MEMS Coastal Wetland Rehabilitation

eDNA Fish Monitoring of Everlasting and Tuckean Swamps

Prepared by: Gavin L. Butler, Leo M. Cameron, Meaghan L. Rourke, Brendan C. Ebner, Chris Bowen, John St Vincent Welch, Natalie Poitras, Camila L. Martins, Max Osborne, Patrick Dwyer, Josh Griffiths & Rachael Impey

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Cover images: Everlasting Swamp (top image) and Tuckean Swamp (bottom image), NSW Fisheries

Preamble

Everlasting and Tuckean swamps are among the largest freshwater wetlands on the New South Wales North Coast. In the post-European settlement era, both swamps were heavily modified, including the installation of extensive networks of drains, channels, floodgates, weirs, levees, and the modification of natural waterways This resulted in major water quality issues, due mainly to leeching of acid-sulphate soils, nutrient imbalances, and infestation by aquatic weeds. The aim of this project was to quantify the fish assemblage within both wetlands and in nearby connected tributaries using a rapid sampling technique, Environmental DNA (eDNA). From February–April 2023, eDNA water samples were collected in and adjacent to Everlasting and Tuckean swamps across six zones, with five sites sampled within each zone at high and low tide. In total, 36 and 42 fish taxa were detected in Everlasting and Tuckean swamps, respectively. The fish assemblages in both wetland complexes comprised species from a wide range of families including coastal marine, estuarine and freshwater species. Despite the degraded state of both systems, the results of the current study suggest fish valuable to recreational and commercial fisheries, as well as several ecologically important species continue to utilise both wetlands. Repeating the eDNA sampling in the future will help to determine if restoration efforts are bringing about changes in the species richness of fishes in both systems. However, for a more complete picture, future surveys should also include a range of complementary sampling techniques to allow a complete understanding of the true benefits of rehabilitation activities, notably regarding waterway connectivity, water quality and food web processes.

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Introduction

Everlasting and Tuckean swamps are among the largest freshwater wetlands on the New South Wales (NSW) North Coast. Everlasting Swamp is located in the lower Clarence River catchment, and Tuckean Swamp is located in the lower Richmond River catchment. Both swamps have a rich Aboriginal history dating back many thousands of years and today remain important areas for the Traditional Owners of the land.

In the post-European settlement era, both swamps were heavily modified, including the installation of extensive networks of drains, channels, floodgates, weirs, levees, and modification of natural waterways. These works were aimed at controlling salinity ingress and to lower floodplain surface water and groundwater tables (Figures 1 & 2). This resulted in major water quality issues due mainly to leeching of acid-sulphate soils, nutrient imbalances, and infestation by aquatic weeds (Pollard and Hannan 1994, Bush et al. 2004, Johnston et al. 2004, Kroon and Ansell 2006, Taffs et al. 2008). Importantly, the modified hydrology at both locations has created landscapes that now frequently generate large volumes of deoxygenated water, impacting fish populations and aquatic ecosystem health within the swamps, the receiving waterways and estuaries, and the broader marine estate.

In 1982, Tuckean Nature Reserve was proclaimed and following additions in 1999, now covers an area around 919 hectares and includes much of the main lower sections of the wetland complex. In 2007, the Everlasting Swamp State Conservation Area (SCA) was declared, and in 2014 National Parks NSW purchased a further ~1700 hectares and created the Everlasting Swamp National Park (NP). Approximately 85% of the Everlasting Swamp is now owned by the NSW Government (NPWS). Conservation and management interventions are ongoing within both wetland complexes, including, private property land acquisition, hydrodynamic modelling and the development of wetland remediation options, investigating ways to better integrate agriculture and environmental management, riparian revegetation, weed management as well as several initiatives to improve water quality. In recent times, there has been discussion around undertaking targeted restoration across both wetland complexes. This has prompted a review of available flora and fauna data. Furthermore, several new surveys of select taxa have been undertaken to bolster knowledge of these ecosystems.

There remains a paucity of recent information regarding the status and makeup of the fish assemblages in and around both swamps (for earlier studies see Kroon and Ansell 2006). Unfortunately, much of the fish assemblage information available has been gathered opportunistically, most often in conjunction with fish kill events (e.g. Walsh et al. 2004). Based on this information and anecdotal reports, both swamps, support dynamic fish assemblages that includes freshwater, estuarine and marine species.

The aim of this project was to quantify the fish assemblage within both swamps and in nearby connected tributaries across all aquatic habitats using a rapid sampling technique, Environmental DNA (eDNA). The technique has been used increasingly for environmental assessments over the last few decades, including for many monitoring programs targeting aquatic and semi aquatic taxa such as fish, amphibians and mammals (e.g. Jerde et al. 2013, Rees et al. 2014, Schenekar et al. 2023). The data in the current survey are intended to provide a baseline against which the success of any future restoration activities can be evaluated.

Figure 1. Sportmans Creek Barrage above the confluence of the Clarence River and Sportmans Creek (Photo NSW NPWS).

Figure 2. Tuckean (Bagotville) Barrage above the confluence of the Richmond River and Tuckean Broadwater.

Methods

Water collections

Water samples were collected by passing 2L of water through a 5 μm self-preserving filter (Thomas et al. 2019) using a Smith-Root eDNA sampler (Thomas et al. 2018) (Figures 5 and 6). Filtration of water samples was undertaken onsite to reduce DNA degradation (Yamanaka et al. 2016). Strict cleaning protocols were employed to minimise contamination among sites. Samples were collected on both a low and a high tidal cycle within one to two days of each other. This approach was used to maximise biodiversity detection and to determine if there was any tide related movement of fishes. A minimum of 10 replicate water samples (minimum five on each tidal cycle), plus a field negative were collected from each zone, with multiple filters used at some sites to allow for the full 2L of water to be processed.

During February–April 2023, eDNA water samples were collected in and adjacent to Everlasting Swamp across six zones with five sites sampled within each zone (Figures 3 and 5). Four zones were located upstream of the Sportsmans Creek Weir and two downstream (Figure 1). The weir is an aged tidal barrage (Figure 1), installed in the 1920s, designed to restrict tidal flows from moving upstream beyond the structure. Investigations undertaken in 2000 found the weir to be 'leaky', with up to 23% of tidal flows passing through the weir at high tide (McElroy, 2000). The Everlasting Swamp sampling sites were:

- Warragai Creek (site code: WCU)
- Sportsmans Creek above the weir (site code: SMU)
- Everlasting Swamp State Conservation Area (site code: ESSCA)
- Everlasting Swamp National Park (site code: ESNP)
- Sportsmans Creek below the weir (site code: SCC)
- Main-stem Clarence adjacent to Sportsmans Creek (near Lawrence) (site code: CAL)

In March 2023, eDNA water samples were collected in and adjacent to Tuckean Swamp across six zones with five sites sampled within each zone (Figure 4). Three zones were located upstream of the Tuckean (Bagotville) Barrage and three downstream (Figure 2). The barrage was constructed in 1971 and is a causeway made up of numerous gated culverts, managed to prevent tidal flows moving upstream of the structure into the Tuckean Swamp. The Tuckean Swamp sampling sites were:

- Tuckean Barrage Eastern Branch (site code: TEB)
- Tuckean Barrage Western Branch (site code: TWB)
- Tuckean Barrage Upstream (site code: TBU)
- Tuckean Barrage Downstream (site code: TBD)
- Tuckean Mid-Channel (site code: TMC)
- Tuckean Richmond Confluence (site code: TRC)

Figure 3. Sampling zones within Everlasting Swamp and adjacent waterways. The six study zones were: Clarence River at Lawrence (CAL), Everlasting Swamp National Park (ESNP), Everlasting Swamp State Conservation Area (ESSCA), Sportsmans Creek below weir (SCC), Sportsmans Creek above weir (SMU) and Warragai Creek (WCU).

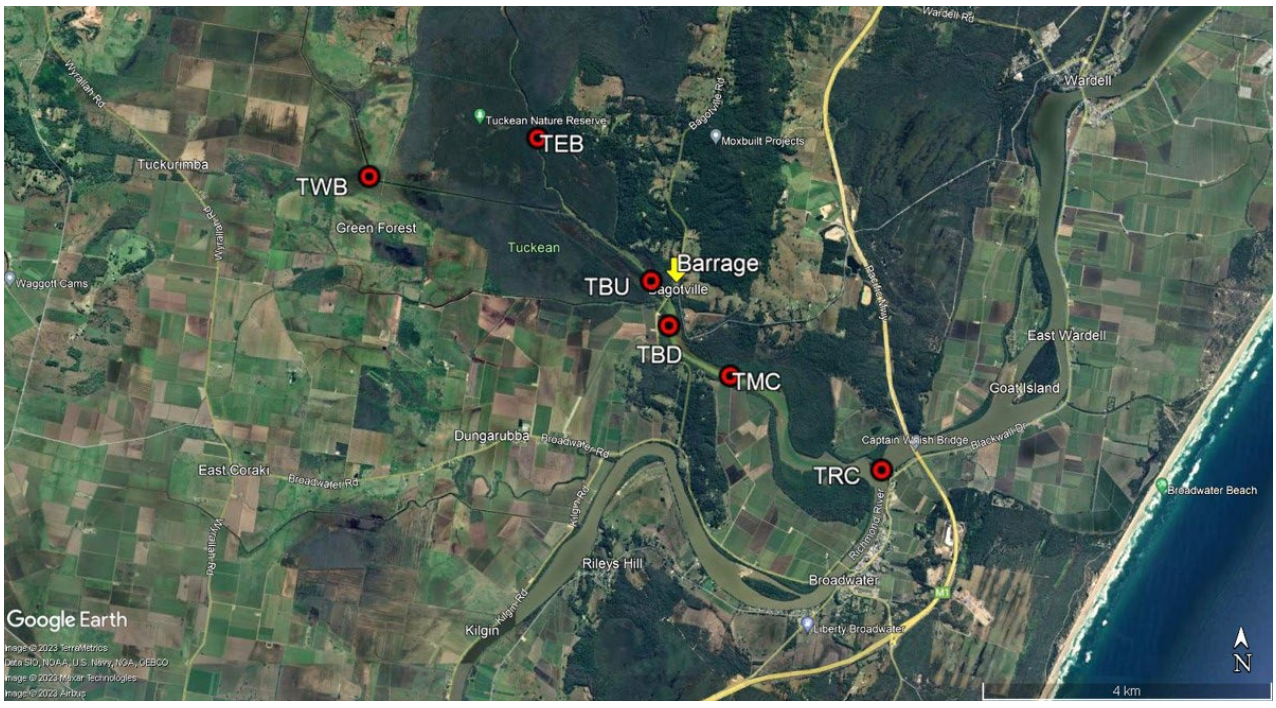

Figure 4. Sampling zones within Tuckean Swamp and adjacent waterways. The six study zones were: Tuckean Barrage Downstream (TBD), Tuckean Barrage Upstream (TBU), Tuckean Barrage Eastern Branch (TEB), Tuckean Mid-channel (TMC), Tuckean Richmond Confluence (TRC) and Tuckean Western Branch (TWB).

Processing water samples

DNA was extracted from filters using a Qiagen PowerSoil Kit which minimises compounds that can inhibit PCR reactions in environmental samples. Library construction involved two rounds of PCR, whereby the first round employed gene-specific primers to amplify the target region and the second round incorporated sequencing adapters and unique barcodes for each sample-amplicon combination included in the library. Negative controls were included during library construction. Negative controls consisted of the extraction negative as well as PCR negatives, in which nuclease-free water was used in place of DNA during both rounds of PCR. Sequencing was carried out on an Illumina sequencing platform.

Following quality control filtering to remove primer sequences, truncated reads, and low-frequency reads, DNA sequences were clustered into Operational Taxonomic Units (OTUs) on the basis of sequence similarity. Taxonomic assignment was performed with VSEARCH software (Rognes et al. 2016), whereby each OTU cluster was assigned a species identity using a threshold of 95% by comparing against a reference sequence database. Where a species could not be assigned (i.e., reference database was deficient and/or taxa were poorly characterised), taxonomic assignments were manually vetted by first obtaining a list of possible species through BLASTN searches against the public repository Genbank (www.ncbi.nlm.nih.gov), followed by elimination of species based on their geographic distributions, using information from the Atlas of Living Australia and other relevant data sources. In cases where an OTU could not be adequately resolved to a single species (e.g., due to shared haplotypes), either a list of multiple species was included, or the OTU is assigned to the lowest taxonomic rank without further classification.

Figure 5. Field staff collecting eDNA water sample in the Everlasting Swamp National Park zone in the Everlasting Swamp study area, March 2023.

Figure 6. Field staff collecting eDNA water sample in the Warragai Creek zone in the Everlasting Swamp study area, April 2023.

Results

In total, 36 and 42 fish taxa were detected in Everlasting Swamp and Tuckean Swamp and their associated waterways, respectively. The fish assemblages comprised species from a wide range of families including coastal marine, estuarine and freshwater species (Tables 1 & 2). Species level confirmation was achievable in most instances, however, only genus or family level resolution was possible for three of the taxa detected in Everlasting Swamp (Table 1) and for four taxa in Tuckean Swamp (Table 2).

Among the six zones, the number of taxa detected ranged from 13 to 23 in Everlasting Swamp (Table 1) and from 9 to 30 in Tuckean Swamp (Table 2). In Everlasting Swamp, the number of species detected was highest in the tidal *Sportsmans Creek Below Weir* zone (*n* = 23) and equal lowest in the *Everlasting Swamp State Conservation Area* and *Warragai Creek* zones (*n* = 13). In Tuckean Swamp, the number of species detected was highest in the *Tuckean Mid Channel* (*n* = 30) and lowest in the above *Tuckean Barrage Eastern Branch* zone (*n* = 9).

Few species were detected across all six zones in either Everlasting or Tuckean swamps. In Everlasting Swamp only two species were detected across all six zones: longfinned eel (*Anguilla reinhardtii*) and flatheaded gudgeon (*Philypnodon grandiceps*) (Table 1). There were six species detected across all zones in Tuckean Swamp: carp gudgeon (*Hypseleotris* spp.), common carp (*Cyprinus carpio*), eastern gambusia (*Gambusia holbrooki*), longfinned eel (*Anguilla reinhardtii*), flatheaded gudgeon (*Philypnodon grandiceps*) and sea mullet (*Mugil cephalus*) (Table 2).

In each swamp complex, there were differences in the species detected in relation to tidal cycle (Tables 1 & 2). In the Everlasting Swamp system, this was most noticeable in the *Sportsmans Creek Below the Weir* and *Warragai Creek* zones. Similarly, there were differences in species level detection at *Tuckean Barrage Upstream* and *Tuckean Mid-Channel* zones within the Tuckean Swamp system.

A small proportion of species were infrequently detected and can be considered rare in the study areas. For example, ornate rainbowfish (*Rhadinocentrus ornatus*) and crescent perch (*Terapon jarbua*) were only detected in one zone within both Everlasting and Tuckean swamps. Similarly, bull sharks (*Carcharhinus leucas*) were only detected in two zones in the Tuckean Swamp study area (*Tuckean Mid Channel* and *Tuckean Richmond Confluence*), with both detections of this species at high tide (Table 2).

Sampling detected the DNA of three alien species (fish not native to Australia) in each swamp. In Everlasting Swamp, goldfish (*Carassius auratus*), common carp and eastern gambusia were detected (Table 1), whilst in Tuckean Swamp, DNA from common carp, eastern gambusia and brown trout (*Salmo trutta*) was detected in samples (Table 2). The brown trout detection was considered as erroneous, due to the high-water temperatures experienced in Tuckean Swamp which would preclude survival of any of the salmonids.

Table 1. Fish detected in and adjacent to Everlasting Swamp from high (H) and low (L) tide cycles (*=tidal or marginally tidal site). Any blank cell under 'common name' represents a detection that could not be identified to species level. The six study zones were: Warragai Creek (site code: WCU), Sportsmans Creek above the weir (site code: SMU), Everlasting Swamp State Conservation Area (site code: ESSCA), Everlasting Swamp National Park (site code: ESNP), Sportsmans Creek below the weir (site code: SCC) and Main-stem Clarence adjacent to Sportsmans Creek (near Lawrence) (site code: CAL). Light grey shading denotes alien species and dark grey shading denotes a potential translocated native species.

		Above Weir					Below Weir	
Species	**Common name**	**WCU (H) (L)**	**SMU (H) (L)**	**ESSCA (H) (L)**	**ESNP (H) (L)**	**Sportsmans Creek Weir**	**SCC* (H) (L)**	**CAL* (H) (L)**
Afurcagobius tamarensis	Tamar goby	× ×	× ×	× ×	× ×		× ×	✓ ✓
Ambassis agassizii	Agassiz's glassfish	✓ ×	✓ ✓	✓ ✓	✓ ✓		× ×	× ×
Ambassis jacksoniensis	Port Jackson glassfish	× ×	× ×	× ×	✓ ✓		✓ ✓	✓ ✓
Anguilla australis	Shortfinned eel	✓ ✓	× ✓	✓ ✓	× ✓		✓ ×	× ×
Anguilla reinhardtii	Longfinned eel	✓ ✓	✓ ✓	✓ ✓	✓ ✓		✓ ✓	✓ ×
Argyrosomus japonicus	Mulloway	× ×	× ×	× ×	× ×		× ×	✓ ×
Bream spp.	Bream	× ×	✓ ✓	× ×	✓ ✓		✓ ✓	✓ ✓
Carassius auratus	Goldfish	✓ ✓	✓ ✓	✓ ✓	✓ ✓		× ×	× ×
Cyprinus carpio	Common carp	✓ ×	× ×	× ×	× ×		× ×	× ✓
Gambusia holbrooki	Eastern gambusia	✓ ✓	✓ ✓	✓ ✓	✓ ✓		✓ ✓	× ×
Gerres subfasciatus	Common silverbiddy	× ×	× ×	× ×	✓ ✓		✓ ✓	✓ ✓
Girella tricuspidata	Luderick	× ×	× ×	× ×	× ×		✓ ✓	✓ ✓
Gobiopterus semivestitus	Glassgoby	× ×	× ×	× ×	× ×		✓ ×	× ×
Goby spp.	Goby	× ×	× ×	× ×	× ×		× ×	✓ ×
Gracilimugil argenteus	Flat-tail mullet	× ×	× ×	× ×	× ×		✓ ✓	✓ ✓
Halfbeak *spp.*		× ×	× ✓	× ×	× ×		✓ ✓	✓ ×
Hyperlophus vittatus	Sandy sprat	× ×	× ×	× ×	× ×		× ✓	✓ ✓
Hypseleotris spp.	Carp gudgeon	✓ ✓	✓ ✓	✓ ✓	✓ ✓		✓ ✓	× ×
Macquaria colonorum or Percalates novemaculeata	Estuary perch or Australian bass	✓ ✓	✓ ✓	× ×	✓ ✓		✓ ✓	× ✓
Monodactylus argenteus	Diamondfish	× ×	✓ ✓	× ×	× ×		× ✓	× ×
Mugil cephalus	Sea Mullet	× ×	✓ ✓	✓ ✓	✓ ✓		✓ ✓	✓ ✓
Nematalosa erebi	Bony bream	✓ ×	× ×	× ×	× ×		× ×	× ×
Philypnodon grandiceps	Flatheaded gudgeon	✓ ×	✓ ✓	✓ ✓	✓ ✓		✓ ✓	✓ ✓
Philypnodon macrostomus	Dwarf flathead gudgeon	× ×	✓ ✓	× ×	✓ ✓		✓ ×	× ×
Platycephalus fuscus	Dusky flathead	× ×	× ×	× ×	✓ ✓		✓ ✓	× ✓
Potamalosa richmondia	Australian freshwater herring	× ×	✓ ✓	× ✓	✓ ✓		× ×	× ×
Pygmy perch spp.	Pygmy perches	× ×	× ×	✓ ×	× ×		✓ ×	× ×
Rainbowfish spp.	Rainbowfish	✓ ✓	✓ ✓	✓ ✓	✓ ✓		× ×	× ×
Redigobius macrostoma	Largemouth goby	× ×	× ×	× ×	× ×		✓ ×	× ×
Retropinna semoni	Australian smelt	× ✓	✓ ✓	✓ ✓	✓ ×		× ×	× ×
Rhadinocentrus ornatus	Ornate rainbowfish	✓ ✓	× ×	× ×	× ×		× ×	× ×
Sea catfish spp.	Catfishes	× ×	✓ ✓	× ×	✓ ✓		✓ ✓	✓ ✓
Selenotoca multifasciata	Spotbanded scat	× ×	× ×	× ×	× ×		✓ ✓	× ✓
Terapon jarbua	Crescent perch	× ×	× ×	× ×	× ✓		× ×	× ×
Trachystoma petardi	Pinkeye mullet	× ×	✓ ✓	× ✓	✓ ✓		✓ ✓	× ✓
Total number of different taxa detected:		**13**	**18**	**13**	**20**		**23**	**19**

Table 2. Fish detected for the six zones in the lower Richmond River catchment from high (H) and low (L) tide periods (*=tidal or marginally tidal). Any blank cell under 'common name' represents a detection that could not be identified to species level. The six study zones include: Tuckean Barrage Eastern Branch (site code: TEB), Tuckean Barrage Western Branch (site code: TWB), Tuckean Barrage Upstream (site code: TBU), Tuckean Barrage Downstream (site code: TBD), Tuckean Mid-Channel (site code: TMC), and Tuckean Richmond Confluence (site code: TRC). Grey shading denotes alien species.

		Above Barrage				Below Barrage		
Species	**Common name**	**TEB (H) (L)**	**TWB (H) (L)**	**TBU* (H) (L)**		**TBD* (H) (L)**	**TMC* (H) (L)**	**TRC* (H) (L)**
Acanthopagrus butcheri	Black bream	× ×	✓ ✓	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Afurcagobius tamarensis	Tamar goby	× ×	× ×	× ✓		✓ ✓	✓ ✓	✓ ✓
Ambassis agassizii	Agassiz's glassfish	× ×	✓ ✓	× ×		× ×	× ×	× ×
Ambassis jacksoniensis	Port Jackson glassfish	× ×	× ×	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Anchovy spp.	Anchovy	× ×	× ×	× ×		× ×	× ×	✓ ×
Anguilla australis	Shortfinned eel	✓ ✓	✓ ✓	× ✓		✓ ✓	✓ ×	× ×
Anguilla reinhardtii	Longfinned eel	✓ ✓	✓ ✓	✓ ✓		✓ ✓	✓ ✓	✓ ×
Argyrosomus japonicus	Mulloway	× ×	× ×	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Carcharhinus leucas	Bull shark	× ×	× ×	× ×		× ×	× ✓	× ✓
Cyprinus carpio	Common carp	✓ ✓	✓ ✓	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Gambusia holbrooki	Eastern gambusia	× ✓	✓ ✓	✓ ✓		✓ ✓	✓ ✓	✓ ×
Gerres subfasciatus	Common silverbiddy	× ×	× ×	× ✓		✓ ✓	✓ ✓	✓ ✓
Girella tricuspidata	Luderick	× ×	× ×	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Gobiopterus semivestitus	Glassgoby	× ×	× ×	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Goby spp.	Goby	× ×	× ×	× ×		× ✓	✓ ×	× ×
Gracilimugil argenteus	Flat-tail mullet	× ✓	× ×	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Hyperlophus vittatus	Sandy sprat	× ×	× ×	× ✓		× ✓	✓ ✓	✓ ✓
Hyporhamphus regularis	Halfbeak garfish	× ×	× ×	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Hypseleotris spp.	Carp gudgeons	✓ ✓	✓ ✓	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Percalates novemaculeata	Australian bass	× ×	✓ ✓	✓ ✓		✓ ✓	× ✓	× ×
Megalops cyprinoides	Oxeye herring	× ×	× ×	× ×		✓ ×	× ×	× ×
Monodactylus argenteus	Diamondfish	× ×	✓ ×	× ✓		× ✓	✓ ✓	✓ ✓
Mugil cephalus	Sea Mullet	✓ ✓	✓ ✓	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Mugilogobius platynotus	Flatback mangrove goby	× ×	× ×	× ✓		× ×	✓ ✓	✓ ✓
Mullet spp.	Mullet	× ×	× ×	× ×		× ×	× ×	× ✓
Nannoperca spp.	Pygmy perches	× ×	× ×	× ×		✓ ×	× ×	× ×
Neoarius leptaspis	Boofhead catfish	× ×	✓ ✓	✓ ✓		✓ ✓	✓ ✓	✓ ✓
Philypnodon grandiceps	Flatheaded gudgeon	× ✓	✓ ✓	✓ ✓		✓ ✓	✓ ✓	✓ ×
Platycephalus fuscus	Dusky flathead	× ×	× ×	✓ ✓		✓ ✓	✓ ✓	× ✓
Potamalosa richmondia	Australian freshwater herring	× ×	✓ ✓	× ✓		× ×	× ×	× ×
Pseudogobius olorum	Blue-spot goby	× ×	× ×	× ×		× ×	✓ ✓	✓ ✓
Rainbowfish spp.	Rainbowfish	× ×	✓ ✓	✓ ✓		× ×	× ×	× ×
Redigobius macrostoma	Largemouth goby	× ×	× ✓	✓ ✓		✓ ✓	✓ ✓	× ×
Retropinna semoni	Australian smelt	× ×	✓ ×	× ✓		× ×	✓ ×	× ×
Rhadinocentrus ornatus	Ornate rainbowfish	✓ ×	× ×	× ×		× ×	× ×	× ×
Salmo trutta	Brown trout	× ×	× ×	✓ ×		× ×	× ×	× ×
Scatophagus argus	Spotted scat	× ×	× ×	× ×		✓ ✓	✓ ✓	✓ ✓
Selenotoca multifasciata	Spotbanded scat	× ×	× ×	× ×		✓ ✓	× ✓	× ×
Sillago flindersi	Eastern school whiting	× ×	× ×	× ×		× ✓	× ×	✓ ✓
Terapon jarbua	Crescent perch	× ×	× ×	× ×		× ×	× ×	× ✓
Trachystoma petardi	Pinkeye mullet	× ×	✓ ✓	× ✓		✓ ✓	× ✓	× ×
Total number of taxa detected:		**9**	**17**	**28**		**29**	**30**	**27**

Tuckean Barrage

Discussion

Everlasting and Tuckean swamps are recognised locally, nationally, and internationally as ecologically important wetlands. However, both wetland complexes have been heavily impacted by coastal floodplain drainage works over the last ~100 years and now function much differently compared to how they did before European settlement (e.g. Pressey and Middleton, 1982; Walsh et al. 2004). Despite their apparent degraded state, the results of the current study suggest several important recreational and commercial fish species are, at times, utilising both wetlands and nearby waterways. These species include dusky flathead (*Platycephalus fuscus*), yellowfin bream (*Acanthopagrus australis*), school whiting (*Sillago flindersi*), Australian bass (*Percalates novemaculeata*), sea mullet (*Mugil cephalus*) and luderick (*Girella tricuspidate*). Whilst an eDNA detection does not discriminate among life-history stages, given that coastal wetlands are known to be important nursery habitats for fish, it is likely juveniles as well as adults of these and of other important species are using both systems. However, whilst several marine and estuarine species were detected within both study areas, very few appear to be penetrating the upstream extremities of either study area, highlighting that connectivity remains limited and needs to be addressed going forward.

Whilst many of the species detected in the current study were spatially and temporally ubiquitous within zones, in some instances tide appeared to have an influence on the occurrence of specific species at some sites. Several possible mechanisms may be at play in these findings, including the nuanced activity patterns or habitat choices among the different species present. For example, crescent perch (*Terapon jarbua*) was only detected at the *Tuckean-Richmond Confluence* site on high tide, which is where juveniles of this species are commonly found, utilising habitats immediately below the tidal limit (e.g. Ebner et al. 2019b; Huang et al. 2022). Similarly, bull shark (*Carcharhinus leucas*) DNA was only detected in the *Tuckean Mid-channel* and *Tuckean Richmond Confluence* sites (Table 2), with juveniles of this species known to track low to intermediate salinity environments which are characteristic of the upper tidal zone (e.g. Heupel & Simpfendorfer 2008). Contrastingly, some small-bodied, less mobile fish species including pygmy perch spp., glass goby (*Gobiopterus semivestitus*), largemouth goby (*Redigobius macrostoma*) and other unidentified goby spp., were only detected at low tide (Table 1). In the case of the gobies, it may be that their highly-site-attached, bottom-dwelling nature and an increase in activity at low tide renders them somehow more detectable at these times, either as a function of their behaviour or as an artefact of the different strata in the water column being sampled at high and low tides. It may also simply be an element of chance if an animal was detected or not and a deeper understanding of species and assemblage behaviour (e.g. observation of fish behaviour and activity) are required to add confidence to interpretations of any tide effects (e.g. Meynecke et al. 2008, Ebner et al. 2019a).

eDNA caveats

The detection sensitivity of eDNA is one of the key rationales underpinning why the technique is being increasingly utilised as a means of determining presence or absence of fish in all types of environments. The technique is proving particularly useful for detecting species which may be in low abundance and/or not easily sampleable, such as identifying isolated populations of threatened species or detecting new incursions of exotic species (Rourke et al. 2023). However, when unexpected or single detections occur, without physical proof the results will always remain questionable. In the current study, the exotic common carp (*Cyprinus carpio*) and the non-endemic

bony herring (*Nematalosa erebi*) were detected at a low number of sites in Everlasting Swamp, with neither species recorded in the Clarence Basin previously. Like most fisheries sampling techniques, the eDNA technique is not without its caveats, including the risk of false positive or negative findings due to factors such as PCR and extraction biases, and the real risk of sampling contamination, both in the field and in the laboratory (Thomsen et al. 2024). As such, following up sampling using more conventional techniques such as electrofishing and netting is required to validate these findings, which will then allow any future management actions to proceed with greater confidence.

Expectedly, the freshwater fish assemblage in both wetland complexes was dominated by relatively common and endemic species but a small number of threatened and rarer species were also detected. Pygmy perch spp. were detected in both systems and given the only known pygmy perch species in the region is the Oxleyan pygmy perch (*Nannoperca oxleyana*), a threatened species with fragmented populations and a high degree of genetic structuring (Hughes et al. 1999; Knight and Arthington 2008), makes this a potentially important funding. However, intensive follow up sampling at Everlasting Swamp using bait-trapping (see Knight et al. 2007 for method justification) failed to catch any *N. oxleyana* (Butler, G.L. unpubl. data). This does not necessarily mean they are not present but that they are potentially in such low numbers that probability of encounter is very low or that the DNA may have originated upstream of the sampling site and been transported downstream by riverine flows. Similarly, the isolated detections of the relatively rare ornate rainbowfish (*Rhadinocentrus* spp.) in each wetland complex also warrants further investigation. The ornate rainbowfish taxon is presently not fully described but it is know there is likely several discrete species throughout its range (Page et al. 2004; Unmack, P., University of Canberra, pers. comm.), meaning that there is a possibility that these detections may be from a new undescribed and potentially threatened species. Both examples and the detection of previously unreported exotic and non-endemic species, highlights the potential value of eDNA as a sampling tool but also that the precautionary principal approach should be adopted for any unexpected detections until they are validated (Rourke et al. 2023).

Conclusion

The current study provides an insight into the fish species that likely now utilise Everlasting and Tuckean swamps and the surrounding waterways. Both swamps are substantial floodplain systems that have been heavily impacted by drainage of surface and ground water, and, the exclusion of tidal flows. The Marine Estate Management Strategy identified coastal wetland drainage and modification of freshwater flows as priority threats and risks, and their rehabilitation must be addressed to maintain environmental, social, cultural, and economic values of the marine estate (NSW Government, 2018). Repeating the eNDA sampling in the future may prove useful to determine if restoration efforts are bringing about changes in the species richness of fishes in both systems. However, key questions pertaining to the life stages and size structure, as well as the abundance of different species, cannot at present be determined using eDNA alone. Complementary sampling using a range of other techniques (e.g. netting, underwater video, electrofishing, stable isotope analysis) needs to be part of any future surveys in order to understand the true outcomes of any rehabilitation activities, notably in regard to waterway connectivity, water quality and food web processes.

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