Spatio-temporal distribution of syngnathid fishes in Cockburn Sound and Owen Anchorage

Theme: Apex Predators and Iconic Species WAMSI Westport Marine Science Program



<u>a</u>

Better science Better decisions

WAMSI WESTPORT MARINE SCIENCE PROGRAM







ABOUT THE MARINE SCIENCE PROGRAM

The WAMSI Westport Marine Science Program (WWMSP) is a \$13.5 million body of marine research funded by the WA Government. The aims of the WWMSP are to increase knowledge of Cockburn Sound in areas that will inform the environmental impact assessment of the proposed Westport development and help to manage this important and heavily used marine area into the future. Westport is the State Government's program to move container trade from Fremantle to Kwinana, and includes a new container port and associated freight, road and rail, and logistics. The WWMSP comprises more than 30 research projects in the biological, physical and social sciences that are focused on the Cockburn Sound area. They are being delivered by more than 100 scientists from the WAMSI partnership and other organisations.

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DATA

Finalised datasets will be released as open data, and data and/or metadata will be discoverable through Data WA and the Shared Land Information Platform (SLIP).

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FRONT COVER IMAGE

Theme: Apex Predators and Iconic Species Front cover image: Scene in Cockburn Sound (Photo by Delphine Chabanne).

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The WAMSI Westport Marine Science Program is a \$13.5 million body of research that is designed to fill knowledge gaps relating to the Cockburn Sound region. It was developed with the objectives of improving the capacity to avoid, mitigate and offset environmental impacts of the proposed Westport container port development and increase the WA Government's ability to manage other pressures acting on Cockburn Sound into the future. Funding for the program has been provided by Westport (through the Department of Transport) and the science projects are being delivered by the Western Australian Marine Science Institution.

1 Spatio-temporal distribution of syngnathid fishes in Cockburn Sound and Owen Anchorage

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West Australian Seahorse *Hippocampus subelongatus*. Photo: Glenn Moore/WA Museum

Project 8.4

Spatio-temporal distribution of syngnathid fishes in Cockburn Sound and Owen Anchorage

Executive Summary

The Syngnathidae (seahorses, pipefishes, seadragons) is a family of fishes mostly found in shallow temperate and tropical waters of all oceans and is one of the most diverse families of coastal fishes in southern Australia. Worldwide, the Syngnathidae comprises around 300 species with at least 45 species in southern Australia. Syngnathid fishes have been identified as iconic species in Cockburn Sound and, as a globally threatened group could be expected to be specifically considered in environmental assessments for developments in coastal waters. Globally, syngnathid fishes face threats from coastal development, over-exploitation and bycatch. In recognition of this concern, all syngnathids are protected Federally and some by various State Acts and have global protections and recognitions as Threatened or Data Deficient. These charismatic species also garner attention from divers, conservationists, naturalists and the general public.

A total of 4839 records of syngnathid fishes from Cockburn Sound and Owen Anchorage was compiled from a range of sources, including historical museum vouchers, published records, historical and contemporary observations from notebooks and diving surveys, historical and contemporary trawl surveys, citizen science observations and environmental DNA assays. The dataset was checked for quality of identification and location data.

Twenty-one species of syngnathid fishes were identified as being present in the area, representing a high diversity for a relatively small embayment. This included two species of seahorse, 16 species of pipefish, one species of pipehorse and two species of seadragon. The amount of data for each species varied considerably from a single record to more than 2600 records. Additional distributional, habitat, biological, life history and breeding data were extracted from extensive literature searches.

At least for those species with sufficient data, the area maintains substantial larval, juvenile and adult populations across the year and is an area where breeding of most, if not all, species occurs. All habitats within the area are utilised by these fishes, although seagrass meadows and reef or reef-like structures support the greatest diversity. Most species are probably patchily distributed in suitable habitat across the whole area.

With a high diversity of species, which are patchily distributed in suitable habitat across the whole area, and present and breeding across the whole year, substantial impacts to syngnathids from disturbances caused by marine development in Cockburn Sound and Owen Anchorage are highly likely. All species known from Cockburn Sound and Owen Anchorage are distributed in suitable habitats across the Perth metropolitan area, and beyond, although the abundance and density of these populations is poorly known. Evidence from around the world shows that syngnathid populations can be impacted detrimentally by development and ongoing infrastructure operations, through direct and indirect interaction, habitat loss, water quality, turbidity and noise. A risk from introduced pests is also possible. While both temporary and long-lasting impacts are documented at relatively local scales, there is a paucity of knowledge about consequences for broader syngnathid populations. Risks and mitigation strategies from global examples are outlined, which are often specific to the unique hydrological, seascape and habitat conditions of the impacted area.

2 Introduction

In 2018, the Western Australian Government committed to investigating the feasibility of a new container port in Kwinana (Westport), within Cockburn Sound, approximately 20 km south of Fremantle. Development projects may have direct, indirect and cumulative impacts on iconic species resulting from construction and ongoing operations. Syngnathid fishes (family Syngnathidae: seahorses pipefishes, seadragons) have been identified as iconic species in Cockburn Sound and are a globally threatened group (Pollom *et al.* 2021). Globally, syngnathid fishes face threats from coastal development, over-exploitation and bycatch (Pollom *et al.* 2021). In recognition of this concern, syngnathids are protected i) Federally (EPBC Act Part 13 [all species]), ii) in Western Australia (Fish Resources Management Act [two species] and Biodiversity Conservation Act [one species]), iii) globally (CITES) [all species] and, iv) many are assessed as Threatened or Data Deficient by the International Union for the Conservation of Nature (Pollom *et al.* 2021). These charismatic species also garner a great deal of attention from divers, conservationists, naturalists and the general public.

The Syngnathidae is a family of fishes mostly found in shallow temperate and tropical waters of all oceans and is one of the most diverse families of coastal fishes in southern Australia (Dawson 1985; Kuiter 2009). Worldwide, the Syngnathidae comprises around 300 species in two subfamilies: Syngnathinae (tail brooders) and Nerophinae (trunk brooders) (Hamilton *et al.* 2017) with around 50 species in southern Australia.

Syngnathids are well known because many species have a complex mating system involving courtship, reproduction and male care tightly linked to sexual selection (Jones and Avise 2001). Female syngnathids transfer eggs to a pouch or pouch-like structure on the male's abdomen or tail. The male provides nutrition, osmo-regulation and aeration to the developing embryos (Wilson *et al.* 2001). The mating systems of species within the family are diverse, including the best known monogamous pair bonds in seahorses and some pipefishes (e.g. Jones and Avise 2001; Sogabe and Yanagisawa 2008; Camins Martinez *et al.* 2023) but a range of other systems are common, including leks (aggregation of competing males to entice female visitation), polyandry (female mates with multiple males) and polygynandry (male mates with multiple females) (e.g. Jones and Avise 2001; Hübner *et al.* 2013; Monteiro *et al.* 2017). However, the mating system remains poorly known, if at all, for most species and especially those in southern Australia. Most species of syngnathid have a relatively low reproductive potential, typically producing fewer than 200 eggs per clutch (Foster and Vincent 2004; Kuiter 2009).

Syngnathid fishes are generally small and highly cryptic. They feed on small invertebrates, primarily pelagic or bentho-pelagic crustaceans, that are sucked up whole through a modified tubular snout (Kuiter 2009). Syngnathids are preyed upon by other fishes, crabs and birds (Kuiter 2009). They are often in small population densities or patchily distributed, yet can add considerably to habitat ecosystem dynamics as well as act as a conduit for energy transfer between habitats (Martin-Smith 2003). They often have small home ranges, or are relatively site attached, at least as adults (e.g. Kvarnemo *et al.* 2021).

Most syngnathids occur in relatively low-energy shallow coastal waters and are especially reliant on habitats that are well represented in Cockburn Sound, including seagrass, filter-feeder communities, shallow detritus, reefs and artificial structures (Kendrick and Hyndes 2003; Kuiter 2009). While syngnathid fishes can be strongly associated with specific habitats, they are typically weak swimmers and have limited ability to move away from resident areas if they are adversely impacted. Marine infill and the construction of associated port infrastructure can remove benthic habitats, including nearshore subtidal communities supporting syngnathids (Vincent *et al.* 2011). The benthic floral and faunal assemblages and the ecological functions of these communities will be assessed by other themes, but the importance of these communities to syngnathid fishes requires investigation. There may be potential for syngnathid fishes to be impacted through habitat loss and dredging activities.

Arguably the best known 'iconic' syngnathid fish in Cockburn Sound is the West Australian Seahorse *Hippocampus subelongatus*. Cockburn Sound is a critical embayment supporting extensive populations with habitat that is underrepresented elsewhere in the species distribution (Atlas of Living Australia 2024, Moore pers. obs). The reproductive biology of this species in Cockburn Sound has been well studied (Jones *et al.* 1998; Kvarnemo *et al.* 2000; Moore 2001; Jones *et al.* 2003; Kvarnemo *et al.* 2006; Kvarnemo *et al.* 2021). Despite this, the distribution, habitat preferences and abundance of this species, both in Cockburn Sound and more broadly, remains poorly known. In addition, Cockburn Sound is potentially home to more than 20 other species of syngnathids, including another species of seahorse, many species of pipefishes and two species of seadragons (Kendrick and Hyndes 2003; Johnson *et al.* 2008; Water Corporation 2019; Hoschke *et al.* 2023; Atlas of Living Australia 2024). The habitat requirements, distributions and abundances of these highly cryptic species, both in Cockburn Sound and more broadly. In some instances, even the true taxonomic identity of species is not clear (Kuiter 2009; Lourie *et al.* 2016).

Quantitative sampling of cryptobenthic fishes is extremely difficult and inference on relative abundance is often inaccurate (e.g. see Kovačić *et al.* 2012; Burnell *et al.* 2015; Bessey *et al.* 2023), especially when the fundamental baseline biodiversity is not well documented. Typically, common species, or those species that are more 'catchable' contribute most to quantitative catches such as trawls and seines (e.g. Kendrick and Hyndes 2003; Dodt 2005; Johnson *et al.* 2008; French *et al.* 2021a; French *et al.* 2021b) with limited data on rare or hard to catch species gathered. In many cases, the most effective approach to understanding cryptic biodiversity is to compile data from multiple sources and the accumulation of species records is best achieved by utilising multiple survey methods across a long timeframe (e.g. Moore *et al.* 2020).

Citizen science initiatives are becoming increasingly popular with non-professionals and have been used to detect biodiversity and map distributions and to detect changes in distribution and climate change effects (e.g. DiBattista *et al.* 2021). The rising interest in SCUBA diving and underwater photography has led to a growth in reporting of syngnathids in particular, which are extremely popular subjects due to their cryptic habits, unusual morphology and mythical status. However, quality control of species identification by non-experts is often of concern. Initiatives that provide image-based observations (e.g. *iNaturalist*, www.inaturalist.org; *Redmap*, www.redmap.org.au) make it possible for experts to confirm, or otherwise, citizen-submitted observations.

Environmental DNA (eDNA) metabarcoding refers to the simultaneous detection of multiple species through the application of taxonomically broad PCR-based assays on environmentally sourced DNA. When used in conjunction with taxonomically verified specimen-anchored genetic sequences, it can characterise whole species assemblages (Gaither *et al.* 2021). In the detection of rare and cryptic species, eDNA methods are emerging as an important supplement to traditional surveying methods and can often be more sensitive and effective (Nester *et al.* 2020; Gaither *et al.* 2021; Bessell *et al.* 2023).

2.1 Aims

Given the paucity of validated baseline biodiversity data for syngnathids in Cockburn Sound, this project aims to provide the first comprehensive summary of current knowledge about which species are present in Cockburn Sound and Owen Anchorage (CS/OA), when they are present, and in which habitats, to interpret the importance of the region for syngnathid species across their broader geographic distributions. It also summarises known life history traits that may influence each species response to disturbance and compiles evidence of the impacts of infrastructure development of syngnathid fishes globally and interprets these in the context of CS/OA. Finally, it addresses some possible general mitigation considerations based on similar disturbances globally. These aims will be achieved by:

(1) a systematic review of syngnathid fishes present in the region, including historical records,

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museum vouchers, literature review, and unpublished research and other catch data;

- (2) collection of additional syngnathid specimens and observations by (i) sampling by other studies, (ii) by targeted sampling in the proposed project area, and (iii) a citizen science program of observational records backed by photographic verification;
- (3) confirmation of species taxonomy by examination of (i) existing voucher specimens held in Australian collections and (ii) of newly collected specimens, and generating molecular barcodes where possible;
- (4) identifying key habitats and distribution for each species; and
- (5) eDNA metabarcoding by targeted sampling in the proposed project area.

3 Review of Literature and Historical Data

Published primary literature on syngnathid fishes was identified principally by interrogating Google Scholar and Researchgate. Unpublished secondary and 'grey' literature was identified by examining citation lists and extensive Google searches. Museum records were collated, and additional records and unpublished survey results were gathered by contacting relevant researchers directly (see below).

Search results were filtered to constrain the data to:

- identify the syngnathid species known from the greater Perth metropolitan area and specifically Cockburn Sound or Owen Anchorage (or predicted to be there);
- existing data and knowledge on those species including spatial distribution, habitat preferences, life history parameters and broader geographical distribution;
- current knowledge on the effects of infrastructure development, as proposed for Westport, on the survival and fitness of syngnathid species globally (see Discussion); and,
- potential mitigation strategies that have proven successful elsewhere (see Discussion).

3.1 Historical accounts of syngnathid fishes in Cockburn Sound and Owen Anchorage.

As the State's authority for faunal taxonomy and biodiversity, the Western Australian Museum (WAM) collection has permanently vouchered specimens of syngnathid fishes from across Western Australia since 1912, including from CS/OA, along with records based on observations (e.g. Hutchins 1994; 2001, Moore unpublished data). Some of these vouchers were derived from other projects cited below (e.g. Kendrick and Hyndes 2003). The WAM collection houses 307 vouchered specimen records from the Perth metropolitan area, including 110 from the area east of Garden and Carnac islands between Fremantle and Cape Peron (Table 1). An additional 42 specimens from the Perth metropolitan area, including CS/OA, are held in collections at the Australian Museum (Sydney) and the Australian National Fish Collection (CSIRO, Hobart).

Early trawl surveys in CS/OA (e.g. Penn 1977) focused on commercially and recreationally harvested species in the deep basin which is dominated by fine mud and silt. These surveys used trawl gear and net sizes that were not designed for, or appropriate for, sampling syngnathid fishes. Although syngnathids can still be a part of the bycatch using such gear (e.g. Kangas *et al.* 2007), early studies did not report any syngnathids (Chittleborough 1970; Penn 1977). The Western Australian Department of Fisheries (now Department of Primary Industries and Regional Development: DPIRD) has been conducting research trawls in Cockburn Sound since the 1970s. However, these surveys were generally focused on monitoring populations of key fishery species. As such, there is unfortunately limited historical data on the presence, identification or abundance of non-target species (D. Yeoh, DPIRD, pers. comm.), although some specimens were lodged with WAM. Similarly, a small Marine Aquarium

Fish Managed Fishery is managed by DPIRD, with specific collection location data understandably commercially confidential. However, at least two species (*H. subelongatus* and *S. argus*), and possibly others, are actively collected in Cockburn Sound (Smith *et al.* 2022).

Dybdahl (1979) conducted extensive trawl and beach seine surveys across Cockburn Sound to document the fish 'resources' (fishes and invertebrates), again with a primary emphasis on commercially and recreationally harvested species. As such, the study also used trawl gear and net sizes that were not appropriate for sampling syngnathid fishes. However, this study did report two species in the bycatch – *Hippocampus tuberculatus* (reported as *breviceps*) and *Stigmatopora argus* (Table 1). The study surveyed multiple locations across the Sound but the report does not provide precise location data for the captured syngnathids. Surprisingly, one of the common Cockburn Sound species, *Hippocampus subelongatus*, was not collected during the trawls. Importantly, Dybdahl (1979) reported that the two species of syngnathid recorded contributed to explaining differences in the faunal composition from different habitats. Despite collecting relative few individuals from extensive trawl and seine effort (*H. tuberculatus* [7 individuals] and *S. argus* [17 individuals]), both species were significant contributors to seagrass catches.

During boat-based seine net surveys of the surf zone, Ayvazian and Hyndes (1995) reported three species of syngnathid fishes from their 'Assemblage E', which included two sites in Cockburn Sound (Table 1). These were typical seagrass species – *Filicampus trigris, Histiogamphelus cristatus* and *S. argus* but it is not clear whether all species were recorded from the Sound itself. Again, *H. subelongatus* was not collected.

Similarly, during beach seines in both seagrass and bare sand areas, five species of Syngnathidae were collected from across Cockburn Sound (Vanderklift 1996; Vanderklift and Jacoby 2003; Table 1). Consistent with previous studies, the abundance of syngnathids in catches was generally low but, these uncommon species often contributed to explaining differences in faunal composition among sites. Again, *H. subelongatus* was not collected. Vanderklift (1996) reported the highest frequency of uncommon species from just a few sites (on the west side of Cockburn Sound) and posited that their absence from the beaches on the eastern shore was driven by reduced detritus loads.

In the most comprehensive study specifically targeting syngnathid biodiversity in the region, Kendrick (2002) and Kendrick and Hyndes (2003) used small mesh trawls in shallow and deeper seagrass meadows (three species of seagrass) and bare sand from Owen Anchorage, Success and Parmelia Banks, between Cockburn Sound and Fremantle. They reported more than 1900 individuals from 14 species including a seadragon (*Phyllopteryx*), a pipehorse (*Idiotropiscis*), two seahorses (*Hippocampus*) and 11 pipefishes (Table 1). The catch was dominated by *S. argus* (67.6% of catch) and *S. nigra* (27.2%) with these species exhibiting somewhat different, but overlapping, habitat preferences. The remaining 12 species were all rarely captured with only one other species, *Vanacampus poecilolaemus*, contributing more than 1% of the catch. Kendrick and Hyndes (2003) also provided evidence that *S. argus* (but not *S. nigra*) exhibits ontogenetic migration between habitats, moving from *Posidonia coriacea* to *P. sinuosa*, at around 132 mm standard length (SL), just before reaching maturity. Males of both species were recorded carrying well developed broods in all seasons. Additional data on breeding remains unpublished. A follow up study detailed the diets of 12 species from the same area (Kendrick and Hyndes 2005).

The distribution of the West Australian seahorse *H. subelongatus* along the eastern shore of CS/OA was recorded during a long-term study on reproductive biology and sexual selection in this species (Jones *et al.* 1998; Kvarnemo *et al.* 2000; Moore 2001; Jones *et al.* 2003; Kvarnemo *et al.* 2006; Kvarnemo *et al.* 2021, Moore unpublished data; Table 1). In addition, sex ratios, home range size and use, mate choice and fidelity and well as reproductive outputs are now well documented for this species, most of which is based on Cockburn Sound populations.

Baker (2002) reported on the distribution and aspects of the biology of two seadragon species in Western Australia, including records from around CS/OA. Precise locality data were obfuscated to

protect these species. However, unpublished data (Dragon Search 2003) suggest that most records are outside of Cockburn Sound (e.g. west side of Garden and Carnac islands, Warnbro Sound and Cape Peron). There is a single record of a leafy seadragon *Phycodurus eques* from the Woodman Point Ammunition Jetty, and a single record of a common seadragon *Phyllopteryx taeniolatus* from northern Cockburn Sound seagrass habitat and numerous records around Fremantle (Table 1).

Only a single species of Syngnathidae, *H. subelongatus,* was reported from a study documenting demersal fauna, based on 23 trawls across seven sites in CS/OA (Johnson *et al.* 2008; Sampey *et al.* 2011; Table 1). Nine individual seahorses were caught across seven of the 23 trawls.

As a purely desktop assessment for the Perth Desalination Plant review, 23 species of Syngnathidae were listed as 'species or species habitat likely to occur within area' of Cockburn Sound based on data held at https://www.environment.gov.au/sprat (Water Corporation 2019; Table 1). However, this approach had no further quality control and consequently included species that have either taxonomic issues or have no other evidence of presence in the area. For example, both *H. subelongatus* and *H. angustus* were included, however this is a consequence of past taxonomic confusion. Both names have been applied to the species in southern Western Australia at various times, but current taxonomy recognises that only the former is found south of Shark Bay (including CS/OA) and the latter north of Shark Bay (Lourie *et al.* 2016; Moore, unpublished data). Consequently, all records of these two species should be combined into *H. subelongatus*. Other species are unlikely to occur; for example, *Solegnathus lettiensis* is only known from offshore trawl grounds between 130 and 180 m deep (Kuiter 2009).

Nester *et al.* (2020) demonstrated that environmental DNA (eDNA) could be used to detect syngnathid fishes in Western Australia and used the method to detect three species in Cockburn Sound (Table 1). Nester *et al.* (2020) was primarily a methodological proof-of-concept and only had sequences for four species rather than a library of sequences from other syngnathid species, so this should not be considered a complete inventory.

Hoschke *et al.* (2023) compiled a list of 24 species of syngnathid fishes from the Perth coast using historical and recent records. Although only a part of the total source data was presented, many additional records for this dataset were based on records or observations within CS/OA (Table 1).

Table 1 Species of Syngmathidae historically reported from Caskburn Sound and Owen Anchorage (1) or reported as possible (y)		
	rn Sound and Owen Anchorage (+) or reported as possible (x)	Table 1 Species of Syngnathidae historically reported from Cockhurn

Species		WAM ¹	Dybdahl ²	Avayzian & Hyndes ³	Vanderklift ^₄	Kendrick & Hyndes⁵	Moore, Kvarnemo ⁶	Baker ⁷	DPIRD ⁸	Water Coorp. ⁹	Nester et al ¹⁰	Hoschke & Whisson ¹¹
Gale's Pipefish	Campichthys galei	+								х		
Tiger Pipefish	Filicampus tigris	+		+		+					+	+
Upside-down Pipefish	Heraldia nocturna									х		
West Aust. Seahorse	Hippocampus subelongatus	+				+	+		+	х	+	+
Knobby Seahorse	Hippocampus tuberculatus	+	+			+	+			х		+
Rhino Pipefish	Histiogamphelus cristatus	+		+	+	+				х		+
Sthn Pygmy Pipehorse	Idiotropiscis australe					+				х		+
Smooth Pipefish	Lissocampus caudalis	+				+				х		
Prophet's Pipefish	Lissocampus fatiloquus	+								х		+
Javelin Pipefish	Lissocampus runa					+				х		
Sawtooth Pipefish	Maroubra perserrata									х		
West. Crested Pipefish	Mitotichthys meraculus	+			+	+				х		
Bonyhead Pipefish	Nannocampus subosseus									х		
Leafy Seadragon	Phycodurus eques	+						+		х		
Ruby Seadragon	Phyllopteryx dewysea	х										
Common Seadragon	Phyllopteryx taeniolatus	+						+		х		+
Pugnose Pipefish	Pugnaso curtirostris	+			+	+				х		+
Gunther's Pipehorse	Solegnathus lettiensis									х		
Spotted Pipefish	Stigmatopora argus	+	+	+	+	+				х	+	+
Widebody Pipefish	Stigmatopora nigra	+				+				х		+
Ringback Pipefish	Stipecampus cristatus	+				+						
Hairy Pipefish	Urocampus carinirostris	+								х		
Mother-of-pearl Pipefish	N Vanacampus margaritifer	+			+	+				х		
Port Phillip Pipefish	Vanacampus phillipi					+				х		
Longsnout Pipefish	Vanacampus poecilolaemus	+				+				х		+

WAM database records and other unpublished observations; 2. Dybdahl (1979); 3. Ayvazian and Hyndes (1995); 4. Vanderklift 1996; Vanderklift and Jacoby 2003; 5. Kendrick (2002), Kendrick and Hyndes (2003); 6. Moore 2001, Jones *et al.* 1998, 2003, Kvarnemo *et al.* 2000, 2006, 2021; Moore unpublished data; 7. Baker (2002); 8. Johnson *et al.* 2008; Sampey *et al.* 2011; Smith *et al.* 2022; 9. Water Corporation 2019; 10.Nester *et al.* (2020); Hoschke & Whisson, unpublished data.

4 Materials and Methods

4.1 Project area

The Cockburn Sound/Owen Anchorage project area (CS/OA), as used here, stretches approximately 24 km north-south and approximately 10 km east-west. It is defined as the area from South Mole, Fremantle (approx. -32.056°) to the southern extent of Cockburn Sound (approx. -32.275°), and from the east side of Garden and Carnac islands and the chain of reefs north of them (approx. 115.662°) to the mainland (approx. 115.766°).

Additional data were collated from across the greater Perth metropolitan area (Yanchep to Mandurah) to understand the distribution of syngnathid fishes across the region.

4.2 Historical Data.

4.2.1 Museums, collections and other observations datasets.

Museum specimens of syngnathid fishes from the Perth metropolitan area were first identified either by accessing the institutional database directly (Western Australian Museum [WAM]) or through the Online Zoological Collections of Australian Museums (OZCAM) via the Atlas of Living Australia (Atlas of Living Australia 2024), and then contacting the staff responsible for collections (Australian Museum, Sydney [AMS]; Australian National Fish Collection, Hobart [CSIRO]). Specimens were re-examined to confirm identifications using current literature and knowledge. Location metadata were scrutinised for accuracy and coordinates corrected as required. A level of accuracy was assigned to each record to be consistent with WAMSI Westport Marine Science Program (WWMSP) Project 4.2.1 (*Spatial distribution and temporal variability in life stages of key fish species in Cockburn Sound*) (high [<200 m], moderate [<500 m], low [>500 m]). Some of these records had associated habitat data. Additional observational data were harvested from unpublished field notebooks. Unpublished data from Dragon Search (2003) were included in the dataset. Specimens of larvae collected from the Perth metropolitan area and held at AMS are included separately in Appendix 1 and used here as evidence of breeding within CS/OA.

4.2.2 Historical trawl dataset.

Kendrick (2002) and Kendrick and Hyndes (2003) published summary data from extensive trawl surveys through Owen Anchorage in 1997 and 1998 (see Section 3). Full methods and data analyses were provided by Kendrick (2002) and Kendrick and Hyndes (2003). Hand-written field notes were made available for this project, which were manually transcribed and quality checked (including date, location, habitat and species). Locations were originally recorded as coordinates at the start and end of short trawls (approximately 50 m or 500 m depending on trawl size; see Kendrick 2002). Given that it is impossible to know at which point along the trawl transect an individual fish was captured, the recorded start point of each trawl was used as the location data for each record. For 658 records, the precise location of the start of each trawl was missing from the field notes. However, during the study, trawls were randomised among grid squares of 500 x 500 m (see Kendrick 2002) and the grid square was identifiable from the field notes. A point approximating the middle of the relevant grid square was used as the location data for each of these records. Coordinates were originally recorded in UTM by GPS and, as part of the current project, were converted to decimal latitude and longitude using ArcGeek coordinate converter for MS Excel (available at: https://giscrack.com/download-exceltemplate-convert-geographic-coordinates-utm). Most of the records from this dataset had associated habitat data (i.e. substrate type and species). Additional records of H. subelongatus were extracted from Johnson et al. (2008). A level of accuracy was assigned to the location data for each record (see Section 4.2.1). Diet and breeding data specific to CS/OA populations was taken from Kendrick and Hyndes 2005) and Kendrick (WAMSI, unpublished data), respectively.

4.3 Contemporary Collections.

4.3.1 SCUBA surveys.

Twenty-four SCUBA-based surveys (from 16 sites) were undertaken in summer and winter in northern Cockburn Sound particularly targeting the area of the proposed Westport development. This included the Kwinana Shelf (18 surveys) and coastal jetties (6 surveys) (Appendix 2). These surveys were 1 hour in duration and loosely followed either a transect tape (open habitat along Kwinana Shelf) or the pylons of the jetty. Surveys focused on searching for cryptic fauna and included gentle disturbance of weed and seagrass to elicit movement of fishes. Using a spray bottle, diluted clove oil was haphazardly applied as an anaesthetic in attempts to sample rubble and reef habitats for cryptic species (under Fisheries Exemption #250966222).

4.3.2 Other projects.

Syngnathid data from sampling for the WWMSP Project 4.2.1 (*Spatial distribution and temporal variability in life stages of key fish species in Cockburn Sound*) were added to the current dataset. Full data analyses and method details are provided in the report for that project, but sampling included a range of vessel-based seines, trawls and sleds, as well as beach seines.

Most of the records from the above WWMSP fish study were not vouchered. However, to ensure accurate identifications of the syngnathids, a set of vouchers of collected species was provided to WAM for confirmation. These newly collected specimens are permanently accessioned into the fish collection of WAM with full metadata and most were DNA barcoded (see Section 4.5.1). All voucher specimen data will be uploaded to the publicly accessible Atlas of Living Australia at the conclusion of the project.

Records of larvae collected by bongo net tows in the WWMSP Project 4.2.2 (*Zooplankton in Cockburn Sound*) are included in Appendix 1 and used here as evidence of breeding within CS/OA.

4.4 Citizen Science.

A 'project' was established within the iNaturalist platform, entitled *Seahorses, pipefishes, seadragons* of Western Australia (<u>https://www.inaturalist.org/projects/seahorses-pipefishes-seadragons-of-western-australia</u>). The project harvested all records submitted to iNaturalist labelled with 'Syngnathidae' and 'Western Australia'. This captured all submissions of syngnathid fishes from Western Australia that were identified (by either the submitter or a member of the iNaturalist community) to family, genus or species in Western Australia.

Historical records on the platform were harvested along with all new submissions. However, to increase uptake, the project was promoted to major dive groups across the Perth metropolitan area. Divers were encouraged to submit sightings and to become a member of the project (to help create a sense of participation). Note that submissions were harvested by the project filters irrespective of whether the submitter was a project member. The iNaturalist platform routinely obfuscates the precise location data, especially for sensitive records (e.g. syngnathids). However, by becoming a member of this project, contributors can mark it 'trusted', which removes location obfuscation and provides access to precise location data for the project manager.

All submissions up to 31 January 2024 were manually checked by the Project leader (Moore) to confirm or change identifications as required. Data were downloaded and the coordinates of all records were visualised on Google Earth. Due to variations in the resolution of location coordinates, the mapped location of each record was compared to the textual description provided by the submitter and adjusted as required. For example, a record might say 'end of Ammunition Jetty', but the mapped position showed the nearby sand-dunes. Where obfuscation was clearly a problem for a record, submitters were contacted directly to request more accurate details. Dubious records that could not be resolved were excluded from the dataset.

4.5 eDNA.

Replicate water samples were collected from the seafloor at six sites (three in summer, three winter) along the Kwinana Shelf (directly adjacent to the proposed Westport footprint. The eDNA metabarcoding workflow largely followed Nester *et al.* (2020) based on a fragment of the 16S rRNA gene. Full details are provided in Appendices 2 and 3.

4.5.1 Genetic barcoding.

Barcode sequences for many of the newly collected specimens (see Section 4.3.2) along with specimens from the existing WAM fish tissue collection were generated using standard extraction, PCR and sanger sequencing in WAM's Molecular Systematics Unit for two genes (COI and 16S; 29 putative species). The COI gene provides standardised species level barcodes consistent with the Barcode of Life database (Ward *et al.* 2009). The 16S rRNA sequences were added to published sequences to create a curated library of 162 syngnathids for sequence matching in the eDNA surveys (see Appendix 3). We used a lowest common ancestor approach so that detections of syngnathid species that were not in our library would still be identifiable as members of the Syngnathidae, albeit with a lower percentage identity, and possibly even to genus.

5 Results

5.1 Compiled Records From All Sources.

A total of 4839 records of adult or juvenile syngnathid fishes from the Cockburn Sound/Owen Anchorage project area were collated from all sources (Table 2). Only 19 records are ambiguous, i.e. identified to family or genus only. Six records were added along the Kwinana Shelf based on eDNA sampling. The majority of the records (79%) were sourced from the historical trawl surveys of Kendrick (2002) and Kendrick and Hyndes (2003), while more than 13% of records were contributed through the citizen science project (Table 2). An additional 1056 observations from the rest of the Perth metropolitan area were also collated to help interpret the data for CS/OA (Table 2). The records ranged from 1913 (four specimens in WAM collection) to January 2024 (Citizen Science). The full dataset is deposited with WAMSI/Westport.

Confidence in the location of each record was generally high, with about 80% of records deemed to have an accuracy of less than 200 m (high). Only 2% of records were deemed to be accurate at a distance of more than 500 m.

Data Source	Туре	Date range	Perth Met Area	tropolitan total	CS/OA only		
			# records	# species	# records	# species	
Museum vouchers	Specimens: vouchered	1913 –2021	336	24	78	16	
Museum & other observations ¹	Observations: photo and non-photo	1989 –2023	69	12	25	8	
Historical trawl data	Specimens: unvouchered ²	1989 –2000	3912	18	3828	18	
Citizen Science project	Observations: photo	2006 –2024	1349	14	679	12	
WWMSP fish study trawls	Specimens: unvouchered	2022 –2023	223	13	223	13	
This project eDNA	DNA	2023	n/a	n/a	6	3	
Total			5889	24	4839	21	

Table 2. Sources of syngnathid fish records contributing to the current dataset for the Perthmetropolitan area and the Cockburn Sound/Owen Anchorage (CS/OA).

¹ Including surveys undertaken as part of the current project and observations from WAM associates; ² Some specimens are vouchered in the Western Australian Museum Fish Collection (but not reported in those data).

5.2 Species of Syngnathid Fishes Confirmed from Cockburn Sound and Owen Anchorage.

Twenty-one species of Syngnathidae are now considered confirmed from the Cockburn Sound/Owen Anchorage project area (listed in Table 3; Figure 1). The most commonly reported syngnathids were two species of the pipefish genus *Stigmatopora* (*S. argus* and *S. nigra*), followed by two seahorses (*Hippocampus subelongatus* and *H. tuberculatus*). For four species, which were previously identified as possibly being in the area (*Heraldia nocturna, Nannocampus subosseus, Phyllopteryx dewysea, Solegnathus lettiensis*; see Table 1), no confirmed records within CS/OA were identified.

5.2.1 Historical records.

All 21 species of syngnathid fishes recorded from CS/OA were represented in historical records, in particular museum records and unpublished/partially published trawl data (Table 2; Figure 1). This ranged from single records for two species (*M. perserrata, U. carinirostris*) to more than 2600 records for *S. argus* (Figure 1).

5.2.2 Contemporary surveys and collections.

Trawls and other benthic sampling for WWMSP Project 4.2.1 (*Spatial distribution and temporal variability in life stages of key fish species in Cockburn Sound*) added 223 specimens from 13 species (Figure 1), particularly from previously poorly sampled areas of the project area. No additional species beyond those identified in historical records were found during the contemporary surveys, however many distributional records for CS/OA were added.

Despite focussed effort searching for cryptic fishes, only a single species of syngnathid fish was observed in 24 dive surveys. *Hippocampus subelongatus* was commonly observed around coastal infrastructure (i.e. 31 individuals in 6 surveys) and was rare in mixed seagrass/reef habitats of Kwinana Shelf (i.e. 2 individuals in 18 surveys) (Appendix 2).

5.2.3 Citizen science records.

As of 31 January 2024, the iNaturalist project had 1555 observations of 35 species of syngnathid fishes from the whole of Western Australia, submitted by 91 different observers. Of these, 1349 observations (14 species) were from the greater Perth metropolitan area (Yanchep to Mandurah) including 679 (12 species) from CS/OA (Fremantle to Point Peron, east of Garden Island) (Table 2; Figure 1).

The species most commonly recorded in CS/OA by citizen science observers was *H. subelongatus*, representing two thirds of all submissions (Figure 1). The pipefish *F. tigris* was the next most commonly reported species (10% of submissions). A small proportion of observations from CS/OA (1.6%), were not able to be identified to species based on the uploaded images.

5.2.4 eDNA.

The 16S_FishSyn_Short assay detected a total of 42 fish taxa from the 30 replicate samples across 6 sites on the Kwinana Shelf. Among these taxa, three were from the family Syngnathidae and all three matched known sequences in our curated library. These were identified in only five of the replicate samples from four sites and were among the most abundant syngnathid species in CS/OA (*H. subelongatus, S. argus, F. tigris;* Appendix 3). All three species were detected from a relatively low number of sequence reads (<250; see Appendix 3).



Figure 1. Number of observations of syngnathid species from Cockburn Sound and Owen Anchorage from the major sources of data (see Table 2). Note the modified *y*-axis and abbreviated column for *Stigmatopora argus* to accommodate the 2608 records from historical trawls.

5.3 Spatial Distribution of Syngnathids in Cockburn Sound and Owen Anchorage.

Notwithstanding differences in sampling effort and spatial focus of the various data sources, syngnathid fishes appear to utilise the whole project area (Figure 2). The greatest density of records was from Owen Anchorage and the northern parts of the project area, primarily from the historical trawl study of Kendrick and Hyndes (1997-1998). Citizen science and other observations were most numerous along the nearshore regions and were concentrated around known popular shore-based diving areas such as various jetties, marinas and Woodman Point. Museum vouchers and recent trawl samples were more evenly distributed across the project area. Species-specific distributions are presented in Section 5.7.

5.4 Habitat Preferences of Syngnathids in Cockburn Sound and Owen Anchorage.

Habitat data were available for 3642 records in CS/OA (Table 3). Most species of Syngnathidae found within CS/OA are broad habitat generalists (Table 3). However, there is a strong preference for seagrasses and or soft substrates in many species. For some species, the preference is very specific to a particular species of seagrass. For example, *M. meraculus* was only reported from *Posidonia coriacea* and adjacent sand or reef areas. In contrast, *V. poecilolaemus* was almost exclusively reported from *P. sinuosa*. Other species, such as *F. tigris* and *Hippocampus* spp. rely heavily on reef or rubble habitats and coastal infrastructure. Species-specific habitat preferences are presented in Section 5.7.

5.5 Temporal Distribution of Syngnathids in Cockburn Sound and Owen Anchorage.

It was possible to determine the month of capture for 4813 (99.6%) of the compiled CS/OA records. For eight species (38%), the number of records was less than 10, making it difficult to assess the temporal use of CS/OA (Table 4). However, for all other species (i.e. with \geq 13 records), the spread of records across months suggests they are resident in CS/OA all year (Table 4). There is no evidence of any species undertaking migrations or seasonal usage of CS/OA. Species-specific usage of CS/OA are presented in Section 5.7.

5.6 Breeding Records of Syngnathids in Cockburn Sound and Owen Anchorage.

For most syngnathid species, aspects of reproduction, including breeding season, are poorly known. There are few specific published records, and anecdotal evidence is often presented in general terms, such as "during summer months" (e.g. Kuiter 2009). Much of the evidence compiled here was taken from literature sources (Table 5) and often applicable to populations outside WA. However, considerable egg-bearing or brooding records specific to CS/OA from Kendrick (unpublished data) are presented for the first time (Table 5), along with larval records from museum collections (AMS) and the WWMSP project *Zooplankton in Cockburn Sound*. (Appendix 1). Detailed species-specific breeding records are presented in Section 5.7.

The most breeding records for syngnathid fishes occur in the warmer months between September and April (Table 5). However, there are some species reportedly breeding in every month and at least five species likely breed in CS/OA across the whole year (*H. tuberculatus, H. cristatus, S. argus, S. nigra, V. poecilolaemus*; Table 5).



Figure 2. Spatial distribution of 4833 syngnathid records from a variety of sources (see Table 2) from Cockburn Sound and Owen Anchorage. Many records overlap and are obscured.

Species	Posidonia coriacea	Posidonia sinuosa	<i>Posidonia</i> undefined	Amphibolis	Undefined/ other seagrass	Algae	Reef	Sponge, sand, mud, rubble	Jetties/ wrecks	n
Campichthys galei					1	1	1	3		6
Filicampus tigris		1		1	+	1	10	3	15	31
Hippocampus subelongatus	2	7			+	+	27	14	374	424
Hippocampus tuberculatus	19	53		1	3	2	6	11	11	106
Histiogamphelus cristatus	53	4		2	1		6	10	1	77
Idiotropiscis australe					1	+	3	2	5	11
Lissocampus caudalis	1		+	4	1		+	2		8
Lissocampus fatiloquus				+	1	2	1	2	1	7
Lissocampus runa		1	+		2^	2	1	+		6
Maroubra perserrata							1		1	2
Mitotichthys meraculus	18				1		3	3		25
Phycodurus eques			+			+	+		1	1
Phyllopteryx taeniolatus	16	4		4	+	1	17	4		46
Pugnaso curtirostris	19	11		8	1	+	+	2	1	42
Stigmatopora argus	513	1438		73	6	5	1	155	11	2202
Stigmatopora nigra	372			17	5	1	4	213	4	616
Stipecampus cristatus	1	1		1	1	+	+			4
Urocampus carinirostris					3^					3
Vanacampus margaritifer		1	+		+	+				1
Vanacampus phillipi	10	11		3	+		+	6		30
Vanacampus poecilolaemus	4	65						2		71
n	1028	1597		114	27	15	81	432	425	3642

Table 3. Habitat preferences for syngnathid fishes from Cockburn Sound and Owen Anchorage based on catch data and/or literature. Numbers indicate records compiled during the present study. + indicates a habitat reported in literature. ^ indicates a species reported from *Zostera*.

Species	Jan	Feb	Mar	Apr	Мау	Jun	Jul	Aug	Sep	Oct	Nov	Dec	n
Campichthys galei		1					1	2		1	1		6
Filicampus tigris	8	16	2	10	10	1	19	7	11	8	11	8	111
Hippocampus subelongatus	44	55	37	70	68	19	54	57	25	57	53	32	571
Hippocampus tuberculatus	28	25	6	4	23	3	11	18	12	11	10	4	156
Histiogamphelus cristatus	9	30		3	2	5	2	19	9	3	11	7	100
Idiotropiscis australe				2		3	3	1	1	2	3		13
Lissocampus caudalis	1	2		1	1		1	2					8
Lissocampus fatiloquus	1				5		1	1		3	1	1	13
Lissocampus runa	1	1			1								3
Maroubra perserrata												1	1
Mitotichthys meraculus	4	7	1	1	4			6	3		7	5	38
Phycodurus eques	1				1								2
Phyllopteryx taeniolatus	5	15	1	2	3		3	3	3	2	4	3	45
Pugnaso curtirostris	12	17	4	2	3			1	2		9	9	59
Stigmatopora argus	298	1020	149	113	302	76	119	278	67	53	130	148	2754
Stigmatopora nigra	69	238		53	33	57	24	77	63	12	63	112	801
Stipecampus cristatus	1	1		1									3
Urocampus carinirostris		1											1
Vanacampus margaritifer		2											2
Vanacampus phillipi	12	13		2	1		2	3			3		36
Vanacampus poecilolaemus	20	30	14	5	5	5	3	3	1		4		90
n	514	1474	214	269	462	169	243	478	197	152	310	330	4813

Table 4. Number of records of species of syngnathid recorded in Cockburn Sound and Owen Anchorage in each month from all sources.

Table 5. Known breeding times for all species recorded in Cockburn Sound and Owen Anchorage. Breeding and/or larval records from within the CS/OA project area are indicated by dark grey. Breeding records inferred from other sources are indicated by light grey.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Campichthys galei ¹												
Filicampus tigris												
Hippocampus subelongatus												
Hippocampus tuberculatus												
Histiogamphelus cristatus												
Idiotropiscis australe												
Lissocampus caudalis ²												
Lissocampus fatiloquus												
Lissocampus runa ¹												
Maroubra perserrata ²												
Mitotichthys meraculus												
Phycodurus eques												
Phyllopteryx taeniolatus												
Pugnaso curtirostris												
Stigmatopora argus												
Stigmatopora nigra												
Stipecampus cristatus ²												
Urocampus carinirostris												
Vanacampus margaritifer ¹												
Vanacampus phillipi												
Vanacampus poecilolaemus												
Confirmed from CS/OA	10	9	4	6	5	4	6	9	8	6	12	3
Including literature sources	15	13	9	9	7	5	6	9	11	13	13	12

¹ no records found; ²reported only as 'summer months'.

5.7 Species Accounts.

The following accounts summarise data compiled for each species in CS/OA from sources used in the current project or from other literature sources. Where possible, data are applicable to Western Australian populations (and in many instances, CS/OA), but data from across a species distribution are also presented. The IUCN Red List of Threatened Species assessments are presented because they represent the global benchmark for evaluating extinction risk. An arbitrary assessment of the status of each species in CS/OA was applied as: rare (< 10 records), uncommon (11-100 records), common (101-500 records), and abundant (>500 records). Common names follow Yearsley *et al.* (2006) and the Australian Fish Names Standard (AS 5300).

5.7.1 Gale's Pipefish Campichthys galei (Duncker, 1909)

Recent synonyms and taxonomic issues: none.

Distribution: Endemic to southern Australia: Shark Bay (WA) to South Australia (Dawson 1985).

Depth/Habitat: 0–18 m. Shallow shell and coral rubble habitats, sparse seagrasses and rocky reefs (Kuiter 2009; Table 3).

Life History: Unknown.

Diet: Unknown.

Size: 6 cm (Kuiter 2009).

IUCN Assessment: Least Concern (Pollom 2016b).

Protections: EPBC, CITES.

Presence in CS/OA: **RARE**. Only six records from CS/OA, spread across the year (Table 4; Figure 3). Two additional records from Perth metropolitan area. Records mostly from northern region (Owen Anchorage) in dedicated trawls but also present in southern Cockburn Sound. Probably distributed in very low abundance throughout the project area in rubble areas with sparse seagrass or algae rather than in the dense seagrass meadows (Table 3).



Figure 3. Records of *Campichthys galei, Pugnaso curtirostris* and *Stipecampus cristatus* from Cockburn Sound and Owen Anchorage. Some records may overlap and be obscured.

5.7.2 Tiger Pipefish Filicampus tigris (Castelnau, 1879)

Recent synonyms and taxonomic issues: none.

- *Distribution*: Endemic to Australia: Port Hedland to Dunsborough (WA), also in Queensland, NSW and South Australia (Moore *et al.* unpublished).
- *Depth/Habitat*: 2–30 m. Seagrass, sand and weed areas as well as sponge, rock and rubble habitats, including around coastal jetties (Dawson 1985; Gray *et al.* 1996; Gray *et al.* 1998; Table 3). Rare in seagrass (Table 3).
- *Life History*: Little known. Eggs or male brooding recorded in CS/OA in February, May and November (Table 5). This is consistent with larvae being detected from November to April (see Appendix 1). Mean brood size 1527 (Kendrick 2002).
- *Diet*: Primarily crustaceans including gammarid (68%) and caprellid (14%) amphipods, mysids (5%) and carid shrimps (4%) (Kendrick and Hyndes 2005).
- Size: 35 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Pollom 2016c).

Protections: EPBC, CITES.

Presence in CS/OA: **COMMON**. With 111 records, *F. tigris* is the fifth most recorded species in CS/OA (Table 4). The spread of records suggests this species is relatively evenly distributed in rubble and soft sediment habitats across the project area and has been found in every month of the year (Table 4; Figure 4). However, this species was reported by divers more than collected in trawls (Figure 1) suggesting it might prefer reef areas, where divers visit and trawls are not undertaken. It was also detected using eDNA by Nester *et al.* (2020) and this project. Nearly 40 additional records from across the Perth metropolitan area were collated, including from the Swan River estuary.



Figure 4. Records of *Filicampus tigris* from Cockburn Sound and Owen Anchorage. Many records may overlap and be obscured.

5.7.3 West Australian Seahorse Hippocampus subelongatus Castelnau, 1873

Recent synonyms and taxonomic issues: Hippocampus angustus. Both names have been applied to the species in southern Western Australia at various times, but current taxonomy recognises that *H. subelongatus* occurs south of Shark Bay (including CS/OA) and *H. angustus* occurs north of Shark Bay (Lourie *et al.* 2016; Moore, unpublished data).

Distribution: Endemic to Western Australia: Augusta to Shark Bay (Moore et al. unpublished).

- *Depth/Habitat*: 1–25 m. Rocky reefs, estuaries and areas of high sediment load, seagrasses, macroalgae such as *Sargassum* spp., and it is often associated with sponges or sea squirts (Moore 2001). Common around jetties, wrecks and artificial habitats in suitable areas, especially in Cockburn Sound (Table 3).
- *Life History*: Breeding season is from October to April and brood time is approximately 3 weeks with a mean brood size of 357 (31–639; Moore 2001, Table 5). Most individuals have multiple broods across a season, each separated by only days (Kvarnemo *et al.* 2000; Moore 2001; Kvarnemo *et al.* 2021). In Cockburn Sound and Swan Estuary, the adult sex ratio is usually female biased (Moore 2001; Kvarnemo *et al.* 2006). Like most seahorses, *H. subelongatus* form monogamous pair bonds during the breeding season and across seasons in some cases (Jones *et al.* 1998). Pair bonds can be broken, with individuals usually (but not always) finding a new partner (Kvarnemo *et al.* 2000). However, there are substantial costs for individuals who switch mates, with a reduced reproductive output caused by a longer inter-brood interval and greater movements, presumably in order to find a new mate (Kvarnemo *et al.* 2000). Pair bonds are maintained by ritualised behaviours and crucial daily early morning greetings (Moore 2001) and the species exhibits size-assortative mating with sexual selection acting on females (Jones *et al.* 2003; Kvarnemo *et al.* 2006). At Palm Beach (Rockingham), home range size was largest for paired females (~93 m² vs ~63 m² for unpaired) than for males (~36 m² for both paired and unpaired) with home ranges overlapping (Moore 2001; Kvarnemo *et al.* 2021). Similar differences were observed for total movement.
- *Diet*: Primarily crustaceans including carid shrimps (26%), gammarids (28%), mysids (21%) and caprellid amphipods (14%) (Kendrick and Hyndes 2005).

Size: 25 cm (Moore et al. unpublished).

IUCN Assessment: Data Deficient (Pollom 2017b).

Protections: EPBC, CITES.

Presence in CS/OA: **COMMON**. CS/OA is one of three strongholds for this species (along with Swan River estuary and Shark Bay). It is rarely observed outside of these three areas. The third most reported species, *H. subelongatus* is resident in CS/OA all year round and is distributed across the whole project area (Figure 5). Abundance and density are largely unknown, however, the species is patchily distributed. Some sites along the eastern side of Cockburn Sound can support remarkably high numbers of individuals. For example, Palm Beach Jetty, an area of 100 m x 25 m with extensive artificial habitat (pylons) supported up to 103 individuals (Moore 2001; Kvarnemo *et al.* 2006; Kvarnemo *et al.* 2021), while other artificial structures may support populations of less than five individuals (Moore 2001, pers. obs.). As the dive surveys for this project demonstrate, *H. subelongatus* is rarely reported from natural habitats. It is not clear whether this reflects a low population density, low detectability or limited search effort in these habitats. However, it appears to be infrequent in dense seagrass meadows (Kendrick and Hyndes 2003; Table 3). This species is predominantly reported by divers through the citizen science project (Figure 1). This species accounted for 59% of all metropolitan Perth syngnathiform catches in the Marine Aquarium Fishery Managed Fishery, caught almost exclusively in CS/OA (Smith *et al.* 2022).

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5.7.4 Knobby Seahorse Hippocampus tuberculatus Castelnau, 1875

- *Recent synonyms and taxonomic issues: Hippocampus breviceps.* There is conjecture regarding the taxonomic status of the species in Western Australia. We follow Kuiter (2001) in recognising the western population as a separate species, noting that work to resolve this taxonomy is underway (Short, Moore *et al.*, unpublished data).
- *Distribution*: Endemic to western and southern Australia. Albany to Onslow (WA). Also South Australia (Moore *et al.* unpublished).
- *Depth/Habitat*: 1–20 m. Sheltered coastal reefs in seagrass and macroalgae (*Sargassum* spp.), reefs and sponge habitats as well as around jetties (Kuiter 2009; Table 3). In CS/OA, reported from *Posidonia sinuosa, P. coriacea* and over sand, but almost absent from *Amphibolis* (Kendrick and Hyndes 2003; Table 3). Juveniles are common on floating weed (Kuiter 2009, WAM unpublished data).
- *Life History*: Little is known about *H. tuberculatus*, however it is likely to have a similar life history to *H. breviceps*, which has been studied by Moreau and Vincent (2004). Breeding primarily during summer months with a 3–4 week brood cycle, producing 50–100 young per brood (Kendrick 2002; Kuiter 2009). However, eggs or male brooding has been recorded in CS/OA across most of the year (Table 5). Populations in Port Philip Bay have roughly equal sex ratios (Moreau and Vincent 2004). *Hippocampus breviceps* reportedly forms small groups (2–5 individuals) during morning social encounters (Moreau and Vincent 2004). Spatial area use is up to 12 m², with females using a significantly larger area than males (Moreau and Vincent 2004). However, only about one third of a study population showed site fidelity across a season (Moreau and Vincent 2004). As with *H. subelongatus*, ritualised greetings are reported, however do not appear to be restricted to monogamous pair bonds (Moreau and Vincent 2004).
- *Diet*: Primarily crustaceans including gammarid (61%) and caprellid (13%) amphipods and harpacticoid copepods (7%) (Kendrick and Hyndes 2005).

Size: 10 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Pollom 2017a), as a synonym of *H. breviceps*.

- Protections: EPBC, CITES.
- *Presence in CS/OA*: **COMMON**. Less commonly reported from CS/OA than *H. subelongatus* (ranked 4th most common; Table 4) but is also substantially smaller in size and possibly less detectable (most records are from trawl surveys; Figure 1). Found all year round in CS/OA (Table 4), *H. tuberculatus* records are concentrated in the northern parts of the project area and it has yet to be reported from near the proposed Westport footprint (Figure 5). In Port Phillip Bay, *H. breviceps* has been reported in high density (0.21/m²) at some sites (Moreau and Vincent 2004), but no similar data exist for *H. tuberculatus*. Large numbers of rafting juveniles are often dip netted on the surface across the Perth metropolitan area during summer (WAM, unpublished data). Targeted in CS/OA by commercial aquarium collectors, with a relatively small take (DPIRD, commercial-in-confidence data).





5.7.5 Rhino Pipefish Histiogamphelus cristatus (Macleay, 1881)

Recent synonyms and taxonomic issues: none.

- *Distribution*: Endemic to Southern Australia: Green Head (WA) to South Australia (Moore *et al.* unpublished).
- *Depth/Habitat*: 1-20 m. Seagrass meadows (*Amphibolis* and *Posidonia*) and adjacent areas of shell, rubble, sand and detritus (Kendrick and Hyndes 2003; Kuiter 2009). Most records from CS/OA are in *Posidonia coriacea* and adjacent sandy areas (Table 3).
- *Life History*: Eggs or male brooding recorded in CS/OA in most months, but probably breeding year-round (Table 5). Mean brood size 178 (Kendrick 2002).
- *Diet*: Primarily crustaceans including gammarid (46%) and caprellid (22%) amphipods and mysid shrimps (15%) (Kendrick and Hyndes 2005).

Size: 28 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Fuji and Pollom 2016a).

Protections: EPBC, CITES.

Presence in CS/OA: **COMMON.** There were 100 records of this species within CS/OA across almost all months (Table 4). Most specimens of this species were caught in trawls in all habitats sampled in CS/OA (Kendrick and Hyndes 2003; Table 3; Figures 1, 6), which were concentrated in the Owen Anchorage area. A few sparse records in Cockburn Sound probably reflects the absence of preferred habitat, although it is known from the Kwinana Shelf (Figure 6). This pipefish is not often sighted by divers, except around Woodman Point (Figures 1, 6). More than 30 additional records from across the Perth metropolitan area were collated, including from the Swan River estuary.



Figure 6. Records of *Histiogamphelus cristatus* from Cockburn Sound and Owen Anchorage.

5.7.6 Southern Pygmy Pipehorse Idiotropiscis australis (Waite & Hale, 1921)

Recent synonyms and taxonomic issues: Acentronura australe

Distribution: Endemic to southern Australia: Waterman's Beach (WA) to South Australia (Moore *et al.* unpublished).

Depth/Habitat: Depth unknown. Appears to prefer sparsely vegetated or unvegetated areas adjacent to reefs or jetties (Kendrick and Hyndes 2003; Table 3). Images of this species from iNaturalist show

sparsely vegetated habitats with rhodophytes and seagrass. *Idiotropiscis lumnitzeri* from NSW inhabits semi-exposed rocky reefs from 6–30 m, with sparse clumps of rhodophytes (Kuiter 2004). *Life History*: Breeding known through spring (August to November; Table 5). Possible site fidelity as

reported for *I. lumnitzeri* in NSW (Kuiter 2004). Mean brood size 45 (Kendrick 2002).

Diet: Unknown, but images of this species sometimes include mysid-like crustaceans.

Size: 5 cm (Moore et al. unpublished).

IUCN Assessment: Data Deficient (Pollom 2016a).

Protections: EPBC, CITES.

Presence in CS/OA: **UNCOMMON**. Thirteen records in CS/OA (primarily from the northern parts of the project area, with the most sampling effort; Figure 7), and more than 30 across the rest of the Perth metropolitan area. Primarily observed by divers and rarely taken in trawls (syngnathid-specific or otherwise), but in very low densities when they are (Kendrick and Hyndes 2003; Figure 1). Most records are during the breeding season (Table 4). With so few records, it is not clear whether this reflects seasonal use of the area or the fact that they are more active during that time.



Figure 7. Records of *Idiotropiscis australe, Phycodurus eques* and *Phyllopteryx taeniolatus* from Cockburn Sound and Owen Anchorage. Some records may overlap and be obscured.

5.7.7 Smooth Pipefish Lissocampus caudalis Waite and Hale, 1921

Recent synonyms and taxonomic issues: none.

Distribution: Endemic to southern Australia: Cervantes (WA) to Victoria (Moore et al. unpublished).

- *Depth/Habitat*: 0-37 m. Shallow algal reefs, rubble and seagrass meadows (*Posidonia coriacea*, *Amphibolis griffithii* and *Zostera* spp.) (Scott 1961; Dawson 1985; Kendrick and Hyndes 2003; Kuiter 2009; Table 3). Like other *Lissocampus* species, also found in floating *Sargassum* (Dawson 1985).
- *Life History*: largely unknown. Breeding season during summer (in Tasmania, Scott 1970) but undocumented in CS/OA (Table 5).
- *Diet*: Primarily crustaceans including harpacticoid (47%) and calanoid (13%) copepods and gammarid amphipods (35%) (Kendrick and Hyndes 2005).

Size: 11 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Pollom and Qian 2016a).

Protections: EPBC, CITES.

Presence in CS/OA: **RARE**. Only eight confirmed records from CS/OA between January and August (Table 4) but probably present all year round and in low abundance in suitable habitat. All confirmed records are from Owen Anchorage and its presence in Cockburn Sound remains unknown (Figure 8). Another four records were collected from elsewhere in the Perth metropolitan area. Reported from less than 1% of seagrass trawls in CS/OA by Kendrick and Hyndes (2003), but likely easily overlooked. Difficult to distinguish from other species of *Lissocampus* from the region and non-vouchered records of all three species that have not been identified by careful examination should be accepted with caution. Rarely observed by divers (Figure 1).

5.7.8 **Prophet's Pipefish** *Lissocampus fatiloquus* (Whitley, 1943)

Recent synonyms and taxonomic issues: none.

- *Distribution*: Endemic to western and northern Australia: Rockingham (WA) around northern Australia to Queensland (Moore *et al.* unpublished).
- *Depth/Habitat*: 0-10 m. Poorly known but similar to *L. caudalis* (Dawson 1985; Kuiter 2009; Table 3), including sand and seagrass (Baker 2006) and in floating weed rafts (Whisson and Hoschke 2021).
- *Life History*: One brooding male recorded in CS/OA in October (Table 5). Mean brood size 26 (Kendrick 2002). Otherwise unknown.

Diet: Unknown, but probably similar to L. caudalis.

Size: 10 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Pollom and Qian 2016b).

Protections: EPBC, CITES.

Presence in CS/OA: **UNCOMMON**. Thirteen records from CS/OA across most of the year (Table 4), but likely easily overlooked. Possibly found in low abundance in suitable habitat across the project area (Figure 8). Another four records from elsewhere in the Perth metropolitan area. Difficult to distinguish from other species of *Lissocampus* from the region and non-vouchered records of all three species that have not been identified by careful examination should be accepted with caution. Rarely observed by divers (Figure 1).

5.7.9 Javelin Pipefish Lissocampus runa (Whitley, 1931)

Recent synonyms and taxonomic issues: none.

Distribution: Endemic to southern Australia: Perth (WA) to NSW (Kuiter 2009).

Depth/Habitat: 1-20 m. Estuaries and coastal areas with rocky reefs, algae, seagrass and sand (Dawson

1977; 1985; Hindell *et al.* 2000; Kuiter 2009; Table 3).

Life History: Unknown (Table 5).

Diet: Unknown, but probably similar to L. caudalis.

Size: 11 cm (Dawson 1977).

IUCN Assessment: Least Concern (Qian and Pollom 2016).

Protections: EPBC, CITES.

Presence in CS/OA: **RARE**. Three records from Owen Anchorage (Table 4; Figure 8), and another six records from Rottnest Island. Difficult to distinguish from other species of *Lissocampus* from the region and non-vouchered records of all three species that have not been identified by careful examination should be accepted with caution. Most records are museum vouchers, which have been confirmed by careful examination (Figure 1).



Figure 8. Records of *Lissocampus* species from Cockburn Sound and Owen Anchorage. Some records may overlap and be obscured.

5.7.10 Sawtooth Pipefish Maroubra perserrata Whitley, 1948

Recent synonyms and taxonomic issues: none.

Distribution: Endemic to southern and eastern Australia: Rottnest Island (WA) to Queensland (Moore *et al.* unpublished).

Depth/Habitat: 3-25 m. Ledges, overhangs and caves on rocky reefs (Kuiter 2009; Table 3).

Life History: individuals sometimes form small aggregations. Brood size around 60 with a 22 day gestation during summer (Kuiter 2009; Table 5).

Diet: Unknown.

Size: 8 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Fuji and Pollom 2016b).

Protections: EPBC, CITES.

Presence in CS/OA: **RARE**. A single observational record from the project area (South Mole; Figure 9) but has been recorded from nearby parts of the Perth metropolitan area so could be more widespread, albeit in very low abundance. Suitable habitat is probably limited to South Mole, Woodman Point and the islands and reefs along the west side of CS/OA. Generally poorly known (Baker 2006).

5.7.11 Western Crested Pipefish Mitotichthys meraculus (Whitley, 1948)

Recent synonyms and taxonomic issues: Some workers consider this species to be in the genus Histiogamphelus.

Distribution: Endemic to WA: Perth to Augusta (Moore *et al.* unpublished).

- *Depth/Habitat*: 0-10 m. Mostly seagrass, primarily *Posidonia coriacea* (Kendrick and Hyndes 2003; Table 3).
- *Life History*: Male brooding recorded in CS/OA in March, April and September (Table 5). Mean brood size 269 (Kendrick 2002). Otherwise unknown.
- *Diet*: Primarily crustaceans especially mysids (80%) and gammarid amphipods (8%) (Kendrick and Hyndes 2005).

Size: 24 cm (Moore *et al.* unpublished).

IUCN Assessment: Data Deficient (Zhang and Pollom 2016).

Protections: EPBC, CITES.

Presence in CS/OA: **UNCOMMON**. Probably an uncommon resident all year in CS/OA (Table 4) utilising suitable habitat across the project area (Figure 9). It is also known from another 29 records across the Perth metropolitan area. Typically sampled by various trawls (Figure 1), this species is increasingly being observed by divers around Fremantle (see iNaturalist project).



Figure 9. Records of *Maroubra perserrata, Mitotichthys meraculus* and *Urocampus carinirostris* from Cockburn Sound and Owen Anchorage. Some records may overlap and be obscured.

5.7.12 Leafy Seadragon Phycodurus eques (Günther, 1865)

Recent synonyms and taxonomic issues: none.

- *Distribution*: Endemic to southern Australia: Jurien Bay (WA) to Victor Harbour (SA) (Moore *et al.* unpublished).
- *Depth/Habitat*: 4-50 m. Rocky reefs, especially with kelp (*Ecklonia*) or other macroalgae and shallow (5–15 m depth) *Posidonia* seagrass meadows (Connolly *et al.* 2002a; Connolly *et al.* 2002b; Kuiter 2009, Connolly *et al.* 2002; Table 3).
- *Life History*: Well-defined home ranges of up to 5 ha (Connolly *et al.* 2002a; Connolly *et al.* 2002b). No diel pattern of movement has been reported and individuals often exhibit long periods with no movement (Connolly *et al.* 2002a; Connolly *et al.* 2002b). *Phycodurus eques* is genetically structured into west and east Australian populations (Stiller *et al.* 2020). Males carry up to 300 eggs from October to January (Kuiter 2009; Table 5).
Diet: Mysids and other crustaceans (Kuiter 2009).

Size: 43 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern with decreasing population trend (Pollom 2017c).

Protections: EPBC, Fish Resources Management Act (WA), Biodiversity Conservation Act (WA), CITES. *Presence in CS/OA*: **RARE**. This species is generally rare around Perth with only two records from CS/OA

(Figure 7) – a museum voucher from Robb's Jetty in 1919 and one from the Ammunition Jetty, Woodman Point in 1998 (Dragon Search 2003). There are a few records from Carnac Island, the west side of Garden Island and Warnbro and elsewhere in the Perth metropolitan area. There is probably limited suitable habitat within Cockburn Sound itself.

5.7.13 Common Seadragon Phyllopteryx taeniolatus (Lacépède, 1804)

Recent synonyms and taxonomic issues: none.

Distribution: Endemic to southern Australia: Geraldton (WA) to Newcastle (NSW), including Tasmania (Moore *et al.* unpublished).

- Depth/Habitat: 1-150 m. Rocky reefs, especially with kelp (Ecklonia) or other macroalgae, and adjacent seagrass (Kuiter 2009). Recorded with Halophila ovalis, Ecklonia radiata, Macrocystis pyrifera, M. angustifolia, Sargassum spp., Posidonia spp., Amphibolis spp., and sponge and sand habitats (Sanchez-Camara et al. 2005; Baker 2009; Table 3).
- *Life History*: Overlapping home ranges from 50–150 m, and up to 50 m wide (Sanchez-Camara and Booth 2004). Breeding (in NSW, at similar latitude to CS/OA) between July and January (Baker 2002; Sanchez-Camara *et al.* 2005). Eggs or male brooding recorded in CS/OA in spring, but probably also across summer months (Table 5). Incubation is around eight weeks with a brood size up to 250, and multiple broods per season (Sanchez-Camara *et al.* 2005; Forsgren and Lowe 2006). Population structuring has been identified, with a single population in the southwest of Australia and reduced genetic diversity around Perth (Stiller *et al.* 2023).

Diet: Primarily crustaceans including mysids (81%), carid shrimps (8%), prawns of the genus *Lucifer* (6%), and other species (Kendrick and Hyndes 2005).

Size: 46 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern with decreasing population trend (Pollom 2017d).

Protections: EPBC, Fish Resources Management Act (WA), CITES.

Presence in CS/OA: UNCOMMON. Phyllopteryx taeniolatus is seen more frequently than P. eques in the Perth metropolitan area. While some sites are well known among divers as population strongholds (especially north of Fremantle), this species is not commonly encountered in CS/OA (Figure 7). There are numerous old museum records around CS/OA but none that are within Cockburn Sound itself. More than 30 specimens were caught in dedicated trawl surveys across a range of habitats in Owen Anchorage, to the north of Cockburn Sound (Kendrick 2002, Kendrick & Hyndes, unpublished data; Figure 7). Individuals were commonly observed around South Mole, Fremantle and Woodman Point in the 1980s and 1990s (Moore, unpublished data) but diver-based observations are now rare in those areas. The citizen science project reported more than 280 records of this species in Western Australia, many of which were around North Fremantle and Point Peron, to the north and south of CS/OA, respectively, but none in Cockburn Sound. The data suggest that *P. taeniolatus* could be considered uncommon in all months (Table 4) in Owen Anchorage and, if it does occur, is a very rare vagrant in Cockburn Sound.

5.7.14 Pugnose Pipefish Pugnaso curtirostris (Castelnau, 1872a)

Recent synonyms and taxonomic issues: none.

- *Distribution*: Endemic to southern Australia: Houtman Abrolhos (WA) to Victoria and Tasmania (Moore *et al.* unpublished).
- *Depth/Habitat*: 11 m. Predominantly in *Posidonia sinuosa* and *P. coriacea* seagrass beds in CS/OA (Table 3), but also in other seagrasses (*Amphibolis griffithii, Zostera* spp.), rocky and algal covered reefs, estuaries, and sandy areas (Dawson 1984; 1985; Kendrick and Hyndes 2003; Kuiter 2009; Table 3). Juveniles often among detritus (Kuiter 2009).
- *Life History*: Little known. Eggs or male brooding in CS/OA at least between August and February (Table 5). Breeding reported as late spring and summer (Bray and Thompson 2022), November to February (in Tasmania, Scott 1970). Brood size is between 30 to 90 eggs (Dawson 1984; Kendrick 2002).
- *Diet*: Primarily crustaceans including gammarid amphipods (42%) harpacticoid copepods (25%), mysids (3%) and tanaids (3%) (Kendrick and Hyndes 2005).
- Size: 19 cm (Moore et al. unpublished).
- IUCN Assessment: Least Concern (Aylesworth and Pollom 2016b).

Protections: EPBC, CITES.

Presence in CS/OA: **COMMON**. Fifty nine records of *P. curtirostris* from across the year were identified, primarily caught during extensive trawling in Owen Anchorage by Kendrick and Hyndes (2003) (Table 4; Figure 3). Probably occurs in low abundance in suitable seagrass habitat throughout CS/OA, similar to that reported in South Australia (Baker 2006).

5.7.15 Spotted Pipefish Stigmatopora argus (Richardson, 1840)

Recent synonyms and taxonomic issues: possibly a species complex (Baker 2006).

- *Distribution*: Endemic to southern Australia: Dongara (WA) to Tasmania and NSW (Moore *et al.* unpublished). Also in New Zealand (Stewart 2015).
- *Depth/Habitat*: 0-10 m. Shallow protected seagrass meadows. Most frequently in broad-leafed species meadows especially *Posidonia sinuosa* but also *P. coriacea* and less frequently *Amphibolis griffithii*, and in deeper water habitats (Hyndes *et al.* 2003; Kendrick and Hyndes 2003; Baker 2006; Browne and Smith 2007; Table 3). Also observed in *Halophila* and *Heterozostera* meadows (see references in Kendrick and Hyndes 2003, Moore pers. obs.) and rafting among detached seagrass and *Sargassum* (Dawson 1985, WAM database). In New South Wales, *S. argus* preferred long seagrass in dense meadows although females were equally common in thinned-out patches (Steffe *et al.* 1989).
- *Life History*: Breeding occurs year-round and up to 41 eggs are carried by the male with a short pelagic stage (Kendrick and Hyndes 2003; Browne and Smith 2007; Parkinson and Booth 2016; Table 4; Appendix 1). Only the largest members of the adult population breed between February and September (Kendrick 2002). A short-lived species (up to 150 days), exhibiting rapid growth and maturation, with males mature at 35 days post hatching (Parkinson and Booth 2016). There is a size-related movement from the narrow-leafed *P. coriacea* to broad-leafed *P. sinuosa* habitats at around 13 cm standard length, just before reaching sexual maturity (Kendrick and Hyndes 2003). Sex ratio was male-biased in New South Wales (Steffe *et al.* 1989).
- *Diet*: Primarily crustaceans including calanoid (68%), cyclopoid (22%) and harpacticoid (8%) copepods, and gammarid amphipods (1%) and ostracods (Steffe *et al.* 1989; Payne *et al.* 1998; Kendrick and

Hyndes 2005) with an ontogenetic shift in composition (Edgar and Shaw 1995).

Size: 26 cm (Moore *et al.* unpublished).

IUCN Assessment: Least Concern with decreasing population trend (Carlyle and Pollom 2016a). *Protections*: EPBC, CITES.

Presence in CS/OA: **ABUNDANT**. This is the most recorded species in CS/OA and is likely abundant year-round in suitable habitat across the whole project area (Table 4; Figure 10). It was especially common in seagrass trawls, representing more than 67% of syngnathid catches (Kendrick and Hyndes 2003). In CS/OA, Kendrick (2002) reported that the density of *S. argus* was lower in winter and spring than in summer. This species has been recorded during most studies in CS/OA, including by eDNA (see Table 1). It was readily collected in high abundance in similar habitats in Geographe Bay (French *et al.* 2021a; French *et al.* 2021b) and is often the most abundant syngnathid in similar habitats, recorded in very high densities (see Martin-Smith 2003). This species is difficult to distinguish from *S. nigra* without careful examination, so some records may be mixed. This species accounted for 11% of all syngnathiform catches in the Marine Aquarium Fish Managed Fishery, probably mostly caught in CS/OA (Smith *et al.* 2022).

5.7.16 Widebody Pipefish Stigmatopora nigra Kaup, 1853

Recent synonyms and taxonomic issues: possibly a species complex (Baker 2006).

- *Distribution*: Endemic to the southern half of Australia: Shark Bay (WA) to Queensland (Moore *et al.* unpublished).
- *Depth/Habitat*: 0-35 m. Seagrass and among floating seaweed and possibly algae (Dawson 1985, WAM database) with a preference for narrow-leafed species (Hyndes *et al.* 2003; Kendrick and Hyndes 2003; Baker 2006; Browne and Smith 2007). In CS/OA, there appears to be a strong preference for *Posidonia coriacea* (e.g. 87% of captures by Kendrick and Hyndes 2003) and adjacent sandy areas (Table 3). They were also reported from deeper habitats, sand and *A. griffithii* but not *P. sinuosa* (Kendrick and Hyndes 2003; Table 3). Elsewhere, they were most abundant in *Zostera* (Browne and Smith 2007) and also among *Posidonia* and bare sand (McClatchie *et al.* 2006). In New South Wales, *S. nigra* preferred long seagrass in dense meadows and there was no difference in preference between the sexes (Steffe *et al.* 1989).
- *Life History*: Breeds year-round and a mean of 117 eggs are carried by the male with a short pelagic stage (Kendrick 2002; Kendrick and Hyndes 2003; Browne and Smith 2007; Parkinson and Booth 2016; Table 5; Appendix 1). A short-lived species (up to 150 days), exhibiting rapid growth and maturation, with males mature at 16–19 days post hatching (Parkinson and Booth 2016). Sex ratio was male-biased in New South Wales (Steffe *et al.* 1989).
- *Diet*: Primarily crustaceans including calanoid (70%), cyclopoid (21%) and harpacticoid copepods (7%), especially planktonic species (Steffe *et al.* 1989; Kendrick and Hyndes 2005; Smith *et al.* 2011). Diet varies depending on habitat density (Smith *et al.* 2011).

Size: 16 cm (Moore *et al.* unpublished).

IUCN Assessment: Least Concern with decreasing population trend (Carlyle and Pollom 2016b). *Protections*: EPBC, CITES.

Presence in CS/OA: **ABUNDANT**. This species was the second most recorded species in compiled records for CS/OA and is found in all months of the year (Table 4). It was especially common in seagrass trawls of Owen Anchorage, representing more than 27% of syngnathid catches by Kendrick and Hyndes (2003) (Figure 10). There are fewer records of *S. nigra* in Cockburn Sound

than in Owen Anchorage (Figure 10), but availability of suitable habitats and sampling effort are both lower. This species is difficult to distinguish from *S. argus* without careful examination, so some records may be mixed.



Figure 10. Records of *Stigmatopora* species from Cockburn Sound and Owen Anchorage. Many records may overlap and be obscured.

5.7.17 Ringback Pipefish Stipecampus cristatus (McCulloch & Waite, 1918)

Recent synonyms and taxonomic issues: none.

- *Distribution*: Endemic to southern Australia: Whitfords (WA) to Tasmania and Victoria (Moore *et al.* unpublished).
- *Depth/Habitat*: 0-15 m. Sheltered reefs with red and brown algae (Dawson 1985) and patchy seagrass habitats (Kuiter 2009), including *Amphibolis griffithii* (Kendrick and Hyndes 2003) and *Posidonia* (Baker 2006) (Table 3).

Life History: Little known. One egg bearing female recorded in CS/OA in January (Table 5). Gestation

is around a month and brood size is 50-100 (Kuiter 1993). Large migrations reported into Port Phillip Bay (Victoria) during the summer breeding season (Kuiter 1993), but there are insufficient data for Western Australian populations to ascertain whether this behaviour occurs more widely.

Diet: Unknown.

Size: 25 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Austin and Pollom 2016a).

Protections: EPBC, CITES.

Presence in CS/OA: **RARE**. Very few records from Western Australia, with three captures in CS/OA (Kendrick and Hyndes 2003; Table 4; Figure 3) and two additional WAM specimens from the Perth metropolitan area. This species is highly cryptic but CS/OA is near the northern limit of this species' known distribution (Moore *et al.* unpublished) so may be low in abundance.

5.7.18 Hairy Pipefish Urocampus carinirostris Castelnau, 1872b

- *Recent synonyms and taxonomic issues: Urocampus guentheri* was described from Shark Bay and may be the correct identity of all WA specimens rather than *U. carinirostris*. The species has two historically reproductively isolated populations on the east coast of Australia, which may represent separate species (Chenoweth *et al.* 2002), however Western Australian specimens were not included in the analysis.
- *Distribution*: Southern and eastern Australia: Perth (WA) to Queensland (Moore *et al.* unpublished). Also in Papua New Guinea (Dawson 1985).
- *Depth/Habitat*: 0-6 m. Shallow protected bays and estuaries, especially around the edges of meadows of species of the seagrasses *Zostera* (Dawson 1985; Howard and Koehn 1985; Gray *et al.* 1996; Chenoweth *et al.* 2002; Table 3). In NSW, the density of *U. carinirostris* individuals decreased significantly with increasing area of *Zostera* meadows but increased significantly along the edges of meadows when compared with the middle (Jelbart *et al.* 2006).
- *Life History*: A prolonged breeding season, with egg carrying males (with up to 50 eggs) observed from September to May (Howard and Koehn 1985). This is consistent with larvae being detected in November and January in Cockburn Sound (Table 5; Appendix 1). This is a short-lived species, with a life cycle of little more than a year (Howard and Koehn 1985). Chenoweth *et al.* (2002) proposed that dispersal might be limited due to poor swimming ability of young, but this is yet to be tested. This species is more commonly collected at night (Gray *et al.* 1998).
- *Diet*: Primarily crustaceans including calanoid (21%), cyclopoid (52%), and harpacticoid (17%) copepods (Howard and Koehn 1985).

Size: 12 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern with a stable population trend (Austin and Pollom 2016b).

Protections: EPBC, CITES.

Presence in CS/OA: **RARE**. Appears to prefer estuaries (Gray *et al.* 1996). Most Western Australian records are from the Swan River estuary, with a few coastal collections (WAM database). Not captured in CS/OA in syngnathid-targeted trawls by Kendrick and Hyndes (2003). A single old (1920) record from Garden Island with dubious location coordinates (Figure 9), and a few records from the Fremantle area suggest it may possibly occur in the restricted and poorly sampled *Zostera* and/or *Heterozostera* seagrass beds of CS/OA.

5.7.19 Mother-of-pearl Pipefish Vanacampus margaritifer (Peters, 1868)

Recent synonyms and taxonomic issues: none.

Distribution: Endemic to southern Australia: Jurien Bay (WA) to Queensland (Dawson 1985).

Depth/Habitat: 2-15 m. Shallow coastal and estuarine seagrass beds (including Heterozostera, Zostera, Posidonia and Halophila) and macroalgae (especially Ecklonia) and floating Sargassum (Dawson 1985) (Table 3). In CS/OA, Kendrick and Hyndes (2003) reported V. margaritifer from Posidonia sinuosa.

Life History: Males are brooding at around 13 cm and have up to 100 eggs per brood (Dawson 1984). No data on breeding season (Table 5). Possibly more active at night (Gray *et al.* 1998).

Diet: Unknown. Possibly similar to other species of *Vanacampus* (Kendrick and Hyndes 2005). *Size*: 16 cm (Dawson 1985).

IUCN Assessment: Least Concern (Rachinski and Pollom 2016a).

Protections: EPBC, CITES.

Presence in CS/OA: **RARE**. Very few records from Western Australia, with two records in CS/OA in February (Table 4; Figure 11). Another three records were found from the Perth metropolitan area, all just outside CS/OA in November or December. Species of *Vanacampus* can be difficult to distinguish from each other without careful examination, so some records of this genus may be incorrect. The two CS/OA records were not vouchered, but the identity of two specimens from nearby are confirmed from WAM vouchers.

5.7.20 Port Phillip Pipefish Vanacampus phillipi (Lucas, 1891)

Recent synonyms and taxonomic issues: possibly a species complex (Baker 2006).

Distribution: Endemic to southern Australia: Cottesloe (WA) to NSW (Moore et al. unpublished).

- Depth/Habitat: 0-25 m. Coastal seagrass beds. In CS/OA, collected from *Posidonia sinuosa, P. coriacea, Amphibolis griffithii* and deeper habitats (Kendrick and Hyndes 2003; Table 3). Reported from *Halophila, Heterozostera, Posidonia, Ruppia,* and *Zostera,* as well as reefs, sand and estuaries (Martin-Smith 2003) and from the edges of eelgrass beds in Western Port (Victoria) (Howard and Koehn 1985) (Table 3).
- *Life History*: Little known. Eggs or male brooding recorded in CS/OA in January, February, August and November (Table 5). Breeding season reported from November to February in Tasmania (Scott 1970). Brood size less than 50 and often around 25 (Dawson 1984; Howard and Koehn 1985; Kendrick 2002; Martin-Smith 2003). Reportedly actively feeding during daylight (Howard and Koehn 1985).
- *Diet*: In CS/OA, this species primarily feeds on crustaceans including gammarid amphipods (45%), harpacticoid copepods (13%) and mysids (7%) (Kendrick and Hyndes 2005). In Western Port (Victoria), the diet was similar but differed slightly in proportions, with calanoid (22%) copepods, gammaridean (28%) amphipods, caprellid amphipods, isopods, ostracods and carid shrimps (Howard and Koehn 1985; Edgar and Shaw 1995). There is an ontogenetic shift in composition of the diet (Edgar and Shaw 1995). Probably has an annual life cycle (Howard and Koehn 1985).

Size: 21 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Rachinski and Pollom 2016b).

Protections: EPBC, CITES.

Presence in CS/OA: **UNCOMMON**. Probably occurs in low abundance across suitable habitat yearround, especially in the northern parts of CS/OA (Table 4; Figure 11). Although both abundance and frequency were low, *V. phillipi* was one of the most collected species in Owen Anchorage by Kendrick and Hyndes (2003). Species of *Vanacampus* can be difficult to distinguish from each other without careful examination, so some records of this genus may be incorrect.

5.7.21 Longsnout Pipefish Vanacampus poecilolaemus (Peters, 1868)

Recent synonyms and taxonomic issues: possibly a species complex (Kuiter 2009).

Distribution: Endemic to southern Australia: Cottesloe (WA) to Victoria (Moore et al. unpublished).

Depth/Habitat: 0-18 m. Coastal seagrass meadows. In CS/OA, this species is almost exclusively caught in *Posidonia sinuosa* meadows (Kendrick and Hyndes 2003; Table 3).

- *Life History*: Little known. Eggs or male brooding recorded in CS/OA across most of the year (Table 5). Approximately 40–50 eggs (Dawson 1985; Kendrick 2002).
- *Diet*: Primarily crustaceans including mysids (68%) and carid shrimps (22%) (Kendrick and Hyndes 2005).

Size: 31 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Pollom and Rachinski 2016).

Protections: EPBC, CITES.

Presence in CS/OA: **UNCOMMON**. Probably common in *P. sinuosa* meadows, but uncommon to rare in other habitats in CS/OA (Table 4; Figure 11). Species of *Vanacampus* can be difficult to distinguish from each other without careful examination, so some records of this genus may be incorrect.



Figure 11. Records of *Vanacampus* species from Cockburn Sound and Owen Anchorage. Many records may overlap and be obscured.

5.7.22 Unlikely species

The following four species have been listed as potentially occurring in CS/OA by one or more sources, but no records were found during the present study and are deemed to be very unlikely in CS/OA.

Upside-down Pipefish Heraldia nocturna Paxton, 1975

- *Recent synonyms and taxonomic issues:* The western population has been proposed as an undescribed species *Heraldia* sp. 1 (Kuiter 2009).
- *Distribution*: Endemic to southern Australia: Geographe Bay (WA) around southern Australia to NSW (Moore *et al.* unpublished).

Depth/Habitat: 2–30 m. Coastal reefs, in caves, ledges and overhangs (Paxton 1975).

Life History: Little known. Nocturnal. Brooding males collected between September and November (Paxton 1975) with up to 100 eggs (Kuiter 2009).

Diet: Unknown.

Size: 11 cm (Moore et al. unpublished).

IUCN Assessment: Least Concern (Jamieson and Pollom 2016).

Protections: EPBC, CITES.

Presence in CS/OA: **NONE**. Listed as potentially occurring in CS/OA by Water Corporation (2019). However, the known distribution of this species only extends north to Geographe Bay.

Bonyhead Pipefish Nannocampus subosseus Günther, 1870

Recent synonyms and taxonomic issues: none.

Distribution: Endemic to WA: Shark Bay to Point Dempster (Esperance) (WAM database).

Depth/Habitat: 0-14 m. Shallow rocky reefs with adjacent sandy area and seagrass (at least *Amphibolis*) and tidepools (Dawson 1985; Kuiter 2009; WAM database).

Life History: Unknown.

Diet: Unknown.

Size: 12 cm (Dawson 1985).

IUCN Assessment: Least Concern (Pollom 2016d).

Protections: EPBC, CITES.

Presence in CS/OA: **NONE**, but possible. Listed as potentially occurring in CS/OA by Water Corporation (2019). Not known from CS/OA, but three specimens from nearby Rottnest Island are in WAM collection so it is possible that it occurs in the area. If so, it is most likely to occur around Garden and Carnac islands. Only known from very few vouchers.

Ruby Seadragon Phyllopteryx dewysea Stiller, Wilson & Rouse, 2015

Recent synonyms and taxonomic issues: none.

Distribution: Endemic to Western Australia: Cottesloe to Great Australian Bight (Moore *et al.* unpublished).

Depth/Habitat: 51-72 m. Found among deep water sponge and algae communities (Rouse *et al.* 2017). *Life History*: Unknown. One male carrying eggs was collected off Albany in October (WAM database). *Diet*: Unknown.

Size: 26 cm (Moore et al. unpublished).

IUCN Assessment: Data Deficient (Aylesworth and Pollom 2016a).

Protections: EPBC, CITES.

Presence in CS/OA: **NONE**. Potentially occurring in the area only because CS/OA falls within the known distribution of this species (WAM database). Little is known of this species, with most records based on beach-cast specimens. Preferred habitat is probably too deep for CS/OA to be suitable, therefore considered very unlikely.

Gunther's Pipehorse Solegnathus lettiensis Bleeker, 1860

- *Recent synonyms and taxonomic issues: Solegnathus guentheri.* Kuiter (2009) considered this to be a separate species, restricted to Western Australia, but the taxonomy is yet to be resolved.
- *Distribution*: Off Garden Island (WA) to north of Bathurst Is (NT). Elsewhere throughout parts of the Indonesian archipelago (Bray and Thompson 2020).

Depth/Habitat: 92-180 m. Offshore trawling grounds (Kuiter 2009; WAM database).

Life History: Unknown.

Diet: Unknown, presumably crustaceans.

Size: 52 cm (Kuiter 2009).

IUCN Assessment: Data Deficient (Pollom 2017e).

Protections: EPBC, CITES.

Presence in CS/OA: **NONE**. Listed as potentially occurring in CS/OA by Water Corporation (2019). However, this was likely based on two public records from the WAM database, collected in 1920 and 1946, with locality information only as 'Garden Island' and 'Coogee Beach' – both beach-cast specimens. Other records of this species are all well offshore in deep water, therefore considered unlikely in CS/OA.

6 Discussion

Australia is home to around 40% of the world's syngnathid fish species, supporting more than 130 described species (and several more undescribed) (Hamilton *et al.* 2017; Bray and Gomon 2024). Southern Australia is particularly notable, where the Syngnathidae is one of the most speciose fish families, represented by 23 genera and 50 known species, of which 90% are endemic to the southern half of the continent (Bray and Gomon 2024).

Cockburn Sound is an unusual marine habitat in south-western Australia. Large sheltered embayments are rare in the region and only Cockburn Sound and Warnbro Sound have natural deep silty basins as well as seagrass-dominated shallows, creating structural and habitat diversity that supports unique biological communities (Wilson *et al.* 1978; Hutchins 1994; Sampey *et al.* 2011). Accordingly, Cockburn Sound/Owen Anchorage is an important coastal embayment for syngnathid fishes. With 21 species now confirmed from an area of around 200 km², this region supports a remarkably high syngnathid diversity. A similar Australian temperate embayment, Gulf St Vincent in South Australia, supports 27 species but, encompassing an area of 6800 km², is 34 times larger than CS/OA (Browne *et al.* 2008). At a similar latitude, Sydney Harbour (estuary) supports 18 species of syngnathids in an area of around 55 km² (DiBattista *et al.*, 2022).

All syngnathids from CS/OA are endemic to Australia. Most (17 species) are endemic to the southern half of Australia, some as far as NSW or Queensland, others only east to South Australia. However, two species (*H. subelongatus* and *M. meraculus*) have much more restricted distributions and are endemic to the temperate west coast of Australia. Hoschke *et al.* (2023) reported 24 shallow water (< 30 m) syngnathid species from the Perth metropolitan area, but the presence of *V. margaritifer* had not been confirmed, so was not included in that total. Based on records across the broader metropolitan area, no species are solely reliant on CS/OA, although it is clearly a stronghold for some (e.g. *H. subelongatus*).

Syngnathids use all habitats within CS/OA to varying degrees, including the pelagic environment, and each habitat type supports a slightly different suite of species and/or life stages. Syngnathid fishes probably have a patchy spatial distribution across the whole area, including the vast soft-sediment basin of central Cockburn Sound. Many species raft in open water on floating vegetation and are at the whim of tides and currents, as are the post-hatching larvae and newborns. Although the greatest diversity and density of syngnathid fishes was recorded from the seagrass meadows, these habitats are probably also the best sampled. There is evidence of ontogenetic movement between habitats in some species (e.g. *S. argus*; Kendrick 2002), which is not necessarily tied to a particular season. Cockburn Sound and Owen Anchorage are inhabited by syngnathid fishes all year round and it is likely that all species are resident across the year, although abundance might fluctuate seasonally.

Most species breed in the spring and summer, but at least some species are breeding in any given month. Species with an open pouch structure often release pelagic larvae as soon as they have hatched, which enter the water column and may remain pelagic for days or weeks (Gomon and Neira 1998). The closed-pouch species (e.g. *Hippocampus*) usually brood the developing embryos for days to weeks before releasing newborn juveniles into the water column, where they likely have a truncated or absent pelagic stage (Sommer *et al.* 2012). During the pelagic stage, many larval and newborn syngnathids may be strongly influenced by currents and wind. This may facilitate movement between habitats or locations, but may also limit dispersal in species with a short pelagic stage (Kendrick 2002; Bertola *et al.* 2020).

6.1 Constraints and Limits of Study

Using data compiled from multiple sources and multiple methods, the dataset presented here is the most comprehensive and quality checked data available, providing a significant improvement on the current sparse knowledge regarding this conservation-listed family of fishes. Despite this, there are

still few records for many species. Given that these compiled data do not reflect comparable sampling methods or effort, the absolute number of records for each species, and the distribution of those records, cannot be considered quantitative and should be interpreted with caution.

Despite considerable interest in the region, there are limited published studies documenting the faunal biodiversity of CS/OA (see summaries in Johnson *et al.* 2008; Water Corporation 2019). Much of the raw biodiversity data are reduced to community level analyses or remain in unpublished reports. This is especially true for syngnathid fishes, which are of low fisheries interest, uncommon and difficult to sample. One thing that becomes clear when compiling data from various studies is that no single method of survey is likely to provide a comprehensive assessment of the spatio-temporal distribution of syngnathids in Cockburn Sound, or anywhere. Furthermore, some methods are unsuitable for syngnathid surveys – e.g. the most comprehensive baited underwater video survey in Cockburn Sound to date did not report any syngnathids (Wakefield *et al.* 2013). Even the success of trawls to sample syngnathids is strongly influenced by gear type, especially mesh size (most trawl meshes are much too large to sample syngnathids effectively), and many are not suitable at all (Kendrick and Hyndes 2003; Dodt 2005; Johnson *et al.* 2008; French *et al.* 2021a; French *et al.* 2021b). All species of pipefish are slender, and several species are 10 cm or less in length, making capture or observation extremely challenging.

Even eDNA sampling only detected three of the larger and more common species. Although the successful identification of syngnathid taxa demonstrates the efficacy of eDNA analysis in detecting target species within aquatic ecosystems, the relatively low number of syngnathid detections compared to the broader fish community raises questions about factors influencing detection specificity. The 16S_FishSyn_Short assay, while designed to target syngnathid taxa, also co-amplifies mtDNA from non-target species due to sequence similarities between syngnathids and other fish taxa. This lack of assay specificity could lead to false-negatives. Moreover, detecting syngnathid eDNA in aquatic environments is inherently challenging. Syngnathid fishes generally have lower biomass compared to other fish taxa, along with low population densities and favour cryptic habitats. Consequently, they are less prone to shedding detectable amounts of DNA into the environment (Nester *et al.* 2020). Lastly, although this is a standard approach (e.g. Nester *et al.* 2020), the 1 L water replicates collected may not have been sufficient to accurately represent the total CS/OA area, potentially limiting syngnathid eDNA detections.

6.2 Vulnerability of Syngnathid Fishes to Disturbance

Due to a range of unique life-history characteristics, these highly specialised teleosts are potentially highly susceptible to disturbance (Foster and Vincent 2004; Camins Martinez *et al.* 2023). Most species of syngnathid tend to inhabit coastal shallow waters and estuaries, which are most impacted by anthropogenic stressors. Furthermore, they are often restricted to habitats that are degraded by those stressors (e.g. seagrass; Table 3). Some syngnathid species are restricted to a single habitat species (e.g. *S. nigra* is found almost exclusively in the seagrass *Posidonia coriacea*; Table 3). Most species have naturally low population densities and, despite seemingly large distribution ranges, many species are patchily distributed. For example, despite a distribution from Cape Leeuwin to Shark Bay, *H. subelongatus* is rarely found outside the three large 'bays' with estuary-like conditions – Cockburn Sound, the Swan River estuary, and Shark Bay (see Species Accounts).

Syngnathids are specialised visual ambush predators, often with a narrow dietary niche dominated by only a few target prey types (see Species Accounts), which means that any impacts on prey populations may have a sizeable flow-on effect to syngnathids. Many species likely have relatively small home ranges, low mobility and limited dispersal (see Species Accounts), suggesting that an individual's capacity to avoid the effects of anthropogenic stressors might be low. In addition, some species are short lived (i.e. < 1-2 years), implying that even a single disturbance event could cause an entire generational failure, in the form of adult deaths (from physical, chemical, habitat or dietary

impacts), loss of larval/juvenile recruitment (from deaths or unsuitable settlement conditions), or disruption of breeding.

Possibly due to the specialised male care in syngnathid fishes, some species have a complex mating system, including ritualised courtships and pair bonds to reduce interbrood intervals and maximise reproductive output (e.g. Kvarnemo *et al.* 2000; Jones and Avise 2001; Moore 2001; Kvarnemo *et al.* 2021). Many species have relatively low fecundity. For example, most species present in Cockburn Sound have between 25 and 200 eggs per brood (see Species Accounts). Brooding males exchange nutrients and gases to the developing embryos so, unlike pelagic spawning species, disturbance to a brooding male may also affect the next generation. Most species have pelagic larvae which could place larvae into yet another disturbed environment (the water column), or, conversely, enhance opportunities for population recovery following disturbance via recruitment from undisturbed populations. Most syngnathid species in CS/OA are spring-summer breeders but peak abundance for many species may be during autumn-winter when generations overlap (Martin-Smith 2003). Note that not all syngnathid species breed in CS/OA during spring and summer. These reproductive attributes suggest that local syngnathids may have limited resilience to poorly timed construction activities, but the extent to which localised impacts might have on a whole species is unknown.

6.3 The Effects of Infrastructure Development on Syngnathid Fishes

Coastal/inshore industrial development involving infill and/or extensive dredging and habitat modification is highly likely to impact local syngnathid populations. After climate change, habitat loss, primarily through coastal infrastructure development and destructive fishing methods, remains one of the most significant pressures facing shallow marine environments supporting syngnathids in Australia (Vincent *et al.* 2011; Harasti 2016; Trebilco *et al.* 2022).

Few studies have directly examined the effects of infrastructure development on syngnathids specifically, but several review papers have considered the overall risks to these fishes across a range of threats. The global extinction risk for all known syngnathids was summarised by Pollom *et al.* (2021). They noted that 6% of syngnathids globally are threatened with extinction and, given that many species were assessed as Data Deficient, that number is likely to be an underestimate. The species recorded from CS/OA fared well in the assessments, with 18 considered to be Least Concern, although four species (*P. eques, P. taeniolatus, S. argus* and *S. nigra*) had evidence of decreasing population sizes across their whole distribution (see Species Accounts). Three species were assessed as Data Deficient (*H. subelongatus, I. australis*, and *M. meraculus*).

Pollom *et al.* (2021) argued that indirect fishing effects (e.g. bycatch and habitat loss, especially from trawling) and direct fishing (e.g. for traditional medicine and aquaria) were the main pressures globally, but large localised habitat loss through infrastructure development, dredging and pollution poses a significant extinction risk for most syngnathids. In Australia, the detrimental impact of fishing bycatch on syngnathid fishes is well documented (e.g. Dodt 2005; Martin-Smith and Vincent 2006; Burnell *et al.* 2015). While the current levels of extraction of several syngnathid species harvested in the region as part of the Marine Aquarium Fish Managed Fishery were assessed to pose negligible risk, Smith *et al.* (2022) determined that life-history traits make them highly vulnerable to over-exploitation. The impact of infrastructure development on syngnathids in Australia is less well documented, at least rigorously. It is likely that habitat loss from development will also exacerbate the effects of fishing pressure (Harasti 2016).

In addition to habitat loss, other anthropogenic environmental changes such as water quality, turbidity, noise or invasive species may have direct and/or indirect effects on either syngnathids or the prey species they target.

6.3.1 Habitat loss

Habitat loss and fragmentation has the potential to impact the populations of all syngnathid fishes, especially those with low abundance. In particular, the loss of benthic habitats that support the highest syngnathid diversity, especially seagrasses, probably impacts both biodiversity and populations of syngnathids the most (e.g. Pollom *et al.* 2021). However, species with habitat specialisation are vulnerable to loss of habitats such as shallow rocky reefs that are restricted and heterogeneous (Harasti 2016). In addition, impacts to non-benthic habitats (i.e. the water column), generally in the form of reduced water quality may also be considered as a 'loss' of suitable habitat, especially for early life stages (see below).

Habitat damage and fragmentation resulting from development, infill 'reclamation', mooring, anchoring, fishing and other anthropogenic pressures has variable impacts on marine fauna (Bell and Westoby 1986; Bell *et al.* 2001) and is still poorly understood across the Syngnathidae. For example, during extensive port development in Malaysia, the population size of the seahorse *Hippocampus kuda* reportedly declined with substantial loss of seagrass habitat (McKenzie *et al.* 2006–2010 as cited in Vincent *et al.* 2011). Destruction of seagrass habitat for two marina developments in Florida, led to measurable population declines of two species of pipefishes (Masonjones *et al.* 2010). Similarly, Stewart (2015) reported that a loss of *Zostera* habitat negatively impacted populations of *S. nigra* in New Zealand estuaries. Areas damaged by unsustainable fishing practices in Philippines coral reefs supported very low densities of a seahorse (Marcus *et al.* 2007). In fragmented seagrass habitats, preferences for large or small patches and for the edges or middle of patches varied among many species of teleosts, including syngnathids (Jelbart *et al.* 2006). Yet in the seahorse *Hippocampus erectus*, habitat structural complexity did not affect foraging success (James and Heck 1994).

Increased disturbance (measured as wave height) in seagrass meadows led to a reduction in the number of adult *Stigmatopora* pipefishes in Port Phillip Bay, Victoria (Moran *et al.* 2003). The same disturbance also resulted in suspension of post-larval and juvenile *Stigmatopora* into the plankton. The authors argued that the resuspension of small individuals and resulting secondary planktonic transport may increase risk of death in post-settlement fishes, possibly through predation, physical injury or lack of suitable prey. Depending on oceanographic conditions, resuspension could either move individuals further offshore and away from suitable habitat or potentially facilitate recolonisation of disturbed areas (Moran *et al.* 2003).

An endangered seahorse (*Hippocampus whitei*) in NSW that is not seagrass-associated suffered catastrophic population declines of between 83 and 97%, which was strongly correlated to a similar degree of loss of the species preferred habitat, especially soft corals and sponges (Harasti 2016). The author reasoned that population recovery was impossible unless habitat remediation was undertaken.

6.3.2 Sediment disturbance and water quality

Changes to water quality such as sediment disturbance, runoff, spills and pollution may affect syngnathids (and other fauna), perhaps at concentrations lower than those accepted under human health guidelines (e.g. Sun *et al.* 2019).

The disturbance of contaminated marine sediments can create indirect deleterious impacts on marine fauna, including the release of harmful chemicals and hypoxic events (Knott *et al.* 2009; Rangel-Buitrago *et al.* 2023). Dredging and other sediment disturbing activities can lead to low oxygen conditions, especially in systems with high nutrient and pollution loads (Commonwealth of Australia 2009; Thompson *et al.* 2021). However, there are few studies demonstrating these impacts on syngnathid fishes specifically. Lower population sizes of several species of pipefishes have been reported from polluted areas compared to similar habitats that are less-polluted (Livingston 1984; Power and Attrill 2003). The effects of hypoxia are usually considered in terms of eutrophication and algal blooms but hypoxia itself has been shown to directly impede feeding success in two species of

pipefishes (Ripley and Foran 2007). This laboratory-based experiment found that feeding was affected as long as the hypoxic conditions prevailed. Hypoxic conditions also negatively affected the intrabrood pouch growth of embryos but not their survival in the pipefish *Syngnathus typhle* (Braga Gonçalves 2010).

Contaminants and heavy metals accumulated in marine sediments are often liberated during marine developments, and CS/OA has historically been documented as a repository of some of these contaminants (e.g. BMT 2018). Seahorses have been shown to act as bioaccumulators of heavy metals, hydrocarbons, microplastics and other chemicals, with resultant deleterious effects on growth, reproduction and overall health (Delunardo et al. 2015; Chipari-Gomes et al. 2017; Sun et al. 2019; Liu et al. 2022a). For example, the effects of the antifouling chemical Tributyltin (TBT) on marine invertebrates (especially gastropods) is relatively well studied (e.g. Shute and Carey 2006); however, this chemical also has significant health impacts on seahorses, with exposure leading to liver damage including tumorigenesis, decreased immunity and increased measures of overall stress (Tang et al. 2021). Similarly, Bisphenol A (BPA) bioaccumulation was positively correlated with exposure in a seahorse, with gene expression effects leading to ovarian cell death and significantly inhibited maintenance of the brood pouch at low environmentally relevant concentrations (Liu et al. 2021). Heavy metals, some of which have been known to be in elevated concentrations in Cockburn Sound (BMT 2018), have been shown to decrease immunity, metabolic efficiency, and increase cell death in seahorses (Liu et al. 2022b). Importantly, the impact of these liberated contaminants on seahorses is likely to occur at concentrations lower than those tolerated under human health guidelines (Sun et al. 2019).

6.3.3 Turbidity

Reduced visibility resulting from increased turbidity could be expected to affect the efficiency of predation by syngnathid fishes, reduce the capacity to detect and avoid predators, as well as disrupt the complex mating systems of these fishes.

Syngnathids have eye structures supporting high visual acuity (Collin and Collin 1999). This is essential for these fishes, which are visual predators that feed by tracking individual crustaceans at short range and sucking them into a tubular snout (Bergert and Wainwright 1997). Mosk *et al.* (2007) demonstrated that the spectral sensitivities of the two most common species in CS/OA (*H. subelongatus* and *S. argus*) were consistent with a visual system adapted to detecting mobile prey across the range of wavelengths found among the background of 'green-water' habitats of seagrass meadows. Light conditions outside these ranges could be expected to lead to reduced feeding success and increased energetic costs of both adults and larvae/juveniles as demonstrated in other fishes (Higham *et al.* 2015). Indeed, low light conditions impeded predation success in the seahorse *H. erectus* (James and Heck 1994) and the pipefishes *Syngnathus fuscus* and *S. floridae* (Ripley and Foran 2007). Increased turbidity is also associated with increased predation risk in both adult and larvae/juvenile fishes because the reactive distance for predator avoidance is reduced (see Higham *et al.* 2015).

The high visual acuity of syngnathid fishes is also important for their elaborate reproductive biology. Most species have ritualised behaviours involving specific body movements and postures as well as colour changes (Vincent 1995; Moore 2001; Sundin *et al.* 2010; Rosenqvist and Berglund 2011). Any disturbance, such as high suspended solids, that hampers light penetration or visibility is likely to negatively impact mate choice, sexual selection and the mating system in general, as well as increase the energetic costs of finding a mate (Sundin *et al.* 2010; Sundin *et al.* 2016; Sundin *et al.* 2017).

Other effects of elevated turbidity on fishes include structural damage to gills resulting in impeded respiratory function (e.g. Wong *et al.* 2013), however prolonged or greater exposure may be tolerated

for species such as temperate syngnathids, which are likely adapted to silty conditions (Lowe *et al.* 2015). No specific data on the effects of elevated turbidity exist for syngnathid fishes.

6.3.4 Noise

Anthropogenic noise can have significant effects on marine fishes, including auditory damage, swimbladder effects, reduced reproductive success, physiological stress and even death (Peng *et al.* 2015; Popper and Hawkins 2019; de Jong *et al.* 2020). Although the response may vary, detrimental impacts can be caused by continuous (e.g. motors and pumps), intermittent (e.g. vessels) and impact (e.g. pile driving) noise (Popper and Hawkins 2019). Navigation exclusion areas have been proposed as a potential and partial mitigation tool (Palma *et al.* 2019a).

Syngnathids are known to be as impacted by noise as much as other fish. Significant stress responses ranging from behavioural changes, weight and condition loss, raised cortisol levels, increased parasite loads and habitat/home range abandonment in response to anthropogenic noise pollution have been documented in syngnathids in both field and laboratory settings (Anderson *et al.* 2011; Palma *et al.* 2019b).

Sounds are produced by at least some syngnathids. Although the role of these sounds is poorly understood, they likely have a communication function during feeding, form an important component of courtship displays and act as a deterrence mechanism against predation (Oliveira *et al.* 2014; Lim *et al.* 2015). Significant deleterious effects of masking of marine animals sounds by anthropogenic noise is well documented (see review in Popper and Hawkins 2019).

6.3.5 Invasive species

The effect of invasive species on syngnathids is under-studied but some examples exist for both exotic vegetation and exotic animals. Habitats dominated by an invasive alga *Caulerpa taxifolia* supported significantly smaller populations of pipefish species compared to native seagrasses in both Queensland (Burfeind *et al.* 2009) and New South Wales (York *et al.* 2006). The nature of the impact may not always be negative. For example, pipefish population densities did not differ between native macrophytes and an invasive alga (*Myriophyllum spicatum*) in Louisiana, U.S.A. (Duffy and Baltz 1998). A native seahorse was reported utilising meadows of an invasive seagrass (*Halophila stipulacea*) in the Caribbean (Pinault *et al.* 2018). However, populations of the seahorse *H. abdominalis* suffered substantial losses in Tasmania, coinciding with the arrival of two exotic marine invertebrates: the seastar *Asterias amurensis* and the crab *Carcinus maenas* (Martin-Smith and Vincent 2005). Syngnathid fishes may also face increased predation risk from invasive species; for example they make up a small but detectable part of the diet of the invasive lionfish *Pterois volitans* in the Caribbean (Morris and Akins 2009).

6.4 Mitigation Actions

While guidance for mitigating the effects of fishing and trade and the effects of fishing bycatch on seahorses in particular are established (e.g. Vincent *et al.* 2011; Pollom *et al.* 2021), there is little written specifically on mitigation efforts to protect syngnathids from development. However, given that the primary risks to these fishes posed by development are similar to those of the marine environment generally (e.g. habitat loss/degradation), mitigation efforts to address those will generally enhance protection for syngnathids (Foster and Vincent 2004; Vincent *et al.* 2011). Significantly, Shokri *et al.* (2009) demonstrated that ranking the conservation value of seagrass beds based on the density and diversity of syngnathids alone, and selecting marine protected areas to represent syngnathid fishes, would concurrently protect other species.

Syngnathid fishes have several life history traits that make them vulnerable to disturbance (see Section 6.2). Vulnerabilities may vary between species and may present across all life stages and across seasons of the year, especially during breeding. This poses a challenge in CS/OA because the syngnathid diversity is very high, with a similarly high diversity in habitat preferences and life histories (including diet and breeding) among the species present. While avoiding the impacts of development on syngnathid fishes is the ideal outcome (Pollom *et al.* 2021), attempting to find a 'one size fits all' approach in CS/OA is likely not achievable.

6.4.1 Habitat Remediation/Restoration/Creation

Where population declines occur as a consequence of habitat loss or degradation, recovery may be impossible unless habitat remediation or restoration is undertaken (e.g. Harasti 2016; McLeod *et al.* 2019; Ayvazian *et al.* 2020; Sievers *et al.* 2024). Restored seagrass habitats can support syngnathids (McLeod *et al.* 2019; Hardison *et al.* 2023) and provide fitness benefits for resident fauna (see Sievers *et al.* 2024). Other restored habitats, such as oyster beds, have been shown to support syngnathids (Ayvazian *et al.* 2020).

Some species might exploit created habitats resulting from infrastructure development, especially when designed using ecological engineering principles (sensu Chapman and Underwood 2011). However, this may be less successful for pipefishes, which are seagrass specialists, than for seahorses, which frequently make use of pylons, moorings and other anthropogenic infrastructure. Artificial rockwalls on the east side of CS/OA, such as South Mole Fremantle and Woodman Point support a diverse array of reef dwelling syngnathids for whom natural reef habitat is limited (iNaturalist observations). However, diversity may be reduced in areas with ongoing disturbance such as vessel activity that results in high turbidity (e.g. from propellers and thrusters; Moore, pers. obs.). Artificial substrates and 'seahorse hotels' have been used to respond to population declines in significantly atrisk endangered seahorses (Claassens et al. 2018; Simpson et al. 2020) but long-term success likely still depends on natural habitat restoration (Sievers et al. 2024). Again, such structures are suited to reef species rather than seagrass inhabitants, which dominate the syngnathid diversity in CS/OA. These structures also probably act as fish aggregating structures rather than increasing biomass per se (Correia et al. 2015), which may make the concentrated fishes even more vulnerable to human disturbance (Claassens et al. 2018). There is limited evidence for successful large-scale artificial replacements for seagrasses (but see Correia et al. 2013), but seagrass meadows have been successfully restored, including in Cockburn Sound (e.g. Sinclair et al. 2013), and may support syngnathids (McLeod et al. 2019; Hardison et al. 2023).

6.4.2 Relocation of impacted syngnathids

The relocation of individuals that are likely to be directly impacted by infrastructure development is a popular approach, especially for threatened fauna. The suitability of a species for relocation is determined by a range of life history parameters including, *inter alia*, habitat associations and suitability of release sites, ease of catching/trapping, resilience to handling/transport, mobility and likelihood of retention at release site.

Syngnathids are highly cryptic and, in Cockburn Sound, most species are either in low population densities or are unreliably sampled (see Species Accounts). so relocation would have limited applicability. For example, Kendrick and Hyndes (2003) showed that trawl methods and mesh sizes affected the species caught. Typically common species may be caught in reasonable numbers (e.g. Kendrick and Hyndes 2003; Dodt 2005; Johnson *et al.* 2008; French *et al.* 2021a; French *et al.* 2021b) with rare or hard to catch species often missed. In fact, even one of the most common species in Cockburn Sound (*H. subelongatus*) was often missed using these methods (Dybdahl 1979; Ayvazian and Hyndes 1995; Vanderklift 1996, Table 1). Trawling may not be ideal for relocations given the

trauma of collection. However, syngnathids can be quite robust to trawling and handling (Kendrick pers. obs., Moore pers. obs.) and this approach might be worth considering where catastrophic habitat loss and death of individuals is inevitable (e.g. dredged channels or spoil and infill sites).

Manual diver-based collection of individuals has been used in the past, but apparently only for seahorses, which are larger and less cryptic than most pipefishes (e.g. DPAW 2014; Harasti 2016). Relocation is certainly warranted for endangered or threatened fauna (e.g. *Hippocampus whitei* in NSW; Harasti 2016; Harasti *et al.* 2022). However, in Cockburn Sound the only species that might be suitable for such relocation effort is *H. subelongatus*, which has no evidence of population decline. The value of relocating one non-threatened species of syngnathid is philosophically subjective when potentially another 20 species of syngnathid, some probably rarer, are not possible to collect. Let alone the dozens of other fishes or the hundreds of species of marine invertebrates and flora that are not as 'iconic' or 'charismatic' and whose conservation status has never been examined. Despite that, syngnathids are protected under the EPBC Act, so any efforts are worthwhile. Indeed, seahorse translocations garner a great deal of community interest:

- https://www.abyss.com.au/en/blog/viewpost/151/relocation-of-the-seahorses-at-manly
- <u>https://manlyobserver.com.au/clontarf-pool-upgrade-in-progress-after-seahorses-relocated/</u>
- <u>https://www.edenmagnet.com.au/story/7894731/eight-adorable-seahorses-found-and-relocated-in-snug-cove-eden/</u>
- <u>https://www.australiangeographic.com.au/news/2010/11/seahorses-return-to-sydney-swimming-baths/</u>

Such manual diver-based collections would not be feasible in seagrass habitats, especially for low density, cryptic species and any attempt would likely be superficial. For example, in 18 dedicated syngnathid surveys using SCUBA on natural mixed reef/seagrass habitat along the Kwinana Shelf in 2022, WAM recorded only two seahorses and no pipefishes, pipehorses or seadragons (see Section 5.2.2, Appendix 2).

Diver-based collection and relocation of seahorses (but not pipefishes) from around jetties in or around the footprint area of the Westport development would be possible. Jetties and other artificial structures are favoured by *H. subelongatus* so populations are often concentrated around these areas. The same is not true for other species of Syngnathidae. Recent WAM surveys suggest that these jetties may each hold H. subelongatus populations of 3-15 individuals during summer (Moore, WAM, unpublished data). If relocation is undertaken, collections would need to be done immediately before impact because individuals will probably recolonise as either adults or juveniles. Relocation efforts should avoid breeding season (Oct-Mar) to prevent breaking the important pair bonds and mating system of this species. There is no need to provide artificial habitat at any release site (c.f. Correia et al. 2013; Correia et al. 2015; Harasti et al. 2022). Firstly, based on past and recent surveys of the impact footprint, the number of individuals to be relocated is probably low. Secondly, there are many suitable release sites away from the impact area that already support mature benthic invertebrate communities and individual seahorses probably move between them naturally (Moore, unpublished data). During extensive prior experiments examining reproduction biology and mating system of H. subelongatus, it proved robust to being handled, transported and moved to new areas (Jones et al. 1998; Kvarnemo et al. 2000; Moore 2001; Kvarnemo et al. 2006, Moore, pers. obs.). Adding a relatively small number of individuals outside of the breeding season, when pair bonds are probably not maintained (Kvarnemo et al. 2000) should not impact the complex mating system. Based on this combined evidence, relocation is likely to be successful.

7 Conclusions/Recommendations

Cockburn Sound and Owen Anchorage support a diverse syngnathid fauna. At least for some species the area maintains substantial larval, juvenile and adult populations across the year and is an area where breeding of most, if not all, species occurs. With a high diversity of species, which are patchily distributed in suitable habitat across the whole area, and present and breeding across the whole year, impacts to syngnathids are inevitable. Although direct data on impacts on populations in CS/OA are lacking, evidence from elsewhere suggests that proponents should anticipate that syngnathid populations in CS/OA are very likely to be impacted detrimentally by the development of Westport and its ongoing operations. These detrimental impacts are likely through direct interaction, habitat loss, reduced water quality and increased turbidity and noise. A risk from introduced pests is also possible.

While some impact is inevitable, it is not clear whether these impacts may have consequences for the broader populations of syngnathids outside CS/OA. All species are distributed in suitable habitats across the Perth metropolitan area, and beyond, although the abundance and density of these populations are poorly known. It is also not clear how far the impacts could reach. It is likely that some of the impacts will be localised or temporary and only affect a subset of the population. Other impacts might be far-reaching or long lasting, affecting much of the CS/OA populations.

Finally, CS/OA is likely to be the site of continued heavy industry development and this study should only be seen as the first step in gaining the knowledge needed to conserve the region's syngnathid fishes. While compiling and improving the existing knowledge on syngnathids in the CS/OA region, this study is far from comprehensive. The ecology and biology of most species remain poorly understood, and four are listed as Data Deficient. More work is needed on these fishes in relation to their future management and conservation given the continued industrial development and activity within CS/OA.

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9 References

- Alberdi, A., Aizpurua, O., Gilbert, M. and Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution* **9**: 134–147.
- Anderson, P.A., Berzins, I.K., Fogarty, F., Hamlin, H.J. and Guillette, L.J. (2011). Sound, stress, and seahorses: The consequences of a noisy environment to animal health. *Aquaculture* **311**: 129–138.
- Atlas of Living Australia. (2024). Atlas of Living Australia. http://www.ala.org.au.
- Austin, D. and Pollom, R. (2016a). *Stipecampus cristatus* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T87568608A115513663.
- Austin, D. and Pollom, R. (2016b). Urocampus carinirostris (errata version published in 2017). The IUCN Red List of Threatened Species **2016**: e.T65375397A115432400.
- Aylesworth, L. and Pollom, R. (2016a). *Phyllopteryx dewysea* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T87568739A115514038.
- Aylesworth, L. and Pollom, R. (2016b). Pugnaso curtirostris. The IUCN Red List of Threatened Species 2016: e.T65373532A67621208.
- Ayvazian, S., Gerber-Williams, A., Grabbert, S., Miller, K., Hancock, B., Helt, W., Cobb, D. and Strobel, C. (2020).
 Habitat benefits of restored oyster reefs and aquaculture to fish and invertebrates in a coastal pond in Rhode Island, US. *Journal of Shellfish Research* 39: 563–587.
- Ayvazian, S.G. and Hyndes, G.A. (1995). Surf-zone fish assemblages in south-western Australia: do adjacent nearshore habitats and the warm Leeuwin Current influence the characteristics of the fish fauna? *Marine Biology* **122**: 527–536.
- Baker, J. (2006). Chapter 4.22 Syngnathid Fishes pp. 469–519. In: S. McClatchie, Middleton, J., Pattiaratchi, C., Currie, D. and Kendrick, G. (Ed.). *The South-West Marine Region: Ecosystems and Key Species Groups*. Report prepared for the National Oceans Office, Canberra.
- Baker, J.L. (2002). Summary of Western Australian Sighting Data to September 2002. Public Report. Dragon Search Community-Based Monitoring Project. 30 pp.
- Baker, J.L. (2009). Dragon Search Public Report Summary of National Sighting Data, 1990 to 2005. Report for Dragon Search Community Based Monitoring Program. Reef Watch. Adelaide, South Australia. 61 pp.
- Bell, J. and Westoby, M. (1986). Variation in seagrass height and density over a wide spatial scale: Effects on common fish and decapods. *Journal of Experimental Marine Biology and Ecology* **104**: 275–295.
- Bell, S.S., Brooks, R.A., Robbins, B.D., Fonseca, M.S. and Hall, M.O. (2001). Faunal response to fragmentation in seagrass habitats: implications for seagrass conservation. *Biological Conservation* **100**: 115–123.
- Bergert, B.A. and Wainwright, P.C. (1997). Morphology and kinematics of prey capture in the syngnathid fishes *Hippocampus erectus* and *Syngnathus floridae*. *Marine Biology* **127**: 563–570.
- Bertola, L.D., Boehm, J.T., Putman, N.F., Xue, A.T., Robinson, J.D., Harris, S., Baldwin, C.C., Overcast, I. and Hickerson, M.J. (2020). Asymmetrical gene flow in five co-distributed syngnathids explained by ocean currents and rafting propensity. *Proceedings of the Royal Society B* **287**: 20200657.
- Bessell, T.J., Appleyard, S.A., Stuart-Smith, R.D., Johnson, O.J., Ling, S.D., Heather, F.J., Lynch, T.P., Barrett, N.S. and Stuart-Smith, J. (2023). Using eDNA and SCUBA surveys for detection and monitoring of a threatened marine cryptic fish. *Aquatic Conservation: Marine and Freshwater Ecosystems* 33: 431–442.
- Bessey, C., Depczynski, M., Goetze, J.S., Moore, G., Fulton, C.J., Snell, M., Parsons, S.K., Berry, O. and Wilson, S. (2023). Cryptic biodiversity: A portfolio-approach to coral reef fish surveys. *Limnology and Oceanography: Methods* 21: 594–605.
- Bleeker, P. (1860). Twaalfde bijdrage tot de kennis der vischfauna van Amboina. Acta Societatis Regiae Scientiarum Indo-Neêrlandicae 8: 1–4.
- BMT (2018). Cockburn Sound-Drivers-Pressures-State-Impacts-Responses Assessment 2017 Final Report. Prepared for Department of Water and Environmental Regulation, the Kwinana Industries Council, the City of Rockingham and the City of Kwinana on behalf of the Cockburn Sound Management Council
- Braga Gonçalves, I. (2010). *Egg size evolution and paternal care in pipefishes*. PhD Thesis, University of Gothenburg. Gothenburg, Sweden. 41 pp.
- Bray, D.J. and Gomon, M.F., (eds.). (2024). *Fishes of Australia*. Museums Victoria and OzFishNet. <u>http://fishesofaustralia.net.au/</u>. Retrieved 10 March 2024.
- Bray, D.J. and Thompson, V.J. (2020). *Solegnathus lettiensis. In. Fishes of Australia*. <u>https://fishesofaustralia.net.au/home/species/1470</u>. Retrieved 10 March 2024.
- Bray, D.J. and Thompson, V.J. (2022). *Pugnaso curtirostris. In. Fishes of Australia*. <u>https://fishesofaustralia.net.au/home/species/3128</u>. Retrieved 10 March 2024.

- Browne, R.K., Baker, J.L. and Connolly, R.M. (2008). Chapter 13. Syngnathids: seadragons, seahorses, and pipefishes of Gulf St Vincent. pp. 162–176. In: S. Shepherd (Ed.). *Natural History of Gulf St Vincent*. Royal Society of South Australia, Adelaide.
- Browne, R.K. and Smith, K. (2007). A new pipefish, *Stigmatopora narinosa* (Syngnathidae) from South Australia. *Memoirs of the Museum of Victoria* **64**: 1–6.
- Burfeind, D.D., Tibbetts, I.R. and Udy, J.W. (2009). Habitat preference of three common fishes for seagrass, *Caulerpa taxifolia*, and unvegetated substrate in Moreton Bay, Australia. *Environmental Biology of Fishes* **84**: 317–322.
- Burnell, O., Barrett, S., Hooper, G., Beckmann, C., Sorokin, S. and Noell, C. (2015). Spatial and temporal reassessment of by-catch in the Spencer Gulf Prawn Fishery. Report to PIRSA Fisheries and Aquaculture. South Australian Research and Development Institute (Aquatic Sciences), Adelaide.
- Callahan, B., McMurdie, P. and Holmes, S. (2017). Exact sequence variants should replace operational taxonomic units in marker gene data analysis. *ISME Journal* **11**: 2639–2643.
- Camins Martinez, E., Stanton, L.M., Correia, M.J. and Vincent, A.C.J. (2023). *Comprehensive review of advances in life history of 35 seahorse species, drawn from community science*. Fisheries Centre Research Report 31. 107 pp.
- Carlyle, C. and Pollom, R. (2016a). *Stigmatopora argus* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T88342897A115514252.
- Carlyle, C. and Pollom, R. (2016b). *Stigmatopora nigra* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T88343011A115515048.
- Castelnau, F.L., de (1872a). Contribution to the ichthyology of Australia. 2. Note on some South Australian fishes. Proceedings of the Zoological and Acclimatisation Society of Victoria 1: 243–248
- Castelnau, F.L., de (1872b). Contribution to the ichthyology of Australia. No. I.—The Melbourne fish market. *Proceedings of The Zoological And Acclimatisation Society of Victoria* 1: 29–242
- Castelnau, F.L., de (1873). Contribution to the ichthyology of Australia. Nos. III thru IX. *Proceedings of the Zoological Acclimisation Society of Victoria* **2**: 37–158.
- Castelnau, F.L., de (1875). Researches on the fishes of Australia. Philadelphia Centennial Expedition of 1876. Intercolonial Exhibition Essays, 1875-6. pp. 1–52.
- Castelnau, F.L., de (1879). Essay on the ichthyology of Port Jackson. *Proceedings of the Linnean Society of New* South Wales **3**: 347–402.
- Chapman, M. and Underwood, A. (2011). Evaluation of ecological engineering of "armoured" shorelines to improve their value as habitat. *Journal of Experimental Marine Biology and Ecology* **400**: 302–313.
- Chenoweth, S.F., Hughes, J.M. and Connolly, R.C. (2002). Phylogeography of the pipefish, *Urocampus carinirostris*, suggests secondary intergradation of ancient lineages. *Marine Biology* **141**: 541–547.
- Chipari-Gomes, A.R., Delunardo, F.A.C., Sadauskas-Henrique, H., Mota, S.B. and de Almeida-Val, V. (2017).
 Genotoxic and biochemical responses triggered by polycyclic aromatic hydrocarbons in freshwater and marine fish: Tambaqui and seahorse as bioindicators pp. 278–304. In: M. Larramendy (Ed.).
 Ecotoxicology and Genotoxicology Non-traditional Aquatic Models. Royal Society of Chemistry, eBook.
- Chittleborough, R.G. (1970). *Conservation of Cockburn Sound (Western Australia). A case study.* Australian Conservation Foundation. Special Publication. 27 pp.
- Claassens, L., Booth, A.J. and Hodgson, A.N. (2018). An endangered seahorse selectively chooses an artificial structure. *Environmental Biology of Fishes* **101**: 723–733.
- Collin, S.P. and Collin, H.B. (1999). The foveal photoreceptor mosaic in the pipefish, *Corythoichthyes paxtoni* (Syngnathidae, Teleostei). *Histology and Histopathology* **14**: 369–382.
- Commonwealth of Australia (2009). *National Assessment Guidelines for Dredging*. Commonwealth of Australia, Canberra. 92 pp.
- Connolly, R.M., Melville, A.J. and Keesing, J.K. (2002a). Abundance, movement and identification of individual leafy sea dragons, *Phycodurus eques* (Pisces: Syngnathidae). *Marine and Freshwater Research* **53**: 777–780.
- Connolly, R.M., Melville, A.J. and Preston, K.M. (2002b). Patterns of movement and habitat use by leafy seadragons tracked ultrasonically. *Journal of Fish Biology* **61**: 684–695.
- Correia, M., Koldewey, H., Andrade, J.P. and Palma, J. (2015). Effects of artificial holdfast units on seahorse density in the Ria Formosa lagoon, Portugal. *Journal of Experimental Marine Biology and Ecology* **471**: 1–7.
- Correia, M., Palma, J., Koldewey, H. and Andrade, J.P. (2013). Can artificial holdfast units work as a habitat restoration tool for long-snouted seahorse (*Hippocampus guttulatus* Cuvier)? *Journal of Experimental Marine Biology and Ecology* **448**: 258–264.

- Dawson, C.E. (1977). Review of the Indo-Pacific pipefish genus *Lissocampus* (Syngnathidae). *Proceedings of the Biological Society of Washington* **89**: 599–620.
- Dawson, C.E. (1984). Synopsis of Australian pipefishes usually referred to the syngnathine (tail-pouch) genera *Syngnathus, Leptonotus* and *Histogamphelus. Memoirs of Museum Victoria* **45**: 71–123.
- Dawson, C.E. (1985). *Indo-Pacific Pipefishes (Red Sea to the Americas)*. Gulf Coast Research Laboratory Ocean Springs (Mississippi). 230 pp.
- de Jong, K., Forland, T.N., Amorim, M.C.P., Rieucau, G., Slabbekoorn, H. and Sivle, L.D. (2020). Predicting the effects of anthropogenic noise on fish reproduction. *Reviews in Fish Biology and Fisheries* **30**: 245–268.
- Delunardo, F., Carvalho, L., Silva, B., Galão, M., Val, A.L. and Chippari-Gomes, A.R. (2015). Seahorse (*Hippocampus reidi*) as a bioindicator of crude oil exposure. *Ecotoxicology and Environmental Safety* **117**: 28–33.
- DiBattista, J., West, K., Hay, A., Hughes, J., Fowler, A. and McGrouther, M. (2021). Community-based citizen science projects can support the distributional monitoring of fishes. *Aquatic Conservation Marine and Freshwater Ecosystems* **31**: 3580–3593.
- Dodt, N. (2005). Syngnathids in the East Coast Trawl Fishery: a review and trawl survey. Department of Primary Industries and Fisheries, Queensland. 31 pp.
- DPAW (2014) Refurbishment of Preston Point Wharf. Determination of Development Application SRT 5696. Part 5. Department of Defense through Sinclair Knight Mertz. [Department of Parks and Wildlife].
- Dragon Search (2003). Summary of Western Australian Sighting Data to September 2002. Internal Report (not for publication). Dragon Search Community-Based Monitoring Project. 32 pp.
- Duffy, K.C. and Baltz, D.M. (1998). Comparison of fish assemblages associated with native and exotic submerged macrophytes in the Lake Pontchartrain estuary, USA. *Journal of Experimental Marine Biology and Ecology* **223**: 199–221.
- Duncker, G. (1909). Pisces, 1. Teil : Syngnathidae pp. 231–250. In: W. Michaelsen and Hartmeyer, R. (Ed.). *Die Fauna Südwest-Australiens. Jena : G. Fischer Vol. 2.*
- Dybdahl, R.E. (1979). Technical report on fish productivity. An assessment of the marine faunal resources of *Cockburn Sound*. Department of Conservation and Environment. Report No. 4. Perth, Western Australia. 96 pp.
- Edgar, G.J. and Shaw, C. (1995). The production and tropic ecology of shallow-water fish assemblages in southern Australia. III. General relationships between sediments, seagrasses, invertebrates and fishes. *Journal of Experimental Marine Biology and Ecology* **194**: 107–131.
- Edgar, R. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26: 2460–2461.
- Edgar, R.C. (2016). UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. *bioRxiv*: 081257.
- Forsgren, K.L. and Lowe, C.G. (2006). The life history of weedy seadragons, *Phyllopteryx taeniolatus* (Teleostei : Syngnathidae). *Marine and Freshwater Research* **57**: 313–322.
- Foster, S.J. and Vincent, A.C.J. (2004). Life history and ecology of seahorses: implications for conservation and management. *Journal of Fish Biology* **65**: 1–61.
- French, B., Wilson, S., Holmes, T., Kendrick, A., Rule, M. and Ryan, N. (2021a). Comparing five methods for quantifying abundance and diversity of fish assemblages in seagrass habitat. *Ecological Indicators* 124: 107415.
- French, B., Wilson, S., Kendrick, A. and Rule, M. (2021b). The mesh size effect: counting long thin fish in seagrass. *Fisheries Research* **242**: 106019.
- Fuji, I. and Pollom, R. (2016a). *Histiogamphelus cristatus*. *The IUCN Red List of Threatened Species* **2016**: e.T65368242A67622410.
- Fuji, I. and Pollom, R. (2016b). *Maroubra perserrata*. *The IUCN Red List of Threatened Species* **2016**: e.T65370433A67622585.
- Gaither, M., DiBattista, J., Leray, M. and von der Heyden, S. (2021). Metabarcoding the marine environment: from single species to biogeographic patterns. *Environmental DNA* **4**: 3–8.
- Gomon, M.F. and Neira, F.J. (1998). Syngnathidae: Pipefishes and seahorses. pp. 122-131. In: F.J. Neira, Miskiewicz, A.G. and Trnski, T. (Ed.). *Larvae of Temperate Australian Fishes*. University of Western Australia Press, Nedlands.
- Gray, C.A., Chick, R.C. and McElligot, D.J. (1998). Diel changes in assemblages of fishes associated with shallow seagrass and bare sand. *Estuarine, Coastal and Shelf Science* **46**: 849–859.
- Gray, C.A., McElligot, D.J. and Chick, R.C. (1996). Intra- and inter-estuary differences in assemblages of fishes associated with shallow seagrass and bare sand. *Marine and Freshwater Research* **47**: 723–735.
- Günther, A. (1865). On the pipe-fishes belonging to the genus Phyllopteryx. Proceedings of the Zoological Society

of London 1865: 327-328, pls. 315-316.

- Günther, A. (1870). Catalogue of the Fishes in the British Museum. Catalogue of the Physostomi, containing the families Gymnotidae, Symbranchidae, Muraenidae, Pegasidae, and of the Lophobranchii, Plectognathi, Dipnoi, [thru] Leptocardii, in the British Museum. Volume 8. British Museum., London. 549 pp.
- Hamilton, H., Saarman, N., Short, G., Sellas, A.B., Moore, B., Hoang, T., Grace, C.L., Gomon, M., Crow, K. and Simison, W.B. (2017). Molecular phylogeny and patterns of diversification in syngnathid fishes. *Molecular Phylogenetics and Evolution* **107**: 388–403.
- Harasti, D. (2016). Declining seahorse populations linked to loss of essential marine habitats. *Marine Ecology Progress Series* **546**: 173–181.
- Harasti, D., Brennan, M. and Booth, D. (2022). Assessing the Effectiveness of Conservation Stocking for the Endangered White's Seahorse *Hippocampus whitei*. *Frontiers in Marine Science* **9**: 867352.
- Hardison, S.B., McGlathery, K.J. and Castorani, M.C.N. (2023). Effects of seagrass restoration on coastal fish abundance and diversity. *Conservation Biology* **37**: e14147.
- Higham, T.E., Stewart, W.J. and Wainwright, P.C. (2015). Turbulence, Temperature, and Turbidity: The Ecomechanics of Predator–Prey Interactions in Fishes. *Integrative and Comparative Biology* **55**: 6–20.
- Hindell, J.S., Jenkins, G.P. and Keough, M.J. (2000). Evaluating the impact of predation by fish on the assemblage structure of fishes associated with seagrass (*Heterozostera tasmanica*) (Martens ex Ascherson) den Hartog, and unvegetated sand habitats. *Journal of Experimental Marine Biology and Ecology* 255: 153– 174.
- Hoschke, A., Whisson, G. and Moore, G. (2023). Complete list of fishes recorded from the Perth Coast (Mandurah to Two Rocks) pp. 262–273. In: G. Whisson and Hoschke, A. (Ed.). *The Perth Coast Fish Book: Identification Guide: Mandurah to Two Rocks.* Aqua Research & Monitoring Services, Perth, Western Australia.
- Howard, R. and Koehn, J. (1985). Population dynamics and feeding ecology of pipefish (Syngnathidae) associated with eelgrass beds of Western Port, Victoria. *Marine and Freshwater Research* **36**: 361–370.
- Hübner, K., Gonzalez-Wanguemert, M., Diekmann, O.E. and Serrão, E.A. (2013). Genetic evidence for polygynandry in the Black-Striped Pipefish Syngnathus abaster: a microsatellite-based parentage analysis. Journal of Heredity 104: 791–797.
- Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S. and Tappu, R. (2016). MEGAN community edition - Interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS Computational Biology* **12**: e1004957.
- Hutchins, J.B. (1994). A survey of the nearshore reef fish fauna of Western Australia's west and south coasts the Leeuwin Province. *Records of the Western Australian Museum Supplement* **46**: 1–66.
- Hutchins, J.B. (2001). Biodiversity of shallow reef fish assemblages in Western Australia using a rapid censusing technique. *Records of the Western Australian Museum* **20**: 247–270.
- Hyndes, G.A., Kendrick, A.J., MacArthur, L.D. and Stewart, E. (2003). Differences in the species- and sizecomposition of fish assemblages in three distinct seagrass habitats with differing plant and meadow structure. *Marine Biology* **142**: 1195–1206.
- James, P.L. and Heck, K.L. (1994). The effects of habitat complexity and light intensity on ambush predation within a simulated seagrass habitat. *Journal of Experimental Marine Biology and Ecology* **176**: 187–200.
- Jamieson, O. and Pollom, R. (2016). *Heraldia nocturna* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T65368085A115424228.
- Jelbart, J.E., Ross, P.M. and Connolly, R.M. (2006). Edge effects and patch size in seagrass landscapes: an experimental test using fish. *Marine Ecology Progress Series* **319**: 93–102.
- Johnson, D.J., Wakefield, C.B., Sampey, A., Fromont, J. and Harris, D.C. (2008). *Developing long-term indicators* for the sub-tidal embayment communities of Cockburn Sound. Swan Catchment Council Project, 01-0506 *T.* Fisheries Research Report. Department of Fisheries, Western Australia. 113 pp.
- Jones, A.G. and Avise, J.C. (2001). Mating systems and sexual selection in male-pregnant pipefishes and seahorses: Insights from microsatellite-based studies of maternity *Journal of Heredity* **92**: 150–158.
- Jones, A.G., Kvarnemo, C., Moore, G.I., Simmons, L.W. and Avise, J.C. (1998). Microsatellite evidence for monogamy and sex-biased recombination in the Western Australian seahorse *Hippocampus angustus*. *Molecular Ecology* **7**: 1497–1506.
- Jones, A.G., Moore, G.I., Kvarnemo, C., Walker, D. and Avise, J.C. (2003). Sympatric speciation as a consequence of male pregnancy in seahorses. *Proceedings of the National Academy of Sciences* **100**: 6598–6603.
- Kangas, M.I., Morrison, S., Unsworth, P., Lai, E., Wright, I. and Thomson, A. (2007). *Development of biodiversity and habitat monitoring systems for key trawl fisheries in Western Australia. Final FRDC Report* 2002/038. Fisheries Research Report 160. 334 pp.

Kaup, J.J. (1853). Uebersicht der Lophobranchier. Archiv für Naturgeschichte 19: 226–234.

- Kendrick, A.J. (2002). *Resource utilisation and reproductive biology of syngnathid fishes in a seagrass-dominated marine environment in south-western Australia*. PhD Thesis. Centre for Fish and Fisheries Research, Murdoch University. Murdoch, Western Australia. 156 pp.
- Kendrick, A.J. and Hyndes, G.A. (2003). Patterns in the abundance and size-distribution of syngnathid fishes among habitats in a seagrass-dominated marine environment. *Estuarine and Coastal Shelf Science* **57**: 631–640.
- Kendrick, A.J. and Hyndes, G.A. (2005). Variations in the dietary compositions of morphologically diverse syngnathid fishes. *Environmental Biology of Fishes* **72**: 415–427.
- Knott, N., Aulbury, J., Brown, T. and Johnston, E. (2009). Contemporary ecological threats from historical pollution sources: impacts of large-scale resuspension of contaminated sediments on sessile invertebrate recruitment. *Journal of Applied Ecology* **46**: 770–781.
- Kovačić, M., Patzner, R.A. and Schliewen, U.K. (2012). A first quantitative assessment of the ecology of cryptobenthic fishes in the Mediterranean Sea. *Marine Biology* **159**: 2731–2742.
- Kuiter, R.H. (1993). Coastal Fishes of South-Eastern Australia. Crawford House, Bathurst, NSW, Australia. 437 pp.
- Kuiter, R.H. (2001). Revision of the Australian Seahorses of the genus *Hippocampus* (Syngnathiformes: Syngnathidae) with descriptions of nine new species. *Records of the Australian Museum* **53**: 293–340.
- Kuiter, R.H. (2004). A New Pygmy Pipehorse (Pisces: Syngnathidae: *Idiotropiscis*) from Eastern Australia. *Records* of the Australian Museum **56**: 163–165.
- Kuiter, R.H. (2009). Seahorses and their relatives. Aquatic Photographics, Seaford, Australia. 333 pp.
- Kvarnemo, C., Andersson, S.E., Elisson, J., Moore, G.I. and Jones, A.G. (2021). Home range use in the West Australian seahorse *Hippocampus subelongatus* is influenced by sex and partner's home range but not by body size or paired status. *Journal of Ethology* **39**: 235–248.
- Kvarnemo, C., Moore, G.I. and Jones, A.G. (2006). Sexually selected females in the monogamous Western Australian seahorse. *Proceedings of the Royal Society B* **274**: 521–525.
- Kvarnemo, C., Moore, G.I., Jones, A.G., Nelson, W.S. and Avise, J.C. (2000). Monogamous pair bonds and mate switching in the Western Australian seahorse *Hippocampus subelongatus*. *Journal of Evolutionary Biology* 13: 882–888.
- Lacepède, B.G. (1804). Mémoire sur plusieurs animaux de la Nouvelle Hollande dont la description n'a pas encore été publiée. *Annales du Muséum National d'Histoire Naturelle. Paris* **4**: 184–211 pls 155–158.
- Lim, A.C.O., Chong, V., Wong, C. and Muniandy, S.V. (2015). Sound signatures and production mechanisms of three species of pipefishes (Family: Syngnathidae). *PeerJ* **3**: e1471.
- Liu, Y., Shang, D., Yang, Y., Cui, P. and Sun, J. (2022a). Bioaccumulation of contaminants in wild seahorses collected from coastal China. *Frontiers in Marine Science* **8**: 1021170
- Liu, Y., Shang, D., Yang, Y., Cui, P. and Sun, J. (2022b). Transcriptomic analysis provides insights into microplastic and heavy metal challenges in the Line Seahorse (*Hippocampus erectus*). *Fishes* **7**: 338.
- Liu, Y., Wu, Y., Qin, G., Chen, Y., Wang, X. and Lin, Q. (2021). Bioaccumulation and reproductive toxicity of bisphenol A in male-pregnant seahorse (*Hippocampus erectus*) at environmentally relevant concentrations. *Science of the Total Environment* **753**: 141805.
- Livingston, R.J. (1984). Trophic response of fishes to habitat variability in coastal seagrass systems. *Ecology* **65**: 1258–1275.
- Lourie, S.A., Pollom, R.A. and Foster, S.J. (2016). A global revision of the Seahorses *Hippocampus* Rafinesque 1810 (Actinopterygii: Syngnathiformes): Taxonomy and biogeography with recommendations for further research. *Zootaxa* **4146**: 001–066.
- Lowe, M.L., Morrison, M.A. and Taylor, R.B. (2015). Harmful effects of sediment-induced turbidity on juvenile fish in estuaries. *Marine Ecology Progress Series* **539**: 241–254.
- Lucas, A.H.S. (1891). On the occurrence of certain fish in Victorian seas, with descriptions of some new species. *Proceedings of the Royal Society of Victoria* **3**: 8–14.
- Macleay, W.J. (1881). Descriptive catalogue of the fishes of Australia. Part 4. *Proceedings of the Linnean Society of New South Wales* **6**: 202–387.
- Marcus, J.E., Samoilys, M.A., Meeuwig, J.J., Villongco, Z.A.D. and Vincent, A.C.J. (2007). Benthic status of nearshore fishing grounds in the central Philippines and associated seahorse densities. *Marine Pollution Bulletin* **54**: 1483–1494.
- Martin-Smith, K. (2003). Role of syngnathids in shallow coastal ecosystems of southeastern Australia pp. 89– 100. In: R.E. Forrest, Scandol, J.P. and Pitcher, T.J. (Ed.). *Towards Ecosystem-based Fishery Management in New South Wales: Proceedings of the Experts and Data Workshop*, Cronulla, Australia.
- Martin-Smith, K.M. and Vincent, A.C.J. (2005). Seahorse declines in the Derwent estuary, Tasmania in the

55 | P a g e

absence of fishing pressure. *Biological Conservation* **123**: 533–545.

- Martin-Smith, K.M. and Vincent, A.C.J. (2006). Exploitation and trade of Australian seahorses and their relatives (Syngnathids). *Oryx* **40**: 141–151.
- Masonjones, H.D., Rose, E., McRae, L.B. and Dixson, D.L. (2010). An examination of the population dynamics of syngnathid fishes within Tampa Bay, Florida, USA. *Current Zoology* **56**: 118–133.
- McCulloch, A.R. and Waite, E.R. (1918). Some new and little-known fishes from South Australia. *Records of the South Australian Museum* 1: 39–78.
- McKenzie, L., Yoshida, R. and Coles, R. (2006–2010). Seagrass-Watch. Available at www.seagrasswatch.org
- McLeod, I.M., Boström-Einarsson, L., Johnson, C., Kendrick, G., Layton, C., Rogers, A.A. and Statton, J. (2019). *The role of restoration for conserving Matters of National Environmental Significance*. Report to the National Environmental Science Programme, Marine Biodiversity Hub. 186 pp.
- Monteiro, N., Carneiro, D., Antunes, A., Queiroz, N., Vieira, M. and Jones, A. (2017). The lek mating system of the worm pipefish (*Nerophis lumbriciformis*): a molecular maternity analysis and test of the phenotype-linked fertility hypothesis. *Molecular Ecology* **26**: 1371–1385.
- Moore, G.I. (2001). *Reproductive Biology of the Western Australian Seahorse Hippocampus subelongatus*. MSc. Thesis. Department of Zoology, University of Western Australia. Nedlands, Western Australia. 140 pp.
- Moore, G.I., Hutchins, J.B. and Swainston, R. (unpublished). *Sea Fishes of Southern Australia*. Western Australian Museum, Perth. In prep.
- Moore, G.I., Morrison, S.M. and Johnson, J. (2020). The distribution of shallow reef fishes of the Kimberley, Western Australia, based on a long-term dataset and multiple methods. *Records of the Western Australian Museum* **Supplement 85**: 105–115.
- Moran, S.M., Jenkins, G.P., Keough, M.J. and Hindell, J.S. (2003). Role of physical disturbance in structuring fish assemblages in seagrass beds in Port Phillip Bay, Australia. *Marine Ecology Progress Series* **251**: 127–139.
- Moreau, M.-A. and Vincent, A.C.J. (2004). Social structure and space use in a wild population of the Australian short-headed seahorse, *Hippocampus breviceps* Peters 1869. *Marine and Freshwater Research* **55**: 231–239.
- Morris, J.A. and Akins, J.L. (2009). Feeding ecology of invasive lionfish (*Pterois volitans*) in the Bahamian archipelago. *Environmental Biology of Fishes* **86**: 389–398.
- Mosk, V., Thomas, N., Hart, N.S., Partridge, J.C., Beazley, L.D. and Shand, J. (2007). Spectral sensitivities of the seahorses *Hippocampus subelongatus* and *Hippocampus barbouri* and the pipefish *Stigmatopora argus*. *Visual Neuroscience* **24**: 345–354.
- Nester, G.M., De Brauwer, M., Koziol, A., West, K.M., DiBattista, J.D., White, N.E., Power, M., Heydenrych, M.J., Harvey, E. and Bunce, M. (2020). Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (family: Syngnathidae). *Environmental DNA* **2**: 614–626.
- Oliveira, T.P.R., Ladich, F., Abed-Navandi, D., Souto, A.S. and Rosa, I.L. (2014). Sounds produced by the longsnout seahorse: a study of their structure and functions. *Journal of Zoology* **294**: 114–121.
- Palma, J., Magalhães, M., Correia, M. and Andrade, J.P. (2019a). Effects of anthropogenic noise as a source of acoustic stress in wild populations of *Hippocampus guttulatus* in the Ria Formosa, south Portugal. *Aquatic Conservation* **29**: 751–759.
- Palma, J., Magalhães, M., Correia, M. and Andrade, J.P. (2019b). Effects of anthropogenic noise as a source of acoustic stress in wild populations of Hippocampus guttulatus in the Ria Formosa, south Portugal. *Aquatic Conservation: Marine and Freshwater Ecosystems* **29**.
- Parkinson, K. and Booth, D. (2016). Rapid growth and short life spans characterize pipefish populations in vulnerable seagrass beds. *Journal of Fish Biology* **88**: 1847–1855.
- Paxton, J.R. (1975). *Heraldia nocturna*, a new genus and species of pipefish (family Syngnathidae) from eastern Australia with comments on *Maroubra perserrata* Whitley. *Proceedings of the California Academy of Sciences* **40**: 439–447.
- Payne, M.F., Rippingale, R.J. and Longmore, R.B. (1998). Growth and survival of juvenile pipefish (*Stigmatopora argus*) fed live copepods with high and low HUFA content. *Aquaculture* **16**: 237–245.
- Peng, C., Zhao, X. and Liu, G. (2015). Noise in the Sea and Its Impacts on Marine Organisms. *International Journal of Environmental Research and Public Health* **12**: 12304–12323.
- Penn, J.W. (1977). *Trawl caught fish and crustaceans from Cockburn Sound*. Department of Fisheries and Wildlife. Report No. 20. Perth, Western Australia. 24 pp.
- Peters, W.C.H. (1868). Über eine neue Nagergattung *Chiropodomys penicillatus,* so wie über einige neue oder weniger bekannte Amphibien und Fische. *Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin* **1868**: 448–460.

Pinault, M., Wickel, J., Nicet, J.-B., Chenoz, M., Montgolfier, B.d. and Fricke, R. (2018). First record of the near threatened native seahorse *Hippocampus reidi* (Teleostei: Syngnathidae) in an ecosystem dominated by the invasive seagrass *Halophila stipulacea* in the Caribbean Sea. *Cybium* **42**: 393–396.

- Pollom, R. (2016a). Acentronura australe (errata version published in 2017). The IUCN Red List of Threatened Species **2016**: e.T61314A115204241.
- Pollom, R. (2016b). *Campichthys galei* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T65364120A115411601.
- Pollom, R. (2016c). Filicampus tigris. The IUCN Red List of Threatened Species 2016: e.T65367453A67624788.
- Pollom, R. (2016d). *Nannocampus subosseus* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T65372227A115430564.
- Pollom, R. (2017a). *Hippocampus breviceps. The IUCN Red List of Threatened Species* **2017**: e.T10063A54904334.
- Pollom, R. (2017b). *Hippocampus subelongatus*. *The IUCN Red List of Threatened Species* **2017**: e.T40773A54906710.
- Pollom, R. (2017c). Phycodurus eques. The IUCN Red List of Threatened Species 2017: e.T17096A67622420.
- Pollom, R. (2017d). *Phyllopteryx taeniolatus*. *The IUCN Red List of Threatened Species* **2017**: e.T17177A67624517.
- Pollom, R. (2017e). Solegnathus lettiensis. The IUCN Red List of Threatened Species **2017**: e.T20316A67623500.
- Pollom, R. and Qian, Y. (2016a). *Lissocampus caudalis. The IUCN Red List of Threatened Species* **2016**: e.T65370051A67624150.
- Pollom, R. and Qian, Y. (2016b). *Lissocampus fatiloquus*. *The IUCN Red List of Threatened Species* **2016**: e.T65370087A67619013.
- Pollom, R. and Rachinski, T. (2016). Vanacampus poecilolaemus. The IUCN Red List of Threatened Species **2016**: e.T65375703A67622370.
- Pollom, R., Ralph, G., Pollock, C. and Vincent, A. (2021). Global extinction risk for seahorses, pipefishes and their near relatives (Syngnathiformes). *Oryx* **55**: 497-506.
- Popper, A.N. and Hawkins, A.D. (2019). An overview of fish bioacoustics and the impacts of anthropogenic sounds on fishes. *Journal of Fish Biology* **94**: 692-713.
- Porter, T. and Hajibabaei, M. (2018). Scaling up: A guide to high throughput genomic approaches for biodiversity analysis. *Molecular Ecology* **27**.
- Power, M. and Attrill, M.J. (2003). Long-term trends in the estuarine abundance of Nilsson's pipefish (*Syngnathus rostellatus* Nilsson). *Estuarine, Coastal and Shelf Science* **57**: 325–333.
- Qian, Y. and Pollom, R. (2016). *Lissocampus runa* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T65370289A115426541.
- Rachinski, T. and Pollom, R. (2016a). Vanacampus margaritifer. The IUCN Red List of Threatened Species **2016**: e.T65375519A67622365.
- Rachinski, T. and Pollom, R. (2016b). Vanacampus phillipi. The IUCN Red List of Threatened Species **2016**: e.T65375571A67622360.
- Rangel-Buitrago, N., Rizzo, A., Neal, W.J. and Mastronuzzi, G. (2023). Sediment pollution in coastal and marine environments. *Marine Pollution Bulletin* **192**: 115023.
- Richardson, J. (1840). On some new species of fishes from Australia. *Proceedings of the Zoological Society of London* **8**: 25–30.
- Ripley, J.L. and Foran, C.M. (2007). Influence of estuarine hypoxia on feeding and sound production by two sympatric pipefish species (Syngnathidae). *Marine Environmental Research* **63**: 350–367.
- Rosenqvist, G. and Berglund, A. (2011). Sexual signals and mating patterns in Syngnathidae. *Journal of Fish Biology* **78**: 1647–1661.
- Rouse, G.W., Stiller, J. and Wilson, N.G. (2017). First live records of the ruby seadragon (*Phyllopteryx dewysea*, Syngnathidae). *Marine Biodiversity Records* **10**: 2.
- Sampey, A., Fromont, J. and Johnston, D.J. (2011). Demersal and epibenthic fauna in a temperate marine embayment, Cockburn Sound, Western Australia: determination of key indicator species. *Journal of the Royal Society of Western Australia* **94**: 1–18.
- Sanchez-Camara, J. and Booth, D.J. (2004). Movement, home range and site fidelity of the weedy seadragon *Phyllopteryx taeniolatus* (Teleostei: Syngnathidae). *Environmental Biology of Fishes* **70**: 31–41.
- Sanchez-Camara, J., Booth, D.J. and Turon, X. (2005). Reproductive cycle and growth of *Phyllopteryx taeniolatus*. *Journal of Fish Biology* **67**: 133–148.
- Scott, E.O.G. (1961). Observations on some Tasmanian fishes. Part X. *Papers and proceedings of the Royal Society* of Tasmania **95**: 49–65.
- Scott, E.O.G. (1970). Observations on some Tasmanian fishes. Part XVIII. *Papers and proceedings of the Royal* Society of Tasmania **105**: 119–143.

- Shokri, M.R., Gladstone, W. and Jelbart, J. (2009). The effectiveness of seahorses and pipefish (Pisces: Syngnathidae) as a flagship group to evaluate the conservation value of estuarine seagrass beds. Aquatic Conservation: Marine and Freshwater Ecosystems **19**: 588–595.
- Shute, S. and Carey, M. (2006). *Investigation into tributyltin (TBT) contamination in Cockburn Sound*. Report prepared for Cockburn Sound Management Council. 9 pp.
- Sievers, M., Connolly, R., Finlayson, K., Kitchingman, M., Ostrowsk, i.A., Pearson, R., Turschwell, M., Adame, M., Bugnot, A., Ditria, E., Hale, R., Silliman, B., Swearer, S., Valdez, S. and Brown, C. (2024). Enhanced but highly variable biodiversity outcomes from coastal restoration: a global synthesis. *One Earth* **7**: 623– 634.
- Simpson, M., Coleman, R.A., Morris, R.L. and Harasti, D. (2020). Seahorse Hotels: Use of artificial habitats to support populations of the endangered White's seahorse *Hippocampus white*i. *Marine Environmental Research* **157**: 104861.
- Sinclair, E.A., Verduin, J., Krauss, S.L., Hardinge, J., Anthony, J. and Kendrick, G.A. (2013). A genetic assessment of a successful seagrass meadow (*Posidonia australis*) restoration trial. *Ecological Management and Restoration* 14: 68–71.
- Smith, K.A., Bissell, A. and Bruce, C. (2022). Ecological Risk Assessment for the Marine Aquarium Fish Resource. Fisheries Research Report No. 323. Department of Primary Industries and Regional Development, Western Australia. 149 pp.
- Smith, T.M., Hindell, J.S., Jenkins, G.P., Connolly, R.M. and Keough, M.J. (2011). Fine-scale spatial and temporal variations in diets of the pipefish *Stigmatopora nigra* within seagrass patches. *Journal of Fish Biology* 78: 1824–1832.
- Sogabe, A. and Yanagisawa, Y. (2008). Maintenance of pair bond during the non-reproductive season in a monogamous pipefish *Corythoichthys haematopterus*. *Journal of Ethology* **26**: 195–199.
- Sommer, S., Whittington, C.M. and Wilson, A.B. (2012). Standardised classification of pre-release development in male-brooding pipefish, seahorses, and seadragons (Family Syngnathidae). *BMC Developmental Biology* **12**: 39.
- Stat, M., John, J., DiBattista, J.D., Newman, S.J., Bunce, M. and Harvey, E.S. (2018). Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conservation Biology* 33: 196–205.
- Steffe, A., Westoby, M. and Bell, J. (1989). Habitat selection and diet in two species of pipefish from seagrass: sex differences. *Marine Ecology Progress Series* **55**: 23–30.
- Stewart, A.L. (2015). Syngnathidae pp. 1050–1062. In: C.D. Roberts, Stewart, A.L. and Struthers, C.D. (Ed.). *The Fishes of New Zealand*. Te Papa Press, Wellington.
- Stiller, J., da Fonseca, R.R., Alfaro, M.E., Faircloth, B.C., Wilson, N.G. and Rouse, G.W. (2020). Using ultraconserved elements to track the influence of sea-level change on leafy seadragon populations. *Molecular Ecology* **30**: 1364–1380.
- Stiller, J., Wilson, N.G. and Rouse, G.W. (2015). A spectacular new species of seadragon (Syngnathidae). *Royal Society Open Science* **2**: 140458.
- Stiller, J., Wilson, N.G. and Rouse, G.W. (2023). Range-wide population genomics of common seadragons shows secondary contact over a former barrier and insights on illegal capture. *BMC Biology* **21**: 129.
- Sun, J., Xia, S., Ning, Y., Pan, X., Qu, J. and Xu, Y. (2019). Effects of microplastics and attached heavy metals on growth, immunity, and heavy metal accumulation in the yellow seahorse, *Hippocampus kuda* Bleeker. *Marine Pollution Bulletin* 149: 511–517.
- Sundin, J., Aronsen, T., Rosenqvist, G. and Berglund, A. (2017). Sex in murky waters: algal-induced turbidity increases sexual selection in pipefish. *Behavioral Ecology and Sociobiology* **71**: 78.
- Sundin, J., Berglund, A. and Rosenqvist, G. (2010). Turbidity Hampers Mate Choice in a Pipefish. *Ethology* **116**: 713-721.
- Sundin, J., Rosenqvist, G., Myhren, S. and Berglund, A. (2016). Algal Turbidity Hampers Ornament Perception, but Not Expression, in a Sex-Role-Reversed Pipefish. *Ethology* **122**: 215–225.
- Tang, L., Zhang, Y.-H., Wang, X., Zhang, C.-C. and Geng Qin, Q.L. (2021). Effects of chronic exposure to environmental levels of tributyltin on the lined seahorse (*Hippocampus erectus*) liver: Analysis of bioaccumulation, antioxidant defense, and immune gene expression. *Science of the Total Environment* 801: 149646.
- Thompson, L., Maiti, K., White, J.R., DuFore, C.M. and Liu, H. (2021). The impact of recently excavated dredge pits on coastal hypoxia in the northern Gulf of Mexico shelf. *Marine Environmental Research* **163**: 105199.
- Trebilco, R., Fischer, M., Hunter, C., Hobday, A., Thomas, L. and Evans, K. (2022). Australia State of the

Environment 2021: Marine. Independent report to the Australian Government Minister for the Environment. Commonwealth of Australia, Canberra. 252 pp.

- Vanderklift, M.A. (1996). *Influence of adjacent seagrass on the fish assemblages off sandy beaches*. MSc Thesis. Centre for Ecosystem Management, Edith Cowan University. Perth, Western Australia. 196 pp.
- Vanderklift, M.A. and Jacoby, C.A. (2003). Patterns in fish assemblages 25 years after major seagrass loss. *Marine Ecology Progress Series* **247**.
- Vincent, A.C.J. (1995). A role for daily greetings in maintaining seahorse pair bonds. *Animal Behaviour* **49**: 258–260.
- Vincent, A.C.J., Foster, S.J. and Koldewey, H.J. (2011). Conservation and management of seahorses and other Syngnathidae. *Journal of Fish Biology* **78**: 1681–1724.
- Waite, E.R. and Hale, H.M. (1921). Review of the lophobranchiate fishes (pipe-fishes and sea-horses) of South Australia. *Records of the South Australian Museum (Adelaide)* **1**: 293–324 figs 239–256.
- Wakefield, C., Lewis, P., Coutts, T., Fairclough, D. and Langlois, T. (2013). Fish assemblages associated with natural and anthropogenically-modified habitats in a marine embayment: comparison of baited videos and opera-house traps. *PLoS One* **8**: e59959.
- Ward, R.D., Hanner, R. and Hebert, P.D.N. (2009). The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology* **74**: 329–356.
- Water Corporation (2019). *Perth Seawater Desalination Plant 2. Environmental Review Document*. Perth, Western Australia.
- Whisson, G. and Hoschke, A. (2021). *The Perth Coast Fish Book*. Aqua Research and Monitoring Services, Perth, Western Australia.
- Whitley, G.P. (1931). New names for Australian fishes. The Australian Zoologist 6: 310-334.
- Whitley, G.P. (1943). Ichthyological notes and illustrations. Part 2. *The Australian Zoologist* **10**: 167–187.
- Whitley, G.P. (1948). New sharks and fishes from Western Australia. Part 4. Australian zoologist 11: 259–276.
- Wilson, A.B., Vincent, A., Ahnesjö, I. and Meyer, A. (2001). Male pregnancy in seahorses and pipefishes (family Syngnathidae): rapid diversification of paternal brood pouch morphology inferred from a molecular phylogeny. *The Journal of Heredity* **92**: 159–166.
- Wilson, B.R., Kendrick, G.W. and Brearley, A. (1978). *The Benthic Fauna of Cockburn Sound, Western Australia. Part 1 : Prosobranch Gastropod and Bivalve Molluscs*. Unpublished report submitted to the W.A.
 Department of Conservation and Environment. Western Australian Museum. Perth, Western Australia.
 131 pp.
- Wong, C.K., Pak, I.A.P. and Jiang Liu, X. (2013). Gill damage to juvenile orange-spotted grouper *Epinephelus coioides* following exposure to suspended sediments. *Aquaculture Research* **44**: 1685–1695.
- Yearsley, G.K., Last, P.R. and Hoese, D.F. (2006). *Standard names of Australian fishes*. CSIRO Marine and Atmospheric Research Paper 009. CSIRO. Hobart, Australia. 64 pp.
- York, P.H., Booth, D.J., Glasby, T.M. and Pease, B.C. (2006). Fish assemblages in habitats dominated by *Caulerpa taxifolia* and native seagrasses in south-eastern Australia. *Marine Ecology Progress Series* **312**: 223–234.
- Zhang, X. and Pollom, R. (2016). *Mitotichthys meraculus* (errata version published in 2017). *The IUCN Red List of Threatened Species* **2016**: e.T65371373A115429077.

10 Appendices

10.1 Appendix 1: Larval Records

Larvae records from 4 species were compiled through the course of the current project (Table A1). This included 13 specimens from 3 identified species from the Australian Museum and 72 specimens from 3 identified species from WWMSP Project 4.2.2 (*Zooplankton in Cockburn Sound*). Some specimens were only identified to the level of genus.

Table A1. Records of syngnathid larvae compiled during the current project from the collection of the Australian Museum, Sydney (AMS) and WWMSP Project 4.2.2 (*Zooplankton in Cockburn Sound*).

Filicampus tigris -32.1647 115.7525 1 2023 AMS Filicampus tigris -32.267 115.683 1 2023 4.2.2 Filicampus tigris -32.1273 115.708 2 2023 4.2.2 Filicampus tigris -32.167 115.71 3 2022 4.2.2 Filicampus tigris -32.167 115.71 3 2022 4.2.2 Filicampus tigris -32.1273 115.708 12 2022 4.2.2 Filicampus tigris -32.1273 115.708 12 2022 4.2.2 Filicampus tigris -32.1273 115.708 12 2022 4.2.2 Filicampus tigris -32.2167 115.75 12 2022 4.2.2 Filicampus tigris -32.2167 115.75 12 2022 4.2.2 Stigmatopora argus -32.167 115.75 12 2022 4.2.2 Stigmatopora argus -32.1273 115.708 11 2022 4.2.2 Stigmatopora argus -32.1273 115.708 1 2022 4.2.2	Species	Latitude	Longitude	Month	Year	Source
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Filicampus tigris -32.15 115.77 2 2023 4.2.2 Filicampus tigris -32.167 115.717 3 2022 4.2.2 Filicampus tigris -32.1273 115.708 11 2021 4.2.2 Filicampus tigris -32.1273 115.708 12 2022 4.2.2 Filicampus tigris -32.1273 115.708 12 2022 4.2.2 Filicampus tigris -32.1273 115.708 12 2022 4.2.2 Filicampus tigris -32.2167 115.75 12 2021 4.2.2 Stigmatopora argus -32.1273 115.708 11 2022 4.2.2 Stigmatopora argus -32.1273 115.708 11 2022 4.2.2 Stigmatopora argus -32.1273 115.708 1 2022 4.2.2 Stigmatopora nigra -32.1273 115.708 1 2023 4.2.2 Stigmatopora nigra -32.167 115.675 3 2023 4.2.2 Stigmatopora nigra -32.167 115.675 4 2022 4.2.2 <td>Filicampus tigris</td> <td>-32.1273</td> <td>115.708</td> <td>2</td> <td>2023</td> <td>4.2.2</td>	Filicampus tigris	-32.1273	115.708	2	2023	4.2.2
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Stigmatopora nigra-32.1167115.675120224.2.2Stigmatopora nigra-32.1273115.708120234.2.2Stigmatopora nigra-32.15115.733120234.2.2Stigmatopora nigra-31.9917115.745319924.2.2Stigmatopora nigra-32.1167115.675320234.2.2Stigmatopora nigra-32.1167115.675320234.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra	Stigmatopora argus	-32.0185	115.7848	-	1985	4.2.2
Stigmatopora nigra-32.1273115.708120234.2.2Stigmatopora nigra-32.15115.733120234.2.2Stigmatopora nigra-31.9917115.745319924.2.2Stigmatopora nigra-32.1167115.675320234.2.2Stigmatopora nigra-32.1167115.675320234.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.755420224.2.2Stigmatopora nigra-32.1167115.755420224.2.2Stigmatopora nigra-32.1167115.755420224.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra	Stigmatopora nigra	-32.1167	115.675	1	2022	4.2.2
Stigmatopora nigra-32.15115.733120234.2.2Stigmatopora nigra-31.9917115.745319924.2.2Stigmatopora nigra-32.1167115.675320234.2.2Stigmatopora nigra-32.1667115.683320234.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.755420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1647115.755620224.2.2Stigmatopora nigra-32.1647115.755620224.2.2Stigmatopora nigra	Stigmatopora nigra	-32.1273	115.708	1	2023	4.2.2
Stigmatopora nigra-31.9917115.745319924.2.2Stigmatopora nigra-32.1167115.675320234.2.2Stigmatopora nigra-32.1667115.683320234.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420234.2.2Stigmatopora nigra-32.1167115.725420234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1647115.755620224.2.2Stigmatopora nig	Stigmatopora nigra	-32.15	115.733	1	2023	4.2.2
Stigmatopora nigra-32.1167115.675320234.2.2Stigmatopora nigra-32.1667115.683320234.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1647115.755520234.2.2Stigmatopora nigra-32.1647115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1167115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1647115.755620224.2.2Stigmatopora nigra-32.1647115.755620234.2.2Stigmatopora nigra-32.1647115.755620234.2.2Stigmatopora nigra-32.1647115.733620224.2.2Stigmatopora nig	Stigmatopora nigra	-31.9917	115.745	3	1992	4.2.2
Stigmatopora nigra-32.1667115.683320234.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420234.2.2Stigmatopora nigra-32.1647115.755520234.2.2Stigmatopora nigra-32.1647115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.675620224.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.15115.7620224.2.2Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620234.2.2Stigmatopora nigra	Stigmatopora nigra	-32.1167	115.675	3	2023	4.2.2
Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420234.2.2Stigmatopora nigra-32.1167115.725420234.2.2Stigmatopora nigra-32.1273115.708420234.2.2Stigmatopora nigra-32.1647115.755520234.2.2Stigmatopora nigra-32.2167115.755520234.2.2Stigmatopora nigra-32.1167115.675620224.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.15115.708620224.2.2Stigmatopora nigra-32.167115.755620224.2.2Stigmatopora nigra-32.167115.725620224.2.2Stigmatopora nigra-32.167115.733620224.2.2Stigmatopora nigra-32.167115.683620224.2.2Stigmatopora nigra-32.1667115.683620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra <td>Stigmatopora nigra</td> <td>-32.1667</td> <td>115.683</td> <td>3</td> <td>2023</td> <td>4.2.2</td>	Stigmatopora nigra	-32.1667	115.683	3	2023	4.2.2
Stigmatopora nigra-32.1167115.675420224.2.2Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420234.2.2Stigmatopora nigra-32.1273115.708420234.2.2Stigmatopora nigra-32.1647115.7525520234.2.2Stigmatopora nigra-32.1647115.755520234.2.2Stigmatopora nigra-32.1167115.755520234.2.2Stigmatopora nigra-32.1167115.675620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.15115.7620224.2.2Stigmatopora nigra-32.1647115.7525620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620234.2.2Stigmatopora nigra-32.1667115.683620234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora nigr	Stigmatopora nigra	-32.1167	115.675	4	2022	4.2.2
Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1167115.725420234.2.2Stigmatopora nigra-32.1273115.708420234.2.2Stigmatopora nigra-32.1647115.7525520234.2.2Stigmatopora nigra-32.167115.755520234.2.2Stigmatopora nigra-32.1167115.675620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.15115.708620224.2.2Stigmatopora nigra-32.167115.755620224.2.2Stigmatopora nigra-32.167115.708620224.2.2Stigmatopora nigra-32.167115.708620224.2.2Stigmatopora nigra-32.1647115.755620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora nigra </td <td>Stigmatopora nigra</td> <td>-32.1167</td> <td>115.675</td> <td>4</td> <td>2022</td> <td>4.2.2</td>	Stigmatopora nigra	-32.1167	115.675	4	2022	4.2.2
Stigmatopora nigra-32.1167115.725420224.2.2Stigmatopora nigra-32.1273115.708420234.2.2Stigmatopora nigra-32.1647115.7525520234.2.2Stigmatopora nigra-32.2167115.75520234.2.2Stigmatopora nigra-32.1167115.755620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1647115.755620224.2.2Stigmatopora nigra-32.1647115.755620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora ni	Stigmatopora nigra	-32.1167	115.725	4	2022	4.2.2
Stigmatopora nigra-32.1273115.708420234.2.2Stigmatopora nigra-32.1647115.7525520234.2.2Stigmatopora nigra-32.2167115.75520234.2.2Stigmatopora nigra-32.1167115.675620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1647115.7525620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora nigra-32.1667115.675720234.2.2Stigmatopora n	Stigmatopora nigra	-32.1167	115.725	4	2022	4.2.2
Stigmatopora nigra-32.1647115.7525520234.2.2Stigmatopora nigra-32.2167115.75520234.2.2Stigmatopora nigra-32.1167115.675620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.167115.7525620224.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1867115.675720234.2.2Stigmatopora nigra-32.167115.675720234.2.2	Stigmatopora nigra	-32.1273	115.708	4	2023	4.2.2
Stigmatopora nigra-32.2167115.75520234.2.2Stigmatopora nigra-32.1167115.675620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1667115.675720234.2.2	Stigmatopora nigra	-32.1647	115.7525	5	2023	4.2.2
Stigmatopora nigra-32.1167115.675620224.2.2Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.15115.7620224.2.2Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1115.733620234.2.2Stigmatopora nigra-32.1667115.683620234.2.2Stigmatopora nigra-32.1115.7620234.2.2Stigmatopora nigra-32.1115.733620234.2.2Stigmatopora nigra-32.2115.7720234.2.2Stigmatopora nigra-32.1167115.675720234.2.2	Stigmatopora nigra	-32.2167	115.75	5	2023	4.2.2
Stigmatopora nigra-32.1167115.725620224.2.2Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.15115.7620224.2.2Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1115.733620234.2.2Stigmatopora nigra-32.1115.733720234.2.2Stigmatopora nigra-32.1115.7720234.2.2	Stigmatopora nigra	-32.1167	115.675	6	2022	4.2.2
Stigmatopora nigra-32.1233115.7267619924.2.2Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.15115.7620224.2.2Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1667115.683620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.2115.7620234.2.2Stigmatopora nigra-32.1667115.675720234.2.2	Stigmatopora nigra	-32.1167	115.725	6	2022	4.2.2
Stigmatopora nigra-32.1273115.708620234.2.2Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.15115.7620224.2.2Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.2115.7620234.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.1167115.675720234.2.2	Stigmatopora nigra	-32.1233	115.7267	6	1992	4.2.2
Stigmatopora nigra-32.1273115.708620224.2.2Stigmatopora nigra-32.15115.7620224.2.2Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.2115.7620234.2.2Stigmatopora nigra-32.2115.7720234.2.2	Stigmatopora nigra	-32.1273	115.708	6	2023	4.2.2
Stigmatopora nigra-32.15115.7620224.2.2Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.2115.7620234.2.2Stigmatopora nigra-32.167115.675720234.2.2	Stigmatopora nigra	-32.1273	115.708	6	2022	4.2.2
Stigmatopora nigra-32.1647115.7525620234.2.2Stigmatopora nigra-32.1667115.683620224.2.2Stigmatopora nigra-32.1833115.733620234.2.2Stigmatopora nigra-32.2115.7620234.2.2Stigmatopora nigra-32.1167115.675720234.2.2	Stigmatopora nigra	-32.15	115.7	6	2022	4.2.2
Stigmatopora nigra -32.1667 115.683 6 2022 4.2.2 Stigmatopora nigra -32.1833 115.733 6 2023 4.2.2 Stigmatopora nigra -32.2 115.7 6 2023 4.2.2 Stigmatopora nigra -32.2 115.7 6 2023 4.2.2 Stigmatopora nigra -32.1167 115.675 7 2023 4.2.2	Stigmatopora nigra	-32.1647	115.7525	6	2023	4.2.2
Stigmatopora nigra -32.1833 115.733 6 2023 4.2.2 Stigmatopora nigra -32.2 115.7 6 2023 4.2.2 Stigmatopora nigra -32.1167 115.675 7 2023 4.2.2	Stigmatopora nigra	-32.1667	115.683	6	2022	4.2.2
Stigmatopora nigra -32.2 115.7 6 2023 4.2.2 Stigmatopora nigra -32.1167 115.675 7 2023 4.2.2	Stigmatopora nigra	-32.1833	115.733	6	2023	4.2.2
<i>Stigmatopora nigra</i> -32.1167 115.675 7 2023 4.2.2	Stigmatopora nigra	-32.2	115.7	6	2023	4.2.2
	Stigmatopora nigra	-32.1167	115.675	7	2023	4.2.2

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Species	Latitude	Longitude	Month	Year	Source
Stigmatopora nigra	-32.1167	115.675	7	2022	4.2.2
Stigmatopora nigra	-32.1273	115.708	7	2023	4.2.2
Stigmatopora nigra	-32.1667	115.683	7	2022	4.2.2
Stigmatopora nigra	-32.1667	115.683	7	2022	4.2.2
Stigmatopora nigra	-32.2	115.7	7	2022	4.2.2
Stigmatopora nigra	-32.25	115.733	7	2023	4.2.2
Stigmatopora nigra	-32.25	115.75	7	2022	4.2.2
Stigmatopora nigra	-32.25	115.75	7	2022	4.2.2
Stigmatopora nigra	-32.15	115.733	8	2023	AMS
Stigmatopora nigra	-32.1833	115.733	8	2023	4.2.2
Stigmatopora nigra	-32.25	115.75	8	2022	4.2.2
Stigmatopora nigra	-32.1167	115.675	9	2021	4.2.2
Stigmatopora nigra	-32.1273	115.708	9	2022	4.2.2
Stigmatopora nigra	-32.1273	115.708	9	2021	4.2.2
Stigmatopora nigra	-32.1667	115.683	9	2021	4.2.2
Stigmatopora nigra	-32.2	115.7	9	2022	4.2.2
Stigmatopora nigra	-32.1273	115.708	10	2021	4.2.2
Stigmatopora nigra	-32.1167	115.725	11	2022	4.2.2
Stigmatopora nigra	-32.1273	115.708	11	2022	4.2.2
Stigmatopora nigra	-32.1273	115.708	11	2022	4.2.2
Stigmatopora nigra	-32.15	115.733	12	2021	4.2.2
Stigmatopora nigra	-32.0185	115.7848	-	1985	4.2.2
Stigmatopora nigra	-32.0394	115.7593	-	1980	AMS
Undifferentiated Stigmatopora	-32.25	115.75	1	2023	4.2.2
Undifferentiated Stigmatopora	-32.1167	115.725	2	2022	4.2.2
Undifferentiated Stigmatopora	-32.1167	115.675	4	2022	4.2.2
Undifferentiated Stigmatopora	-32.1273	115.708	4	2023	4.2.2
Undifferentiated Stigmatopora	-32.15	115.7	4	2022	4.2.2
Undifferentiated Stigmatopora	-32.15	115.733	4	2023	4.2.2
Undifferentiated Stigmatopora	-32.1833	115.733	6	2023	4.2.2
Undifferentiated Stigmatopora	-32.1167	115.675	7	2023	4.2.2
Undifferentiated Stigmatopora	-32.15	115.733	10	2022	4.2.2
Undifferentiated Stigmatopora	-32.2167	115.717	10	2022	4.2.2
Undifferentiated Stigmatopora	-32.1167	115.675	11	2021	4.2.2
Undifferentiated Stigmatopora	-32.15	115.733	12	2021	4.2.2
Undifferentiated Stigmatopora	-32.15	115.733	12	2022	AMS
Undifferentiated Stigmatopora	-32.25	115.733	12	2021	AMS
Urocampus carinirostris	-32.0074	115.8509	1	1987	AMS
Urocampus carinirostris	-32.0074	115.8509	1	1987	AMS
Urocampus carinirostris	-32.0229	115.8557	1	1980	AMS
Urocampus carinirostris	-32.0229	115.8557	1	1980	AMS
Urocampus carinirostris	-31.965	115.85	11	1986	AMS
Urocampus carinirostris	-31.965	115.85	11	1986	AMS
Urocampus carinirostris	-31.9875	115.8207	-	1984	AMS
Urocampus carinirostris	-31.9875	115.8509	-	1984	AMS

10.2 Appendix 2: Dive Survey and eDNA Sampling Sites



Figure A1. Location of dive surveys (circles) and eDNA sampling (diamonds). Those sites with evidence of the presence of syngnathids (observation or eDNA) are indicated by a closed icon. See also Table A2.

Site	Date	Latitude	Longitude	Туре	Species recorded
Ammunition Jetty	14/12/2022	-32.1241	115.7590	Dive (x2)	H. subelongatus
Kwinana Shelf 6	8/03/2022	-32.1414	115.7343	Dive (x2) eDNA	- H. subelongatus, S. argus
Kwinana Shelf 1	1/03/2022	-32.1472	115.7341	Dive (x2) eDNA	-
Kwinana Shelf 2	1/03/2022	-32.1501	115.7342	Dive (x2) eDNA	-
Kwinana Shelf 5	8/03/2022	-32.1528	115.7343	Dive (x2)	-
Kwinana Shelf 10	30/08/2023	-32.1538	115.7509	Dive	
Kwinana Shelf 11	30/08/2023	-32.1586	115.7353	Dive	H. subelongatus
Kwinana Shelf 3	1/03/2022	-32.1643	115.7365	Dive (x2) eDNA	- F. tigris
Kwinana Shelf 12	30/08/2023	-32.1731	115.7372	Dive	-
Kwinana Shelf 4	8/03/2022	-32.1738	115.7379	Dive (x2)	-
Kwinana Shelf 9	25/08/2023	-32.1853	115.7401	Dive	H. subelongatus
Kwinana Shelf 8	25/08/2023	-32.1939	115.7427	Dive eDNA	- S. argus
Kwinana Shelf 7	25/08/2023	-32.1985	115.7444	Dive eDNA	- F. tigris
Kwinana Bulk Jetty	7/01/2022	-32.2086	115.7650	Dive (x2)	H. subelongatus
Grain Terminal	10/12/2022	-32.2566	115.7480	Dive	H. subelongatus
Palm Beach Jetty	8/01/2023	-32.2760	115.7206	Dive	H. subelongatus

Table A2. Dive survey and eDNA sampling sites in Cockburn Sound and Owen Anchorage, arranged by latitude. Dives with two independent surveys are indicated by the suffix (x2). See also Figure A1 and Appendix 3.

10.3 Appendix 3: Environmental DNA (eDNA) Sampling

(see Section 5.2.4)

10.3.1 Methods.

Water samples were collected from six sites along the Kwinana Shelf, adjacent to the Westport footprint and/or the potential dredged channels (Table A2; Figure A1). The habitat was mixed seagrass, algae and low broken reef surrounded by sparse filter feeder communities in 8–10 m.

Sample collection & filtration

Water samples (5x 1 L replicates) were collected from each site using sterile Nalgene bottles that were opened underwater just above the substrate at the sampling site and then immediately closed following sampling. Water samples were frozen at -80°C until filtration (within one week) and filtered using a Sentino peristaltic pump onto 0.45 μ m pore size 47 mm filter membranes (Pall Life Sciences) Filter membranes were frozen at -20°C until DNA extraction.

DNA extraction and metabarcoding

Water samples were extracted using a DNeasy Blood and Tissue kit (Qiagen) and a modified protocol (Stat et al. 2018). Extraction controls (i.e. no sample) were implemented for every site and extracted alongside the water samples. Metabarcoding was performed in duplicate on each DNA extract and control. DNA was amplified to target Syngnathidae taxa using mitochondrial 16S markers: 16S_FishSyn_ShortF 5' GACGAGAAGACCCTGTGGAGC 3' 16S_FishSyn_ShortR 5' CCGYGGTCGCCCCAAC 3' (Nester et al. 2020). The assay gPCR reactions (25 mL) consisted of the following: 2.5 mM/L MgCl₂ (Applied Biosystems, USA), 1× PCR Gold buffer (Applied Biosystems), 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems), 0.25 mM/L dNTPs (Astral Scientific, Australia), 0.4 mg/ml bovine serum albumin (Fisher Biotec, Australia), 0.4 µmol/L forward and reverse primer, 0.6 µl of a 1:10,000 solution of SYBR Green dye (Life Technologies, USA) and 4 μ l of template DNA. gPCR amplifications were performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Waltham, Massachusetts). To reduce the likelihood of index-tag switching and chimera production, multiple unique forward and reverse fusion tag combinations were added to the qPCR products, each consisting of an adapter sequence, gene specific primers, and a unique multiple identifier (MID). A 'no template' control was also included in each qPCR to detect any cross-contamination between samples (n=16). Additionally, a positive control (*H. subelongatus* tissue) was used in duplicate. Thermocycler conditions were as follows: 95°C for 5 m, 50 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 45 sec, completed by a 72°C elongation step for 10 min. Extraction and negative controls showed no sign of amplification and were therefore excluded from downstream analyses.

Resulting amplicons were pooled in approximate equimolar ratios, size-selected using a Pippin Prep (Sage Science) and purified using a QIAquick PCR Purification Kit (Qiagen). The final library was quantified using a QIAxcel Advanced System (Qiagen) and a Qubit Fluorometric Quantitation machine (Thermo Fisher) and sequenced on an Illumina Miseq platform using a 300 cycle Miseq V2 Reagent Kit and custom sequencing primers at Curtin University in Perth, Western Australia.

Bioinformatics & taxonomic assignment

Sequences with 100% matches to Illumina sequencing adapters, index barcodes and template specific primers were retained for downstream analysis using Geneious v. 10.2.6. Usearch v. 10 (Edgar 2010) was used to quality filter and discard reads with error rates of 1%, short reads (<50 bp) and chimeras. Resulting sequences were dereplicated into unique sequences and denoised into zero-radius operational taxonomic units (ZOTUs; denoised OTUs that aim to report correct biological sequences at a higher resolution than OTUs (Edgar 2016; Callahan *et al.* 2017). Curated databases for select gene

regions of target taxa enhance the taxonomic range of current metabarcoding databases and the likelihood of correct taxonomic assignment. A custom 16S rRNA syngnathid database was created using 162 NCBI GenBank sequences and in-house syngnathid sequences. ZOTUs were then compared to our custom 16S Syngnathid database using the Basic Local Alignment Search Tool for nucleotides (BLASTn) on the Setonix system (Pawsey Supercomputing Centre, Western Australia). We used a lowest common ancestor approach so that detections of syngnathid species that were not in our library would still be identifiable as members of the Syngnathidae, albeit with a lower percentage identity, and possibly even to genus.

ZOTUs with BLASTn parameters of E value above 10^{-5} , percentage identity below 94% and query coverage per subject below 99% were removed to decrease uncertainty surrounding ZOTU taxonomic assignment (Alberdi *et al.* 2018; Porter and Hajibabaei 2018). Taxonomic identities of ZOTUs were assigned and visualised in MEGAN v6 (MEtaGenome ANalyzer; Huson *et al.* 2016) using the LCA (lowest common ancestor) parameters: min bit score 100.0 and reports restricted to the top 10% of matches.

10.3.2 Results

The 16S_FishSyn_Short assay detected a total of 42 fish taxa from the 30 replicate samples across 6 sites on the Kwinana Shelf. Among these taxa, three were from the family Syngnathidae and all three matched known sequences in our curated library. These were identified in only five of the replicate samples from four sites and were among the most abundant syngnathid species in CS/OA (*H. subelongatus, S. argus, F. tigris*; Section 5.2.4). All syngnathid species were detected from a relatively low number of reads (<250; Table A3). Only two additional taxa attributable to the Order Syngnathiformes (both in Mullidae) — *Upeneichthys vlamingii* (Bluespotted Goatfish) and *Upeneus tragula* (Bartail Goatfish) — were detected (number of reads 1171–7143 reads).

Table A3. Syngnathid species and the number of reads detected from water samples along KwinanaShelf using eDNA.

	Sites (Replicate #)				
Species	3 (4)	6 ₍₂₎	6 (5)	7 ₍₃₎	8 (3)
Filicampus tigris	246			132	
Hippocampus subelongatus			142		237
Stigmatopora argus		53			173

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