproductive cycles is linked to the variability in factors such as photoperiod, water temperature and increased food availability, which are sometimes mediated through changes in discharge (e.g. Trippel and Chambers 1997, King *et al*. 2003, Zeug and Winemiller 2008). Tropical and sub-tropical regions exhibit smaller variations in annual photoperiod and temperature than temperate regions. However, some studies suggest that fishes still exhibit seasonal changes in reproduction and recruitment, but the cyclic nature is more likely to be linked to water level, rainfall or water conductivity changes (e.g. Welcomme 1985, Baumgartner *et al*. 2008, Reynalte *et al*. 2012).

Rivers in northern Australia are characterised by their wet-dry climatic cycles (also referred to as tropical savannah climate) (Peel *et al*. 2007, Warfe *et al*. 2011). Most discharge in these rivers occurs over only a few months as a result of monsoonal rains in the wet season, contrasting with the remaining months where little or no rainfall, and low- or zero-flow, occurs during the dry season (Petheram *et al*. 2008, Warfe *et al*. 2011, King *et al*. 2015). River flow is highly seasonal and annually predictable, but considerable interannual variability occurs in the timing, magnitude, duration and frequency of key hydrological events (King *et al*. 2015). Whilst the flow regimes in many of the regions' rivers are

relatively unmodified at present (Woinarski *et al*. 2007, Pusey *et al*. 2011, Warfe *et al*. 2011), growing interest in expanding agriculture and mining industries is likely to place increasing pressure on the water resources of the region (Hart 2004, Blanch 2008, Douglas *et al*. 2011, COA 2015).

Northern Australia's wet-dry tropical rivers contain a rich diversity of aquatic biota (Douglas *et al*. 2011), with 111 freshwater fish species recorded to date from a variety of taxonomic and life history groups (Pusey *et al*. 2017). In contrast to the southern catchments of Australia, ecological studies on freshwater fishes in the wet-dry tropics are limited (Pusey *et al*. 2011). In particular, there is a dearth of knowledge on the reproductive biology or early life history of these fishes (Pusey *et al*. 2004, King *et al*. 2013). Indeed, few studies have attempted to sample the early life stages in these rivers, such that even knowledge of suitable sampling methods is limited. Whilst there is some inferred knowledge of reproductive biology of fishes in these rivers derived for cosmopolitan species from other regions (e.g. Beumer 1979, Pusey *et al.* 2001, 2002, 2004, Close *et al*. 2005, Balcombe *et al*. 2007, Godfrey *et al.*, 2016) and from recruitment strength analyses (e.g. Pusey *et al*. 2018); the most comprehensive research to date on fish reproductive biology in the wild was undertaken by Bishop *et al.* (2001) in the ephemeral Alligators Rivers Region, Northern Territory in 1978/79. This study (Bishop *et al.* 2001) examined the fish assemblages and simple reproductive measures based on length-frequency analysis and gonad staging. Analysis of the spawning phenology described in this study suggests that while some species spawned all year round (*Oxyeleotris lineolatus*, *Craterocephalus stercusmuscarum* and *Glossamia aprion*), the majority of species spawned around the onset of the wet season (Bishop *et al*. 2001, King *et al*. 2013). Breeding at this time presumably enables young fishes to rapidly grow, develop and disperse by taking advantage of the increased food and habitat available during periods of extensive floodplain inundation (Bishop *et al*. 2001, Warfe *et al*. 2011). Indeed, while much emphasis has been placed on the importance of the wet season period for spawning and recruitment (e.g. Bishop *et al*. 2001, Pusey and Kennard 2009, Warfe *et al*. 2011); knowledge on the timing, peak abundance, variability and environmental correlates of spawning for most species in the region remains poor (Stewart-Koster *et al*. 2011, King *et al*. 2013).

This study aimed to (i) provide an initial insight into the spawning phenology and occurrence of early life stages (larval and juvenile) of freshwater fishes in a large floodplain river in

northern Australia's wet-dry tropics (Daly River, Northern Territory), and (ii) trial the utility of commonly used methods for sampling the early life stages fish in these logistically challenging systems. Unlike other studies, we directly sampled fish early life stages throughout all hydrological seasons (including the wet season), over one full hydrological cycle in the river. With regards to the first aim of the study, we hypothesised that fish spawning would occur throughout the annual wet-dry cycle, but that there would be contrasting spawning phenologies among species. We also hypothesised that the four trialled methods would capture species with various life history and behavioural traits, and therefore all methods would be required to adequately describe the larval fish assemblage.

Methods

The Daly River catchment is a perennial, groundwater fed river, in the wet-dry tropics of northern Australia (river length ~ 320 km, catchment area ~ 52,500 km², mean annual rainfall 1070 mm, mean annual stream-flow ~ 270 m³/sec (Begg *et al*. 2001, CSIRO 2009, Fig. 1). Low-density cattle-grazing (50%) and conservation / natural environment (42%) are the two dominant land use types in the catchment, with approximately 10% of the catchment cleared for other intensive land uses including agriculture, urbanisation and mining (Law and Blanch 2009). Rainfall in the region is highly seasonal with up to 95% of rainfall occurring in the wet season (November – April) and negligible rainfall occurring during the dry season (May-October). Like other tropical rivers, the annual occurrence of high and low flow periods is predictable, but there is high inter-annual variability in flow timing, magnitude and duration of the four hydrological seasons: (i) wet season (high flow, flooding period), (ii) wet-dry transition season (declining flows, floodplain run-off), (iii) dry season (stable, low flow period) and (iv) dry-wet transition season (episodic rainfall or flow events) (see Warfe *et al.* 2011, King *et al*. 2015). There are no major impoundments (dams or weirs) in the Daly River catchment; however groundwater is extracted from bores, which affects dry season river discharge (DLRM 2016).

This study occurred in the lower freshwater (non-tidal) reaches of the river, upstream of the Daly River Crossing (Fig. 1); this site is representative of the lower freshwater non-tidal reaches of the river. The crossing itself is a low-level road crossing, with large culverts allowing flow downstream, and is easily overtopped with increased water levels during the dry-wet transition season and wet season periods. The river in this reach is largely sand-bed dominated, but is also dispersed with cobble beds and bedrock outcrops. The channel

contains deep pool (up to 5m) and run sequences (~1-2m deep), and a variety of shallower, slackwater habitats occur along the littoral margins. Large woody debris is common throughout the reach, and riparian vegetation includes *Melaleuca* spp. and *Eucalyptus* spp., and dense understorey stands of *Phragmites karka* along the river margins. The freshwater fish fauna of the Daly River catchment is species rich compared with many other Australian river systems, with at least 75 species recorded in the freshwater reaches, with a diversity of life history and migration strategies (Pusey *et al.* 2011).

Sampling of fish early life stages (larvae and juveniles) was conducted at ~six weekly intervals from May 2013 to July 2014 (a total of 11 sampling trips), throughout one full hydrological wet-dry annual cycle. Sampling was conducted using four standard early life stage sampling methods: drift net, tow net, light trap and sweep net electrofishing (SNE); to ensure a diverse range of species, behaviours and life history strategies were being sampled (Humphries *et al*. 2002, King 2004). Drift and tow nets were used to sample the deeper, faster flowing mid-channel habitats; while SNE and light trap methods were used to sample littoral habitats. Drift nets were constructed of 500 µm mesh, were 1.5 m long with a 0.5 m diameter mouth opening, and tapered down to a removable collection container. A General Oceanics Inc. (Florida, USA) flow meter was attached in the mouth of each drift net to enable raw catch data to be adjusted to a standard volume of filtered water (1000 m^3). Drift nets were used to sample the deeper, faster flowing mid-channel habitats. On each sampling occasion, three drift nets were tied to immovable instream woody debris and deployed for a period of 3 hours during the day (1500-1800) and 12 hours overnight (2000-0800). The tow net was constructed of 500 µm mesh, tapering to a collection jar, with a General Oceanics Inc. (Florida, USA) flow meter attached to the mouth of the net to enable raw catch data to be adjusted to a standard volume of filtered water (1000 m^3). The net was towed ~10m behind the boat (travelling in an upstream direction) in the mid-channel open water for three minutes during the day. The tow net was briefly trialled in this study, but was very ineffective (no fish captured in 20 samples), and tow net sampling was discontinued after the second trip. Fish catches from tow nets are not considered in further analysis.

The SNE method (King and Crook 2002) was used to sample larval fish in slackwater and slow water habitats. SNE sampling was conducted during the day (0800-1730) and the same habitats were then sampled again at night (2000-2230) during all trips except Trip 1. Habitats were not disturbed between the collections of the day and night-time samples, and these two

sampling events were treated as independent in the analysis, as fish were able to move freely between sampled and non-sampled habitats for a number of hours between each sampling event. The number of sampled locations varied slightly per trip, with at least 10 during the day and 10 at night. Ten modified quatrefoil light traps (5mm slot width, Humphries *et al*. 2002) were deployed on each trip to sample larval fish from structurally dense, still-slow velocity habitats, which are difficult to sample with the other active methods. Light traps were deployed near dusk (~1830) and retrieved early the next day (~0800). Water quality was recorded at each sampling location during the day and night with measurements of pH, conductivity ($mS.cm^{-1}$), dissolved oxygen ($mg.L^{-1}$), dissolved oxygen concentration (%) , temperature (^0C), and turbidity (NTU) taken using a Horiba™ U10 Water Quality Checker (Horiba Ltd., Japan).

Any captured fish that could be confidently identified in the field were recorded and released. The remaining sample was placed in an overdose of clove oil solution (at least 6 drops per 1 litre) for 5 minutes to euthanise any fish present. The whole sample was then retained and preserved with 95% ethanol prior to further processing in the laboratory. Fish were sorted from debris and examined under a dissecting microscope to identify species and development stage, with the transition from larva to juvenile delineated by the acquisition of the adult complement of segmented rays on all fins and the loss of the pre-anal fin fold (Kelso and Rutherford 1996, Serafini and Humphries 2004). Juvenile fish were identified to species level using published descriptions and keys (Allen and Burgess 1990; Allen *et al*. 2002; Pusey *et al*. 2004). Larvae were identified using published descriptions where available (Ivantsoff *et al.* 1988; Bishop *et al*. 2001; Pusey *et al*. 2004; Close *et al*. 2005: Simon *et al.* 2012); however, descriptions of early life stages of Australian tropical freshwater fish are lacking. To assist in identification for some species, we developed a specimen reference library consisting of either known specimens from local aquarium facilities (pers comm. David Wilson, Aquagreen), or a known development series was established from a positively identified juvenile to the smallest collected larva. Some genera were grouped where we couldn't be confident of species specific characteristics (e.g. *Melanotaenia* spp., *Craterocephalus* spp.).

The occurrence of fish larvae and juveniles of a given species across sampling periods was used to construct simple spawning phenologies. Estimated spawning periods across hydrological seasons are presented both as the number of species occurring as larvae only,

and larvae and juveniles combined (where the timing of juvenile occurrence was lagged by one month to account for development, as an estimate of spawning period). Juvenile data was not used in any other statistical analysis. Patterns and significance in fish larval assemblages, species richness and total abundance were analysed using non-metric multidimensional scaling (NMDS) and using a 3-way nested PERMANOVA for the factors Season, Method and Trip (nested in Season) in PRIMER 7™ (Clarke and Warwick 2001). Larval fish assemblage data were analysed as the relative abundance of a given species per replicate sample. Drift net data were standardised for the volume of water filtered, but SNE and light trip methods were analysed as uncorrected data (i.e. only standardised for field effort). Sampling trips were categorised into four hydrological seasons according to the shape of the hydrograph (following King *et al*. 2015; see above and Table 1). Note that sampling effort was not equal between sampling seasons (dry n=5, dry-wet n=1, wet n=2, wet-dry n=3). Fish data were log10(x+1) transformed, and an NMDS plotted on the basis of a Bray–Curtis similarity matrix. A 3-way nested PERMANOVA was also conducted on assemblage data, species richness and total abundance, principally to test for differences between Season and Method. Unrestricted permutations of data were performed for all analyses, with 999 permutations for the test. Dispersion of significant treatments was tested using PERMDISP, and interpretations adjusted if required (see Anderson et al. 2008). Sample discrimination (SIMPER) was used to investigate which species provided the greatest contribution to the groups (Clarke and Warwick 2001).

Results

Hydrology and the measured water quality parameters varied substantially during the study period, operating in a cyclical manner (Fig. 2). During the dry season, discharge and turbidity were at their lowest levels, while pH and temperature were low early in the season and increased during the late dry season. High discharge wet season flows also resulted in low dissolved oxygen and pH, while turbidity and conductivity was at their highest levels (Fig. 2).

Larval and juvenile fish were captured all year round, in all hydrological seasons (Table 1, Fig. 3). A total of 1,134 larvae and 1,038 juvenile fish, from 22 species and a further eight unidentified species, were captured during this study (Table 1). The most commonly captured species as larvae were *Craterocephalus* spp*., Amniataba percoides,* and *Melanotaenia* spp.. The highest combined species richness of larvae and juveniles were present in the wet season (23 species) followed by the wet-dry (17), dry (16) and dry-wet (8) seasons (Fig. 3a). Using

combined larvae and adjusted juvenile occurrence, the majority of species were estimated to spawn all year round (11 species, Figure 3b; *Ambassis* spp., *Amniataba percoides, Craterocephalus* spp., *Glossamia aprion, Glossogobius* spp., *Leptachirus triramus, Melanotaenia* spp., *Oxyeleotris lineolatus , Oxyeleotris selheimi, Strongylura krefftii and Toxotes chatareus*). Nine species spawned in the wet season only (Figure 3b) (*Mogurnda mogurnda, Neosilurus ater, Syncomistes butleri, UNID Neosilurus* spp., *UNID sp. B, D, E, F, H*). Only one species, *Ophisternon gutturale*, was predicted to spawn in the dry season only based on captures of juveniles; however, the catch of this species was very low (n=2) (Fig. 3b), and the interpretation of spawning time for this species should be treated with caution.

Larval fish assemblage structure, total abundance and species richness differed significantly among Season, Method, Trip (Season) and Season x Method interaction (Table 2, Fig. S1 and S2, available in supplementary material). The larval assemblage, total abundance and species richness all differed significantly between the two transition periods for both the drift net and SNE sampling methods (PERMANOVA post-hoc results, $p<0.05$) and between the wet and dry season ($p<0.05$), but not for any other period or method combination. The larval assemblage differed most significantly between the two transition periods (pairwise tests, wet-dry and dry-wet, $P<0.001$), and the dry and wet season ($p<0.05$). Larval assemblage differences between all seasons were characterised only by varying contributions of *Craterocephalus* spp. and *Melanotaenia* spp. (SIMPER analysis).

The larval assemblage differed significantly between drift net and both SNE and light trap collection methods ($P<0.05$), but not between SNE and light traps. The larval assemblage differences in drift net catches were characterised by *A. percoides, Craterocephalus* spp., and *UNID sp.C.* (SIMPER analysis in order of percent contribution). The mean number of larvae and species richness for all methods was lowest in the early dry season, but steadily increased throughout the dry and into the dry-wet season transition period (Fig. 4, Fig S.1). The mean number of larvae and number of species collected peaked in the wet season for drift nets and SNE samples (Fig. 4) and the dry-wet season for light traps (Fig. 4). Whilst all methods collected a range of species as both larvae and juveniles, the highest number of species and individuals were collected by SNE method, while light traps captured the lowest number of species and did not collect additional species collected in other methods (Table S1).

Discussion

The wet season in tropical rivers is an important hydrological phase, whereby flood waters annually inundate previously dry floodplains, generating large amounts of nutrients and food resources to support the river's food webs (Junk *et al*. 1989, Warfe *et al*. 2001). Perhaps in part as a consequence of this, much emphasis has been placed on the likely importance of the wet season for spawning and recruitment of freshwater fishes in Australia's wet-dry tropics (e.g. Bishop *et al*. 2001, Staunton-Smith *et al*. 2004, Pusey and Kennard 2009, Warfe *et al*. 2011). Presumably at this time, young fish would grow and develop rapidly, taking advantage of the increased food and habitat available, and be able to disperse to more favourable rearing habitats if required (Bishop *et al*. 2001, Warfe *et al*. 2011). However, breeding during flood conditions also has some inherent risks, such as stranding, poor water quality and physical damage from high velocity waters (see e.g. King *et al*. 2003). In this study, spawning occurred throughout all hydrological seasons and water quality conditions, with the highest diversity and abundance of larvae occurring during the wet season. However, high numbers of species were also recorded spawning in the dry, wet-dry and dry-wet transition seasons.

Whilst flooding may be an important cue to initiate spawning and to support successful recruitment for some fish in wet-dry tropical rivers, this study demonstrates that not all species require a flow stimulus to spawn. Sixteen species spawned during low flow, dry season conditions, where little hydrological or water quality variation occurred. Although the larval and juvenile fish assemblage varied over the dry season, the small-bodied opportunistic species (*sensu* Winemiller and Rose 1992), *Craterocephalus* spp. and *Melanataenia* spp., always dominated the assemblage. Spawning and recruitment of some species during low flow, dry season conditions, has also been reported in other tropical and sub-tropical, (e.g. Milton and Arthington 1983, Pusey *et al.* 2001, 2002, Godfrey *et al*. 2016) and temperate rivers (Turner *et al*. 1994, Humphries *et al*. 2002, Lorig *et al*. 2013). The low flow recruitment hypothesis (Humphries *et al*. 1999) postulated that some species are able to utilise low flow conditions for spawning and recruitment by using shallow, slackwater habitats which may favour successful recruitment by providing: shelter from predators, warmer temperatures that enhance growth and higher concentrations of suitable prey than other habitats (see also King 2004, Zeug and Winemiller 2008). Whether these mechanisms govern the use of dry season conditions for spawning was not examined in this study and would be a worthwhile theme for future research.

The spawning phenologies exhibited by fish in the Daly River varied substantially among species and among life history strategies. A high number of species spawned during the wet season, however only nine species (*M. mogurnda, N. ater, S. butleri, UNID Neosilurus* spp*., UNID sp. B, D, E, F, H*) were found to spawn in the wet season only. Similarly, potentially only one species (*O. gutturale)*, spawned solely during the dry season (but was collected only as 2 juveniles). Whilst not a comprehensive study of spawning phenology for these species due to the spatial (one site) and temporal (six week sampling frequency, 1 year only) limitations of the sampling program, this study highlights important spawning time discrepancies if spawning times are transferred from other systems, such as the Australian wet tropics. For example, *T. chatareus*, *H. fuliginosus* and *L. unicolor* that have previously been reported to spawn in the wet season only (Pusey *et al*. 2004, 2018, Simon *et al*. 2012), were collected in this study throughout a range of hydrological conditions, indicating a potentially more prolonged spawning time for these species than previously recorded.

This study recorded a high number of species seemingly undertaking an aseasonal spawning strategy (spawning not limited to a particular season). Aseasonal spawning is likely to be an uncommon reproductive strategy in freshwater fishes globally, but may be more common in tropical regions that lack marked changes in temperature and photoperiod among seasons. Aseasonal spawners are likely to have a high degree of flexibility or nonspecific spawning requirements, and may have a recruitment advantage over species that spawn over a brief or discrete time period as their young are more likely to encounter optimal rearing conditions (Humphries *et al*. 2013). Kerezsy et al. (2011) also reported the predominance of continual recruitment strategies for fish in Australian arid zone rivers, where some fishes were able to recruit all year round irrespective of water level conditions. Aseasonal spawning has been previously reported for *O. lineolatus*, *Craterocephalus* spp. and *G. aprion* in the nearby Alligator Rivers system, Northern Territory (Bishop *et al*. (2001). The current study supports this assignment for these species, and suggests that a further eight species (*Ambassis* spp*., A. percoides, Glossogobius* spp*., L. triramus, Melanotaenia* spp*., O. selheimi, S. krefftii, T. chatareus*) may be aseasonal spawners in the Daly River. Melanotaenia rainbowfishes have previously been described as having a very flexible spawning period, capable of spawning and recruiting during both dry and wet season conditions in a range of different flow habitats across northern Australia (Bishop *et al*. 2001, Pusey *et al*. 2001, 2004, 2018, Godfrey *et al*. 2016). However, while the larval and juvenile collections in this study suggest that *A. percoides* and *Glossogobius spp.* spawn throughout the year, Pusey *et al*. (2018) noted these

two species as only dry season spawners in an upstream, intermittent tributary of the Daly River. This discrepancy may indicate varied spawning patterns in different habitat types, and may be due to the restricted dry season sampling period and sampling focus on larger bodied individuals by Pusey *et al*. (2018), or false attribution of spawning time from juveniles captured in our study; further research is required to explore these discrepancies further.

The environmental conditions in wet-dry tropical rivers in northern Australia vary immensely throughout the year and hydrological seasons (see Warfe *et al*. 2011 for further discussion, Fig. 2.). This, along with the number of major predators in these systems (saltwater crocodiles and sharks), makes effective sampling of fish early life stages very challenging, and could perhaps partly explain the lack of this type of research in the region. A range of different sampling gears (e.g. seine and drift nets, traps, electrofishing), are commonly used to sample the early life stages of fish in freshwater systems, with each gear type having its own inherent biases and limitations towards particular environments, behaviours and life stages (Humphries *et al*. 2002, King and Crook 2002, King 2004). As a consequence, many studies use a combination of sampling gears to more accurately reflect assemblage structure. Prior to this study, the only recorded study to our knowledge that sampled fish larvae in this region used plankton nets and beam trawls during the dry season only (Berra and Neira 2003). Studies in tropical rivers elsewhere have often sampled dry season periods only and have limited the range of sampling methods, often only to plankton nets (e.g. Pusey *et al*. 2002, Baumgartner *et al*. 2008).

Of the four methods trialled in this study, drift nets, light traps and SNE method captured larvae and juveniles from a range of species with differing life history strategies, and were able to be safely and efficiently used under varied environmental conditions throughout the year. Tow nets, however, failed to capture any larvae during the first two trips and were therefore removed from the sampling protocol. We are unclear why this occurred but may be due to habitat type avoidance or sampling interference from the boat. Drift nets and the SNE method captured both the highest number of larvae and highest species richness, although the larval assemblages did differ across the methods between seasons. Drift nets are widely used in riverine larval studies (e.g. Humphries *et al*. 2002, King *et al*. 2016) and sample fishes that are either actively drifting or accidentally entrained in the faster flowing waters. SNE and light traps are able to effectively sample early life stages that utilise still or slow flowing habitats, and use both active and passive collection techniques respectively. Light traps had

similar successes to SNE at all times of year except for in the wet season, when they caught less individuals and species. It is possible that reduction in light trap catch during the wet season may be the result of very turbid water during the high flows, reducing the efficiency of the light in attracting fish into the trap. Conversely, juvenile fish were sometimes observed to actively swim out of range of the electric field before they could be stunned, which became more apparent in clear water potentially because they could see the net/operator (King and Tyler, pers. obs.). These potential sampling biases highlight that environmental conditions need to be carefully considered when selecting sampling methods, and the simultaneous use of both methods in this study may have accounted for the deficiency of the other. We therefore recommend that future studies in these systems should continue to utilise a range of sampling gears over a variety of habitat types.

This study, while limited in its spatial and temporal sampling extent, provides valuable new information about the potential spawning phenology for many freshwater species in wet-dry tropical rivers. Spawning was recorded throughout all hydrological seasons and water quality conditions. This highlights the diverse range of environmental characteristics required for successful fish reproduction; and the need for water management to consider both wet and dry season flow management rules to minimise the impact of flow regime alterations on spawning of freshwater fishes in wet-dry tropical rivers. This study emphasises the importance of the dry season for spawning of some fish species. This finding emphasises the importance of maintaining base flows in perennial systems such as the Daly River, and suggests that excessive groundwater or surface water extraction during the dry season may affect the recruitment success of some species. Given the increasing amount of water harvesting and extraction throughout northern Australian rivers (COA 2015, King *et al*. 2015), further and more detailed research is urgently required on the flow requirements for fish spawning and recruitment to support science-based environmental flow provisions into the future.

Conflicts of Interest

The authors declare no conflicts of interest.

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Table 1: Occurrence of total larvae (TL) (black bars) and total juveniles (J) (hatched bars) throughout the sampling period. All methods are included; W-D = wet-dry, Dry = dry, Wet = wet, D-W = dry-wet hydrological season.

Year		2013						2014					Total	
Sampling Month		1 May	11 Jun	23 Jul	9 Sep	28 Oct	26 Nov	22 Jan	24 Feb	17 Mar	28 Apr	15 Jul		
Hydrological season		W-D	Dry				D-W	Wet		W-D		Dry	TL	J
Ambassis spp.	TL												71	
	J													288
Amniataba percoides	TL												130	
	J													121
Arramphus sclerolepsis	J													5
Craterocephalus spp.	TL												566	
	J													30
Glossamia aprion	TL												7	
	J													24
Glossogobius spp.	J													18
Hephaestus fuliginosus	J													7
Hypseleotris compressa	TL												36	
	J													65
Leptachirus triramus	J													14
Leiopotherapon unicolor	J													37
Melanotaenia spp.	TL												107	
	L													100
Mogurnda mogurnda	J													3
Nematalosa erebi	TL												1	
	J													17
Neoarius graffei	J													4
Neosilurus ater	TL												1	
	J													2
Neosilurus hyrtlii	J													12
Ophisternon gutturale	J													2
Oxyeleotris lineolatus	TL												6	
	J													9
Oxyeleotris selheimi	J													6
Strongylura kreffti	J													19
Syncomistes butleri	TL												1	
	J													5
Toxotes chatareus	TL												5	
	J													250
UnID *Heramphidae spp.*	TL												2	
UnID *Neosilurus spp.*	TL												29	
UnID spB	TL												1	
UnID spC	TL												49	
UnID spD	TL												42	
UnID spE	TL												24	
UnID spF	TL												4	
UnID spH	TL												52	
													GRAND TOTAL	**1134**	**1038**

Table 2. PERMANOVA results (significance *P<0.05, **P<0.01, ns=not significant) for assemblage, total abundance and species richness of larvae only.

		Assemblage		Total Abundance		Sp. Richness	
Factors	df	MS	P	MS	P	MS	P
Season	3	8412.2	*	8701.7	*	9367	*
Method	2	4269.6	*	635.55	ns	549.72	ns
Trip (Season)	7	2697.8	**	1658.3	*	1124.4	**
Season x Method	6	3497.4	**	2518.3	**	2411.1	**
Trip (Season) x Method	14	798.8	*	416.17	ns	464.81	ns

Fig. 1. Map of the Daly River catchment showing sampling site location at Daly River Crossing.

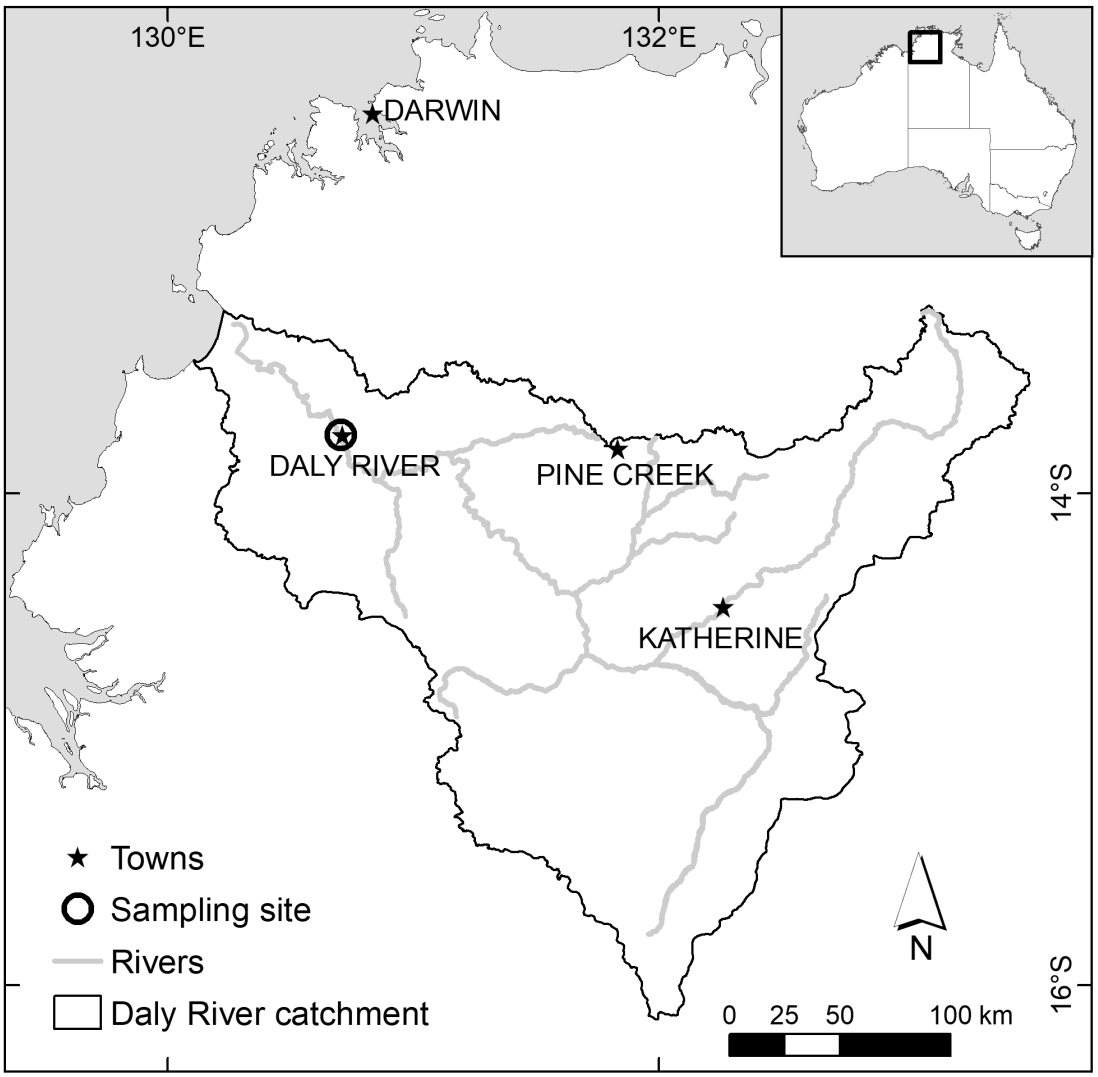


Fig. 2. (a) Discharge and mean water quality variables, (b) pH, (c) temperature, (d) dissolved oxygen, (e) conductivity, (f) turbidity across the sampling period. Shading depicts different hydrological seasons. SE values too small to be displayed.

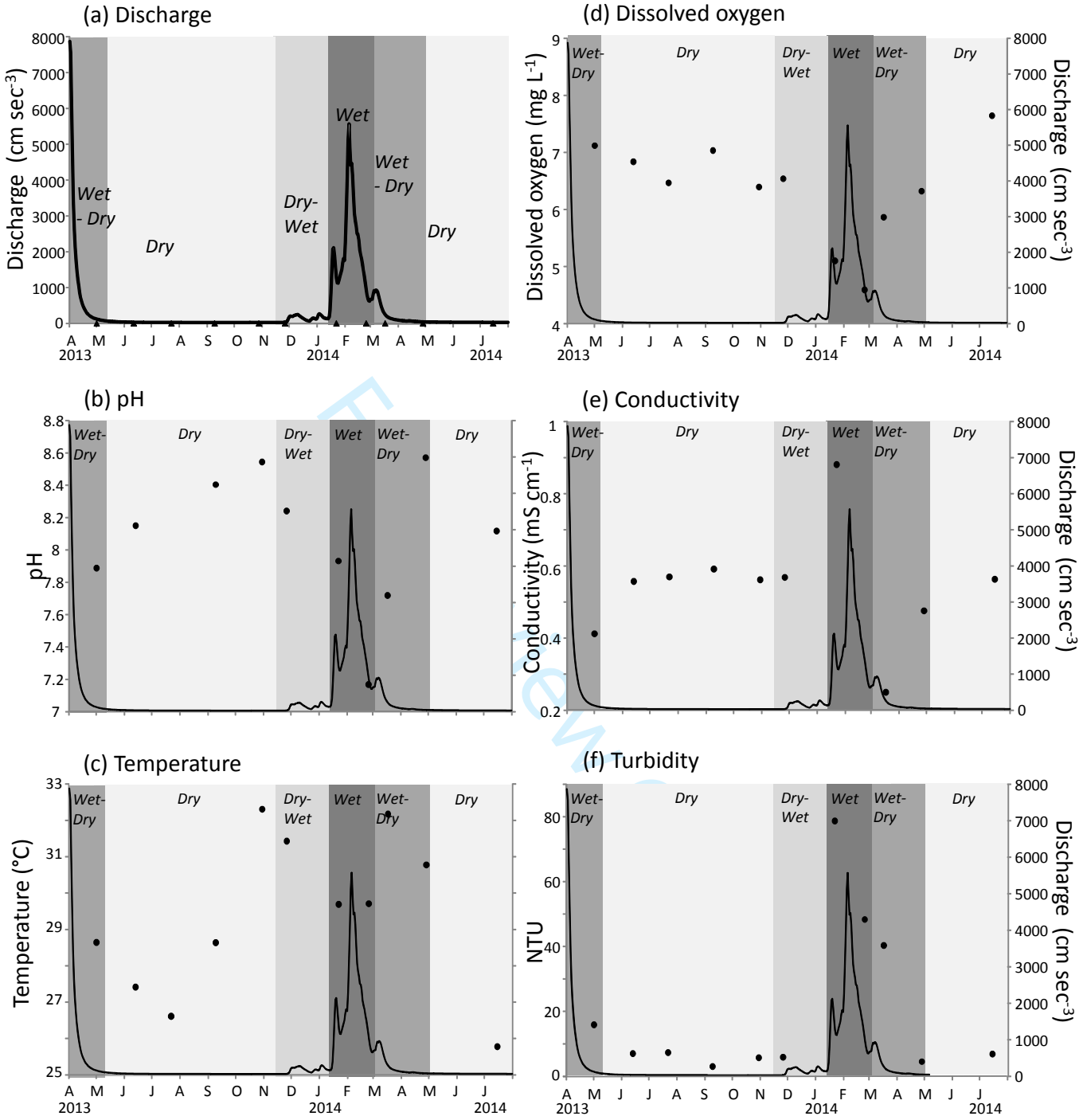
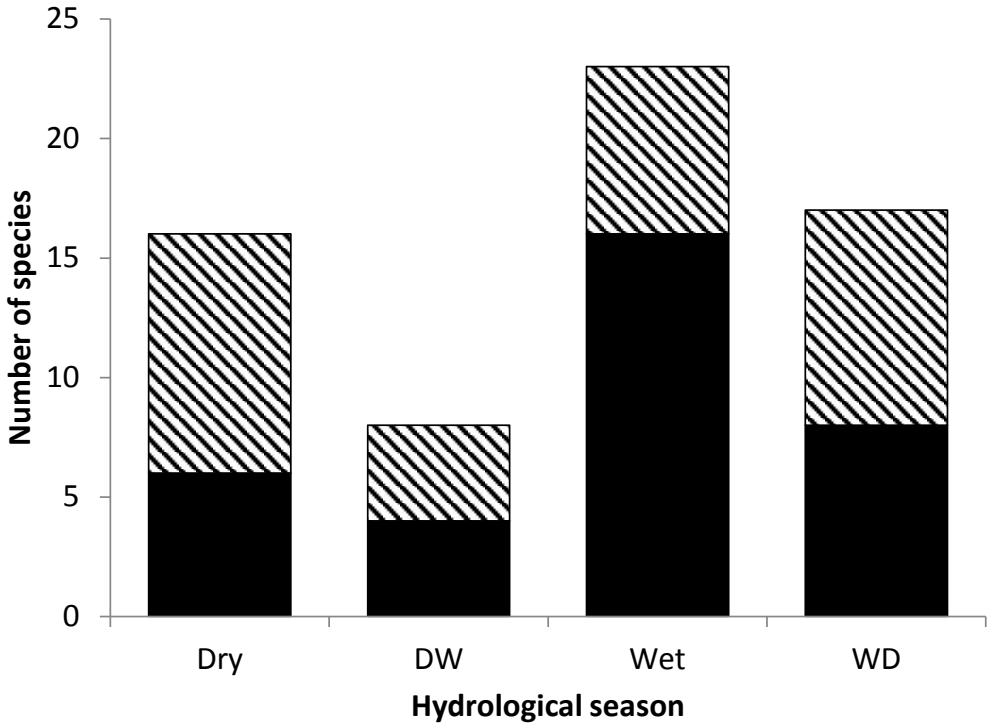


Fig. 3. (a) Number of species estimated to be spawning in each hydrological season (b) Number of species for each estimated spawning period. Black bars represent number of species collected as larvae only, hatched bars as the adjusted occurrence of juveniles (adjusted by one month prior to collection) as an estimate of spawning time, white bars number of species as sum of larvae and adjusted juvenile catch. Note: the number of sampling trips is not even throughout the hydrological period. Wet or W = wet season, WD = wet-dry transition, Dry or D = dry season, DW = dry-wet transition (see Figure 2).

(a)



(b)

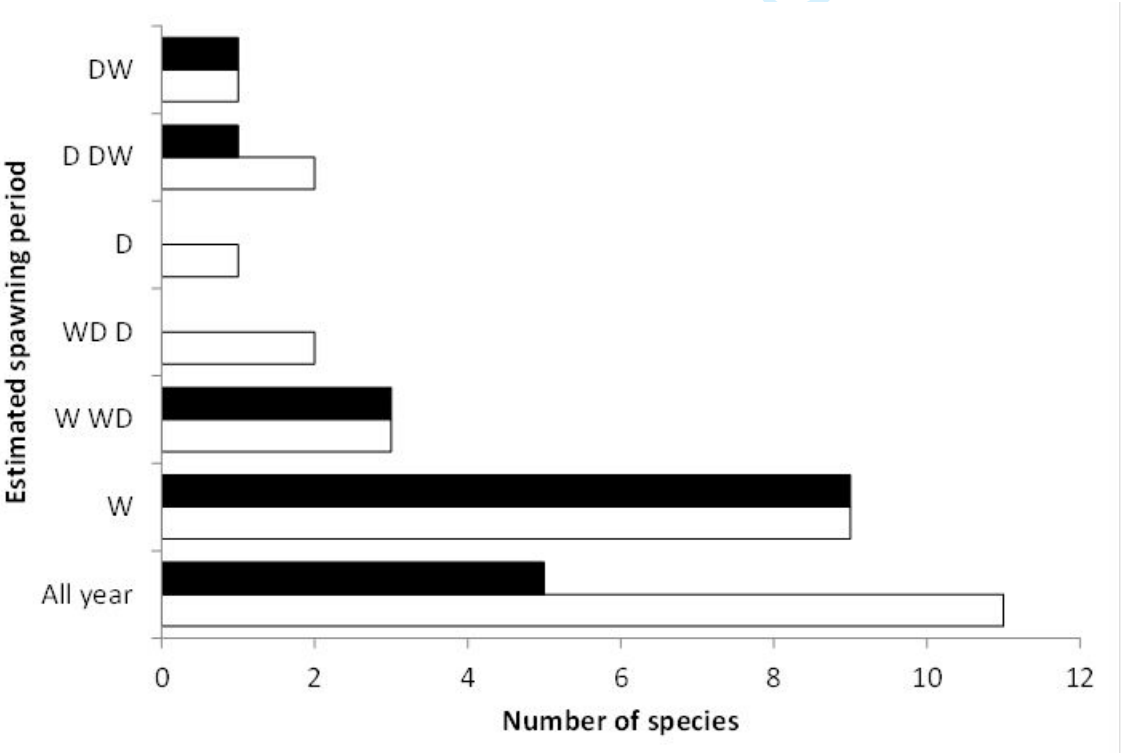
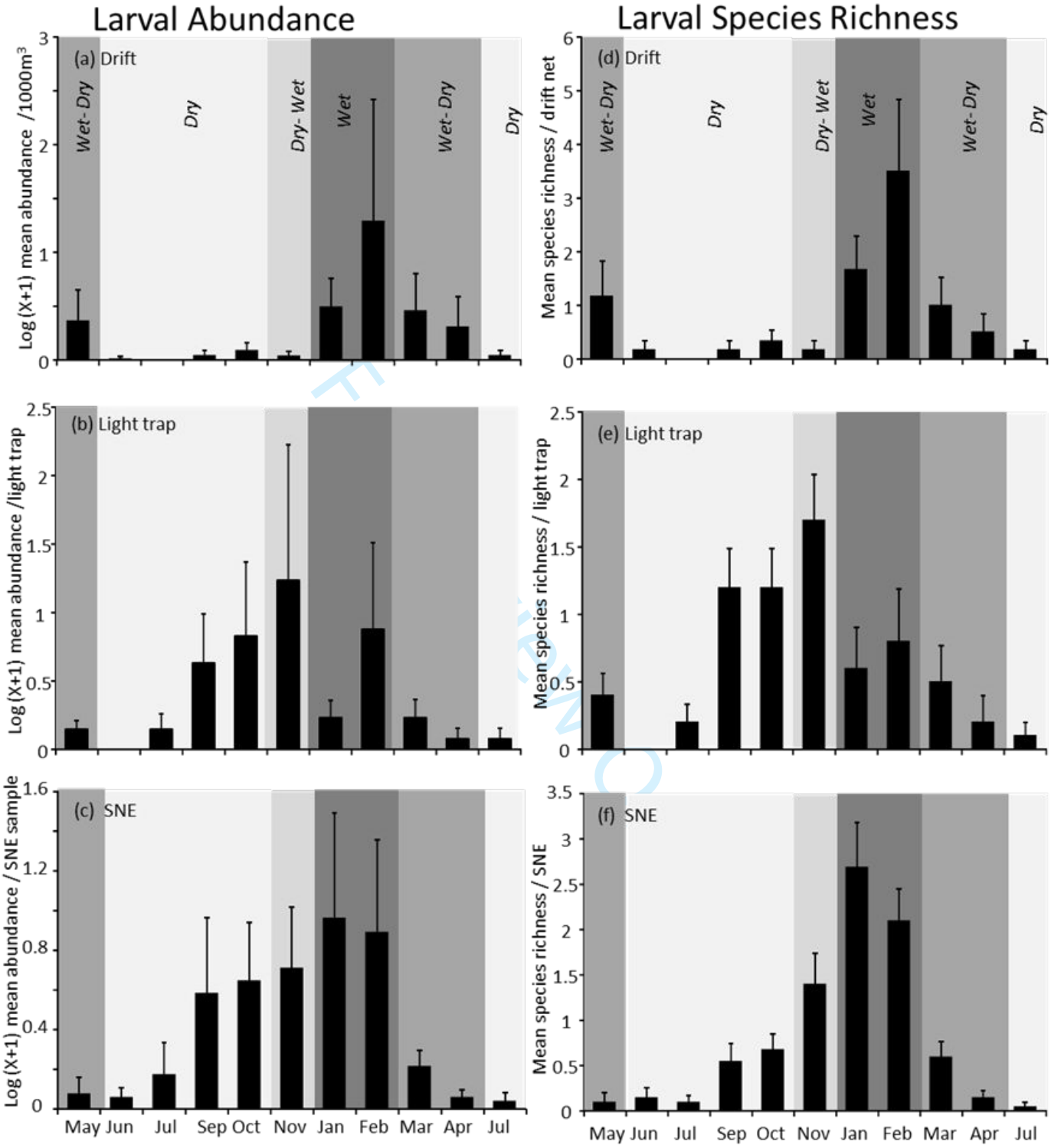


Fig. 4. Mean Log (x+1) (+SE) total larval abundance (a-c) and mean (+SE) species richness (d-f) across sampling trips for each method (a,d) drift net, (b,e) light trap and (c,f) sweep net electrofisher (SNE). Background shading depicts hydrological seasons.



Supplementary Material

Table S.1. Raw number and percentage of individuals across collection methods. TL = total larvae, J = juvenile, D = Drift, LT = Light Trap, SNE = Sweep net electrofishing.

		Raw Numbers of Individuals				Percent		
		D	LT	SNE	Grand Total	D	LT	SNE
Ambassis spp.	*TL*	27	3	41	71	38	4	58
	J	64	186	38	288	22	65	13
Amniataba percoides	*TL*	58	63	9	130	45	48	7
	J	3	5	113	121	2	4	93
Arramphus sclerolepsis	*J*	0	0	5	5	0	0	100
Craterocephalus spp.	*TL*	8	233	325	566	1	41	57
	J	0	7	23	30	0	23	77
Glossamia aprion	*TL*	1	3	3	7	14	43	43
	J	1	9	14	24	4	38	58
Glossogobius spp.	*J*	0	0	18	18	0	0	100
Hephaestus fuliginosus	*J*	1	0	6	7	14	0	86
Hypseleotris compressa	*TL*	0	14	22	36	0	39	61
	J	0	8	57	65	0	12	88
Leptachirus triramus	*J*	1	0	13	14	7	0	93
Leiopotherapon unicolor	*J*	0	0	37	37	0	0	100
Melanotaenia spp.	*TL*	9	23	75	107	8	21	70
	J	3	21	76	100	3	21	76
Mogurnda mogurnda	*J*	0	0	3	3	0	0	100
Nematalosa erebi	*TL*	1	0	0	1	100	0	0
	J	8	0	9	17	47	0	53
Neoarius graeffei	*J*	0	0	4	4	0	0	100
Neosilurus ater	*TL*	1	0	0	1	100	0	0
	J	1	0	1	2	50	0	50
Neosilurus hyrtlii	*J*	5	0	7	12	42	0	58
Ophisternon gutturale	*J*	1	0	1	2	50	0	50
Oxyeleotris lineolatus	*TL*	6	0	0	6	100	0	0
	J	0	0	9	9	0	0	100
Oxyeleotris selheimi	*J*	0	0	6	6	0	0	100
Strongylura kreffti	*J*	0	0	19	19	0	0	100
Syncomistes butleri	*TL*	1	0	0	1	100	0	0
	J	4	0	1	5	80	0	20
Toxotes chatareus	*TL*	4	0	1	5	80	0	20
	J	9	7	234	250	4	3	94
UniID Heramphidae spp.	*TL*	1	0	1	2	50	0	50
UnID Neosilurus spp.	*TL*	27	0	2	29	93	0	7
UniID spB	*TL*	1	0	0	1	100	0	0
UniID spC	*TL*	12	3	34	49	24	6	69
UniID spD	*TL*	17	2	23	42	40	5	55
UniID spE	*TL*	1	0	23	24	4	0	96
UniID spF	*TL*	0	0	4	4	0	0	100
UniID spH	*TL*	52	0	0	52	100	0	0
UnID Damaged	*TL*	39	34	41	114	34	30	36
Total number		**367**	**621**	**1298**	**2286**	**16**	**27**	**57**
Species Richness		**21**	**9**	**28**	**30**	**70**	**30**	**93**

Fig. S.1. Log (X+1) abundance of all larvae captured on each sampling trip in (a) drift nets, (b) light trap and (c) SNE samples.

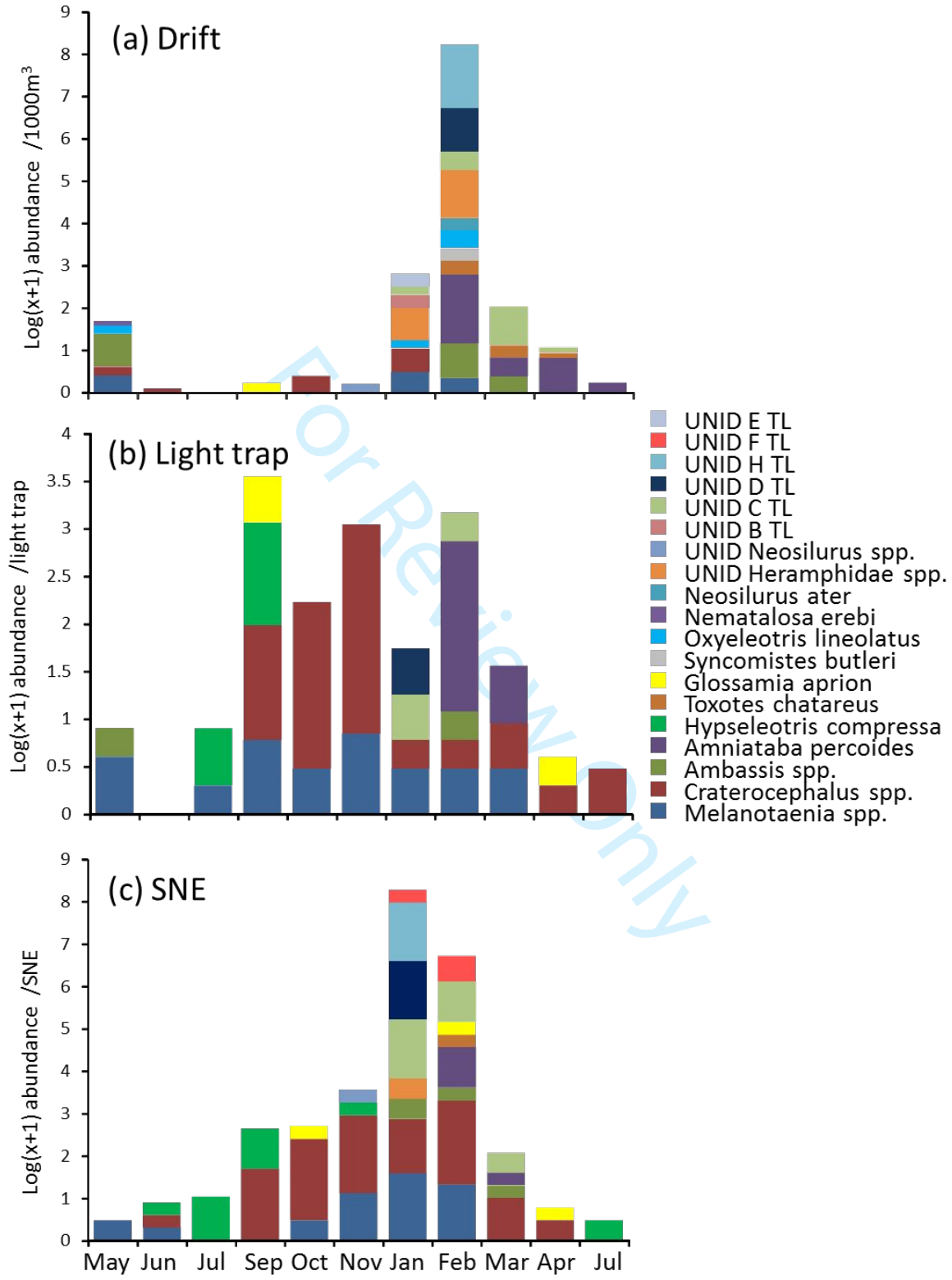
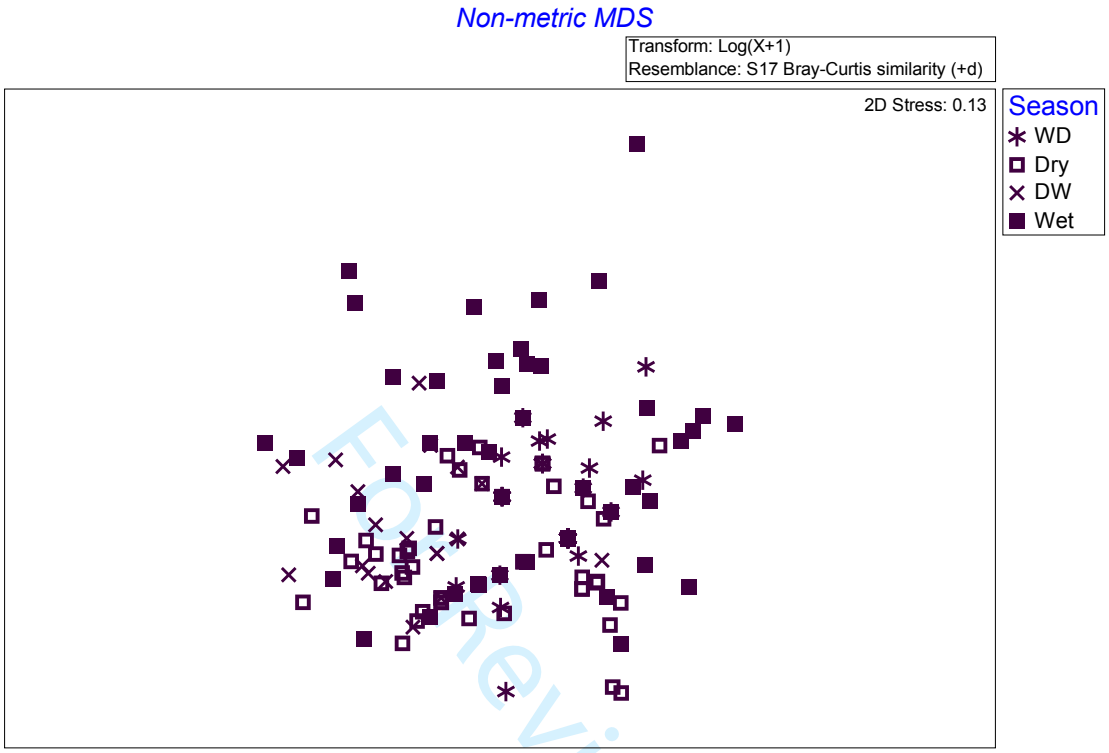


Fig. S.2. Non-metric MDS plots for larval abundance (log(x+1)) showing (a) season and (b) method.

(a)



(b)