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A Remarkable Diversity of Syngnathid Fishes in a Highly Modified Large Coastal Embayment in the Southeastern Indian Ocean

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Abstract

Southern Australia supports a high diversity of fishes in the family Syngnathidae, which is recognised as globally threatened, facing threats from coastal development, over-exploitation and bycatch. In southwestern Australia, Cockburn Sound houses a large industrial port and naval defence assets with plans to substantially expand the port facilities but also sits within a recognised syngnathid 'hotspot'. Using a broad array of data 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, we compiled 4839 records of syngnathid fishes from the embayment. Twenty-one species of seadragons, seahorses or pipefishes were confirmed from an area of around 200 km^2, representing a high diversity by area on a global scale. This remarkable diversity exists despite the bay being a highly modified system. Many species are likely to face conservation concerns within the bay, but others may benefit from the addition of artificial structures. There is clearly a need to protect the diversity and mitigate detrimental impacts from development and ongoing infrastructure operations, including direct and indirect interaction, habitat loss, water quality, turbidity, noise and introduced pests.

Keywords: seahorse; pipefish; seadragon; Cockburn Sound; threatened; infrastructure development

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1. Introduction

The Syngnathidae (seahorses, pipefishes, seadragons) is a family of fishes mostly found in shallow temperate and tropical waters of all oceans and due to population densities, habitat requirements and life history characteristics of its species, is considered to have high conservation value. Syngnathid fishes are generally small and highly cryptic and inhabit shallow coastal waters, where they are especially reliant on habitats such as seagrass,

filter-feeder communities, shallow detritus, reefs and artificial structures [1–4]. They can be strongly associated with specific habitats and often have small home ranges or are relatively site-attached, at least as adults [5], and may have limited ability for dispersal away from those habitats [6,7]. They primarily feed on pelagic or bentho-pelagic crustaceans and are preyed upon by other fishes, crabs and birds [1,8]. Syngnathids are often in low 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 [9]. Many species have a complex mating system involving courtship, reproduction and male care linked to sexual selection [10] and most species have a relatively low reproductive potential, often producing fewer than 200 eggs per clutch [1,6].

Globally, syngnathid fishes face threats from coastal development, over-exploitation, bycatch and climate change [11,12], largely due to their life-history characteristics, specific habitat requirements and limited dispersal mechanisms. In recognition of this concern, syngnathids have various levels of local, national and international protections and many are assessed as Threatened or Data Deficient by the International Union for the Conservation of Nature (IUCN, [11]). Despite the conservation concerns for syngnathids, many species occupy highly modified coastal waters adjacent to substantially urbanised or industrialised areas. Conservation issues are likely to be more pronounced in regions where high levels of endemism coincide with high levels of urbanisation. For example, of the ~250 syngnathid species worldwide, at least 45 species occur in southern Australia, making it one of the most diverse families of coastal fishes in the region [1,13]. Around 88% of those 45 species are restricted to southern Australia, forming a global "hot spot" of endemism [14]. Yet, in the region, the syngnathid fauna commonly occurs in highly modified harbours such as Sydney Harbour, Port Phillip and Cockburn Sound [15–17].

Understanding the potential influences of coastal modifications on the biodiversity of fishes with high conservation value requires access to comprehensive datasets. Quantitative sampling of cryptobenthic fishes is difficult and inference on relative abundance is often inaccurate [18–20], especially when the fundamental baseline biodiversity is not well documented. Typically, common species or those that are more 'catchable' contribute most to quantitative catches in trawls and seines [2,21–24] with limited data on rare or hard-to-catch species gathered. Often, the most effective approach to understanding cryptic biodiversity is to compile data from multiple sources and accumulating species records is best achieved by utilising multiple survey methods across a long timeframe [25]. Combining traditional historical data from museums and other research programs with emerging tools such as citizen science and environmental DNA (eDNA) may provide a comprehensive approach to estimating biodiversity. Citizen science initiatives are becoming increasingly popular [26–28], particularly through the rising interest and accessibility of underwater photography. However, quality control of species identification by non-experts is often of concern. Initiatives that provide image-based observations (e.g., iNaturalist, Redmap) make it possible for experts to identify citizen-submitted observations, while maintaining publicly accessible data. In addition, eDNA metabarcoding may characterise whole species assemblages when combined with taxonomically verified specimen-anchored genetic sequences [29,30]. For rare and cryptic species, eDNA methods can be an important supplement to traditional survey methods and can sometimes be more sensitive and effective [29,31–33].

In this study, we use multiple historical datasets, contemporary surveys, citizen science and eDNA, to provide the first comprehensive summary of current knowledge about which syngnathid species are present in Cockburn Sound, a coastal area highly modified by heavy industrial, shipping and naval defence activities over many decades [17] with plans for future expansion [34,35]. It provides a "model" system of a highly modified coastal area in

temperate southern Australia within an endemic hotspot for Syngnathidae. We believe this is the first dedicated assessment of the syngnathid biodiversity in highly modified coastal systems within temperate Australia. This assessment allows us to examine resource use, including habitats and food, and life history characteristics to interpret the importance of the region as a refuge for syngnathid species across their broader geographic distributions, and assess potential winners and losers related to the past and future modifications of this coastal system. It also reduces data deficiencies for assessing "data deficient" species by IUCN.

2. Materials and Methods

2.1. Project Area and Survey History

Cockburn Sound and the adjacent Owen Anchorage (CS/OA) is a large coastal embayment in southwestern Australia stretching ~24 km north–south and ~10 km east–west (Figure 1). It comprises the area from Fremantle (approx. −32.056°) to the southern end 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°). The area supports many habitats typically used by syngnathid fishes and has long been known as an important refuge for many species. However, the habitat requirements, distributions and abundances of these highly cryptic fishes, both in Cockburn Sound and more broadly, is poorly understood. In some instances, even the true taxonomic identity of species is not clear [1,36].

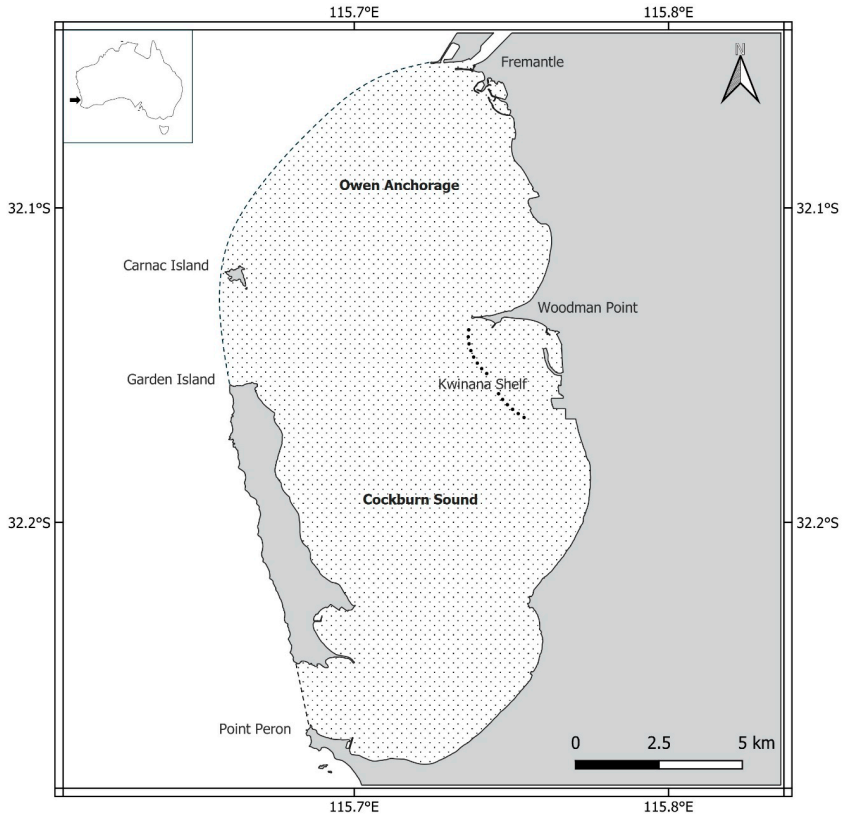

Figure 1. Location of Cockburn Sound and Owen Anchorage in southwestern Australia. Project area is shaded.

Commercial trawl studies in CS/OA reported no [37,38] or very few [22,39,40] syngnathids in the bycatch. More targeted seine studies detected up to five species, which are among the most common in the region [41–43] and, although the abundance of syngnathids

in catches was generally low, these uncommon species often contributed to statistical differences among sites. In the most comprehensive study specifically targeting syngnathid biodiversity in the region, Kendrick [44] and Kendrick and Hyndes [2] used small-mesh beam trawls to collect 1900 individuals from 14 species, with two species comprising nearly 95% of the catch. A follow up study detailed the diets of 12 of those species [45]. Baker [46] reported on two seadragon species using data from Dragon Search [47] with a single record of each from northern Cockburn Sound seagrass habitat and other records around Fremantle. Nester et al. [32] detected three common syngnathid species in Cockburn Sound using eDNA. A list of 24 species of syngnathid fishes was compiled from Perth coast waters [48], with many based on records or observations within CS/OA. Cockburn Sound's best known syngnathid, the West Australian Seahorse *Hippocampus subelongatus*, was the subject of a long-term study on reproductive biology, mating system and sexual selection, e.g., [5,49–51]. A small, managed fishery for aquaria exists in CS/OA, with a negligible-to-small harvest of at least two species (*H. subelongatus* and *Stigmatopora argus*; [52]) but data are commercially confidential.

2.2. Historical Data

Museum specimens of syngnathid fishes from CS/OA housed at the Western Australian Museum (WAM), Australian Museum (AMS) and the Australian National Fish Collection (CSIRO) were re-examined by an experienced fish taxonomist [GIM] using relevant literature and comparison with voucher specimens to confirm identifications. Extensive literature searches were undertaken to identify published primary literature and unpublished secondary and 'grey' literature on syngnathid fishes in the region. Location metadata were checked and corrected as required and associated habitat data were extracted. Additional observational data were harvested from unpublished field notebooks and unpublished data were extracted from Dragon Search [47].

Egg-bearing or brooding records specific to CS/OA were taken from Kendrick [44] and field notebooks (Kendrick, unpublished data). Specimens of larvae collected historically from the Perth metropolitan area (in AMS collection) as well as contemporary collections (see below) were used as evidence of breeding. Additional breeding evidence was extracted from literature sources but often applicable to populations outside of WA.

Hand-written field notes of extensive trawl surveys through Owen Anchorage in 1997 and 1998 [2,44] 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; [44]). 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 original study, trawls were randomised among grid squares of 500 × 500 m [44] and the grid square was identifiable from the field notes. A point approximating the centre of the relevant grid square was used as the location data for each of these records. Most of the records from this dataset had associated habitat data. Additional trawl records of *H. subelongatus* were extracted from Johnson et al. [22]. Diet data specific to CS/OA populations was taken from Kendrick and Hyndes [45].

2.3. Contemporary Surveys

Syngnathids were sampled using a range of vessel-based seines, trawls and sleds, as well as beach seines as part of general fish diversity surveys (full details in [53]). Vouchers of collected species were identified and DNA barcoded (see below). Records of larvae collected by Bongo net tows in CS/OA [54] are used here as evidence of breeding within CS/OA.

Twenty four SCUBA-based surveys (at 16 sites) were undertaken in Cockburn Sound, including the Kwinana Shelf (18 surveys) and coastal jetties (6 surveys) [55]. These surveys were 1 h in duration and loosely followed either a transect tape (in open habitat along Kwinana Shelf) or the pylons of a jetty. Surveys targeted 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 detect cryptic species in rubble and reef habitats.

2.4. Citizen Science

A 'project' was established within the iNaturalist platform in 2021 (https://www.inaturalist.org/projects/seahorses-pipefishes-seadragons-of-western-australia). The project harvested all historical and new records submitted to iNaturalist labelled with 'Syngnathidae' and 'Western Australia'. To increase uptake, the project was promoted to major dive groups. 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 project filters irrespective of whether the submitter was a project member. However, members could make the project 'trusted', which removed location obfuscation that is routinely applied to threatened species by the iNaturalist platform.

All submissions up to 31 January 2024 were manually checked and identified. Data were downloaded and the coordinates of all records 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.

2.5. eDNA

Replicate 1 L water samples (n = 5 per site) were collected from the seafloor by SCUBA at six sites along the Kwinana Shelf (see Supplementary Material S1). The eDNA metabarcoding workflow largely followed Nester et al. [32]. Briefly, samples were filtered onto 0.45 µm pore-size membranes (47 mm Pall Life Sciences filters, Corporation, New York, NY, USA) using a Sentino peristaltic pump. Filters were stored at −20 °C until DNA extraction using a modified DNeasy Blood and Tissue kit protocol (QIAGEN, Hilden, Germany) [56]. Metabarcoding targeted a short fragment of the mitochondrial 16S rRNA gene using broad-range fish primers known to amplify Syngnathidae taxa (16S_FishSyn_Short; [32]). To enhance taxonomic assignments, a custom 16S reference library was constructed using Sanger sequences from 29 putative syngnathid species. Bioinformatic processing included quality filtering, chimera removal, and denoising into ZOTUs using USEARCH (v10). ZOTUs were compared to both GenBank and the custom 16S reference library using BLASTn (version 2.16.0) and visualised in MEGAN v6 under LCA parameters. ZOTUs with <94% identity, <99% coverage, or E-values > 10^{-5} were excluded from downstream analyses. Full laboratory protocols and database details are provided in Supplementary Material S1.

2.6. Species Accumulation

To evaluate inventory completeness, species accumulation curves were generated with data pooled by decade. The accumulation of species was first plotted against the number of records (following a $\log_{10}$ transformation). The number of species was then predicted using permuted non-parametric species estimators. Species accumulation curves were calculated using three estimates of species richness (Sobs, Chao 1, Jackknife 1) in PRIMER v.7 [57] using 999 permutations. Permuted estimators avoid assumptions about underlying species

abundance distribution and estimate the 'true' number of species and thus the likelihood of undiscovered species [58,59].

3. Results

3.1. Compiled Records from All Sources

A total of 4839 records of adult or juvenile syngnathid fishes from CS/OA were collated from all sources (Table 1). Only 19 records were ambiguous, i.e., identified to family or genus only. Six records were added along the Kwinana Shelf based on eDNA sampling. Most of the records were sourced from historical trawl surveys [2,44], while more than 13% of records were contributed through the citizen science project (Table 1). An additional 1056 observations from the rest of the Perth metropolitan area were also collated to help interpret the data for CS/OA (Table 1). The records ranged across more than a century from 1913 (four specimens in WAM collection) to 2024 (Citizen Science). Confidence in the location of each record was generally high, with about 80% of records deemed to have an accuracy of ≤200 m (high) and 98% of records had an accuracy of ≤500 m.

Table 1. Syngnathid fish records contributing to the current dataset for the Perth metropolitan area and Cockburn Sound/Owen Anchorage (CS/OA). See Methods for data sources.

Data Source	Type	Date Range	Perth Metropolitan Area		CS/OA Only	
			# Records	# Species	# Records	# Species
Museum vouchers	Specimens: vouchered	1913–2021	336	24	78	16
Museum and other observations	Observations: photo and non-photo	1989–2023	69	12	25	8
Historical trawl data	Specimens: unvouchered	1989–2000	3912	18	3828	18
Citizen Science	Observations: photo	2006–2024	1349	14	679	12
Trawls	Specimens: vouchered and unvouchered	2022–2023	223	13	223	13
eDNA	DNA	2023	n/a	n/a	6	3
Total			5889	24	4839	21

3.2. Species Confirmed from Cockburn Sound and Owen Anchorage

Twenty-one species of Syngnathidae are now considered as confirmed from CS/OA (Table 2; Figure 2). The most reported syngnathids were two species of the pipefish genus *Stigmatopora* (which were the dominant species in our largest data source—historical trawls), followed by two seahorses, *Hippocampus* (one of which was the dominant species reported by citizen scientists). No confirmed records were identified for four species that were previously identified as possibly present in CS/OA (*Heraldia nocturna*, *Nannocampus subosseus*, *Phyllopteryx dewysea*, *Solegnathus lettiensis*; [60]). Fifteen species were confirmed as present in CS/OA during time of the project (2021–2024) with the last reported collection of the other six species ranging from 1987 to 1998 (Table 3). These six species are among the rarest, with one to six records each (Table 3). Three of the 21 species (*Hippocampus subelongatus*, *Mitotichthys meraculus* and *Idiotropiscis australe*) are considered Data Deficient on the IUCN Red List while the remaining species are listed as Least Concern.

Table 2. Occurrence of syngnathid fishes in different habitats from Cockburn Sound and Owen Anchorage based on catch data and/or literature. Numbers indicate records compiled during present study. + indicates a habitat reported in literature (see Supplementary Material S2). IUCN Red List status is Least Concern (LC) or Data Deficient (DD). ˆ *Zostera*.

Species	IUCN Red List	*Posidonia coriacea*	*Posidonia sinuosa*	*Posidonia* Undefined	*Amphibolis*	Undefined/ Other Seagrass	Algae	Reef	Sponge, Rubble	Jetties, Wrecks
Campichthys galei	LC					1	1	1	3	
Filicampus tigris	LC		1		1	+	1	10	3	15
Hippocampus subelongatus	DD	2	7			+	+	27	14	374
Hippocampus tuberculatus	LC	19	53		1	3	2	6	11	11
Histiogamphelus cristatus	LC	53	4		2	1		6	10	1
Idiotropiscis australe	DD					1	+	3	2	5
Lissocampus caudalis	LC	1		+	4	1		+	2	
Lissocampus fatiloquus	LC				+	1	2	1	2	1
Lissocampus runa	LC		1	+		2 ˆ	2	1	+	
Maroubra perserrata	LC							1		1
Mitotichthys meraculus	DD	18				1		3	3	
Phycodurus eques	LC			+			+	+		1
Phyllopteryx taeniolatus	LC	16	4		4	+	1	17	4	
Pugnaso curtirostris	LC	19	11		8	1	+	+	2	1
Stigmatopora argus	LC	513	1438		73	6	5	1	155	11
Stigmatopora nigra	LC	372			17	5	1	4	213	4
Stipecampus cristatus	LC	1	1		1	1	+	+		
Urocampus guentheri	LC					3 ˆ				
Vanacampus margaritifer	LC		1	+		+	+			
Vanacampus phillipi	LC	10	11		3	+		+	6	
Vanacampus poecilolaemus	LC	4	65						2	

Table 3. Number of records of species of syngnathid recorded in Cockburn Sound and Owen Anchorage in each month from all sources. The year each species was last recorded is indicated.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Last
Campichthys galei		1					1	2		1	1		6	1997
Filicampus tigris	8	16	2	10	10	1	19	7	11	8	11	8	111	2023
Hippocampus subelongatus	44	55	37	70	68	19	54	57	25	57	53	32	571	2024
Hippocampus tuberculatus	28	25	6	4	23	3	11	18	12	11	10	4	156	2023
Histiogamphelus cristatus	9	30		3	2	5	2	19	9	3	11	7	100	2023
Idiotropiscis australe				2		3	3	1	1	2	3		13	2023
Lissocampus caudalis	1	2		1	1		1	2					8	2023
Lissocampus fatiloquus	1				5		1	1		3	1	1	13	2022
Lissocampus runa	1	1			1								3	1998
Maroubra perserrata												1	1	1990
Mitotichthys meraculus	4	7	1	1	4			6	3		7	5	38	2024
Phycodurus eques	1				1								2	1998
Phyllopteryx taeniolatus	5	15	1	2	3		3	3	3	2	4	3	45	2022
Pugnaso curtirostris	12	17	4	2	3			1	2		9	9	59	2023
Stigmatopora argus	298	1020	149	113	302	76	119	278	67	53	130	148	2754	2023
Stigmatopora nigra	69	238		53	33	57	24	77	63	12	63	112	801	2023
Stipecampus cristatus	1	1		1									3	1998
Urocampus guentheri		1											1	1987 ^
Vanacampus margaritifer		2											2	2023
Vanacampus phillipi	12	13		2	1		2	3			3		36	2023
Vanacampus poecilolaemus	20	30	14	5	5	5	3	3	1		4		90	2022

^ larval record (see Supplementary Material S3).

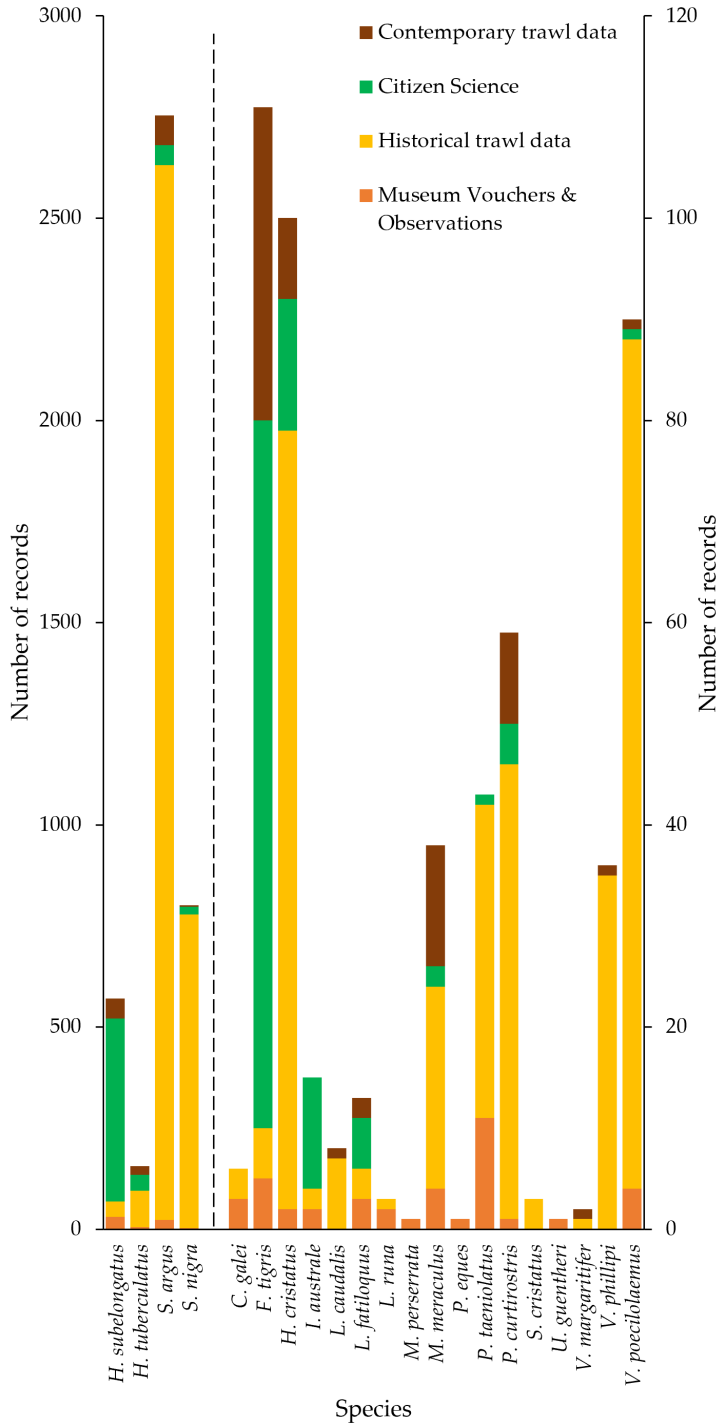

Figure 2. Number of observations of syngnathid species from Cockburn Sound and Owen Anchorage from the major sources of data. Frequently recorded species to left of dotted line use primary *y*-axis and less commonly recorded species to right of dotted line use secondary *y*-axis.

All 21 species of syngnathid fishes recorded from CS/OA were represented in historical records (nearly 4000), especially museum records and unpublished/partially published trawl data (Figure 2). This ranged from single records for two species (*Maroubra perserrata, Urocampus guentheri*) to more than 2600 records for *Stigmatopora argus* (Figure 2). Contemporary trawls and other benthic sampling added 223 specimens from 13 species (Figure 2), particularly from previously poorly sampled areas of CS/OA. No additional species beyond those identified in historical records were found during the contemporary surveys; however, many distributional records within CS/OA were added.

Despite focused 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). The iNaturalist citizen science project had 679 observations of 12 species of syngnathid fishes from CS/OA (Table 1; Figure 3). The species most recorded in CS/OA by citizen science observers was *H. subelongatus*, representing two-thirds of all submissions (Figure 2). The pipefish *Filicampus tigris* was the next most commonly reported species (10% of submissions). A small proportion of citizen science observations from CS/OA (1.6%) could not be identified to species based on the uploaded images.

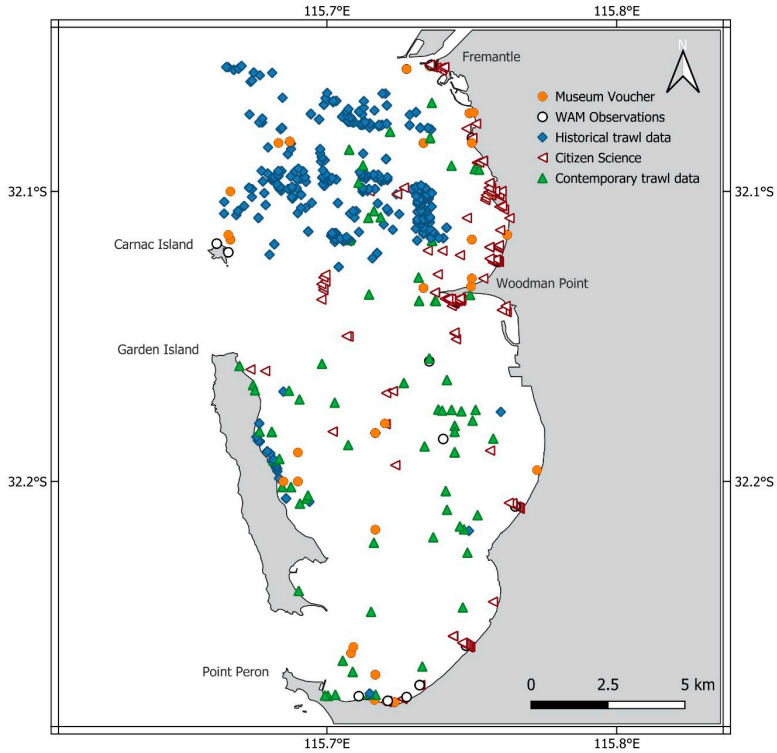

Figure 3. Spatial distribution of 4833 syngnathid records from Cockburn Sound and Owen Anchorage. Many records overlap and are obscured.

The 16S eDNA assay detected 42 fish taxa from the 30 replicate samples across 6 sites on the Kwinana Shelf (see Supplementary Material S1). Among these taxa, three were from the family Syngnathidae and all matched known sequences in our library and were among the most recorded syngnathid species in CS/OA (*H. subelongatus*, *S. argus*, *F. tigris*). Syngnathids were identified in only five of the 30 replicate samples from four sites and all three species were detected from a relatively low number of reads (<250).

3.3. Species Richness and Inventory Completeness

Species accumulation was influenced by effort across time (Figure 4). Species were added to the inventory incrementally in the first part of the 20th century, but this jumped dramatically in the 1990s when a spike of more than 3800 records added another 11 species. Despite more than 1000 new records in the 21st century, no additional species were recorded. Actual and permuted species accumulation curves reached asymptote, suggesting that the inventory is nearly complete (Figure 4B).

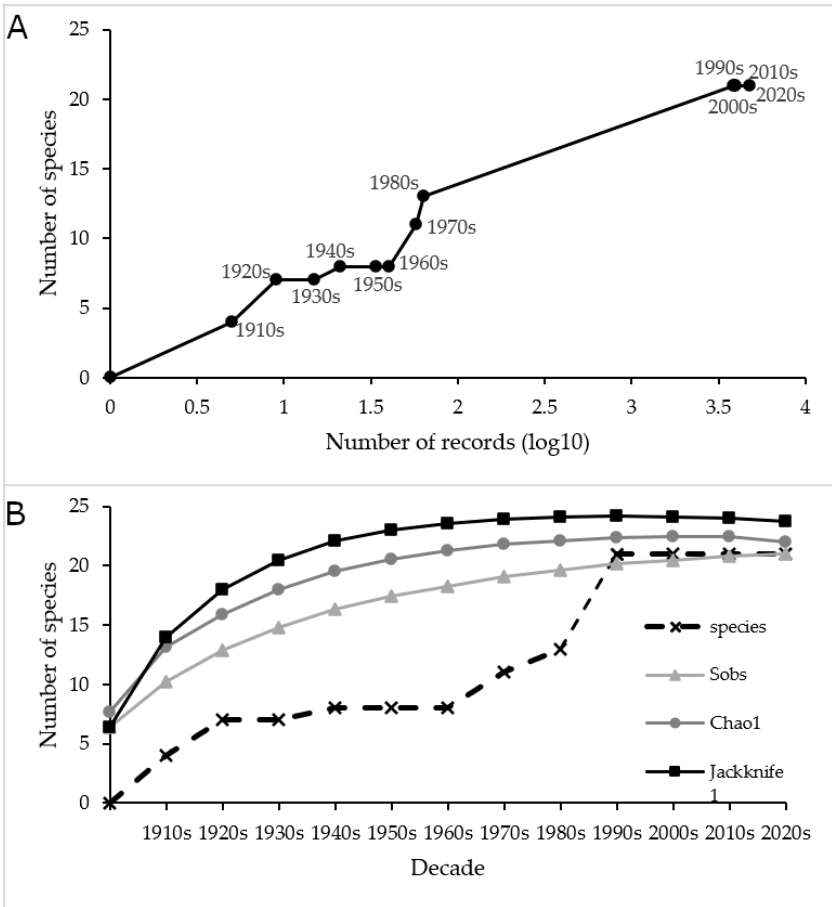

Figure 4. Species accumulation curves for syngnathid records from Cockburn Sound and Owen Anchorage. (**A**). Species accumulation with number of individual records plotted by decade. (**B**). Species accumulation over time (by decade): actual (×) and permuted (■, ●, ▲).

3.4. Spatio-Temporal Distributions

Notwithstanding differences in sampling effort and spatial focus of the various data sources, syngnathid fishes appear to use almost all of CS/OA (Figure 3). The greatest density of records was from Owen Anchorage and the northern parts of Cockburn Sound, which reflects a sampling bias from historical trawls [2]. 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 CS/OA. Species-specific data are presented in Supplementary Material S2.

Habitat data were available for 1322 records in CS/OA (Table 2). Most species of Syngnathidae found within CS/OA are broad habitat generalists; however, the occurrence of many species is strongly linked with seagrasses and habitats associated with soft substrates (e.g., sponge 'gardens' over sand and mud; Table 2; Supplementary Material S2). *Stigmatopora argus* had far more occurrences over seagrass meadows (particularly *Posidonia sinuosa* and *P. coriacea*) and other structured habitats (e.g., sponges), while *S. nigra* tended to occur more frequently in *P. coriacea* and other structured habitats (e.g., sponges). The occurrence of some species is very specific to a species of seagrass. For example, *Mitotichthys meraculus* was only reported from *P. coriacea* and adjacent sand or reef areas and *Vanacampus 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, with *H. subelongatus* particularly prevalent on the latter.

It was possible to determine the month of capture for 4814 (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 3). However, for all other species (with ≥13 records), the spread of records across months suggests continuous occurrence in CS/OA across the year (Table 3).

3.5. Breeding Records

The most breeding records for syngnathid fishes in CS/OA occur in the warmer months between September and April (Table 4; Supplementary Material S2). 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 4).

Table 4. Known breeding times for all species recorded in Cockburn Sound and Owen Anchorage. Breeding and/or larval records from within Cockburn Sound and Owen Anchorage are indicated by (√) in dark grey. Breeding records inferred from other sources are indicated by (*i*) in light grey. Data sources for each species are provided in Supplementary Material S3.

Species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Campichthys galei [1]												
Filicampus tigris	*i*	√	*i*	*i*	√						√	*i*
Hippocampus subelongatus	√	√	√	√						√	√	√
Hippocampus tuberculatus	√	√	*i*	√	*i*	√	√	√	√	√	√	*i*
Histiogamphelus cristatus	√	√	*i*	√	√	*i*	√	√	√	√	√	*i*
Idiotropiscis australe								√	*i*	*i*	√	
Lissocampus caudalis [2]	*i*	*i*										*i*
Lissocampus fatiloquus										√		
Lissocampus runa [1]												
Maroubra perserrata [2]	*i*	*i*										*i*
Mitotichthys meraculus			√	√					√			
Phycodurus eques	*i*									*i*	*i*	*i*
Phyllopteryx taeniolatus	*i*						√	√	√	*i*	√	*i*
Pugnaso curtirostris	√	√						√	√	*i*	√	*i*
Stigmatopora argus	√	√	√	√	√	√	√	√	√	√	√	√
Stigmatopora nigra	√	√	√	√	√	√	√	√	√	√	√	√
Stipecampus cristatus [2]	√	*i*										*i*
Urocampus guentheri	√	*i*	*i*	*i*	*i*				*i*	*i*	√	*i*
Vanacampus margaritifer [1]												
Vanacampus phillipi	√	√						√	*i*	*i*	√	*i*
Vanacampus poecilolaemus	√	√	*i*	*i*	√	√	√	√	√	*i*	√	*i*
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; [2] reported only as 'summer months'.

4. Discussion

Cockburn Sound/Owen Anchorage is an important coastal embayment for syngnathid fishes on a global scale. With 21 confirmed species from an area of around 200 km^2, this small region supports a remarkably high syngnathid diversity. For example, coastal waters of the entire continent of Europe (>550,000 km^2) supports only 19 species [12]. Furthermore, this single bay in southwestern Australia represents almost half of the species recorded

across the entire coast of southern Australia. While Gulf St Vincent in South Australia, a similar environment to CS/OA, supports 27 species, its ~6800 km^2 is 34 times larger in area [61]. Similarly, Port Phillip in Victoria may support up to 19 species in an area of ~1930 km^2 [62]. At a similar latitude, Sydney Harbour (estuary) supports 18 species of syngnathids in an area of around 55 km^2 [15]. However, the species recorded in these harbours may be underestimated, as they have not been assessed or quantified to the level we have been able to achieve by compiling a broad suite of datasets and verifying Syngnathidae diversity in CS/OA. Similar to other southern Australian bays, CS/OA has been highly modified from human activities since European settlement [17,63]. Despite those modifications, the region supports a high diversity of seahorse and pipefish species, with some species likely impacted detrimentally [64] while others may benefit from the addition of coastal infrastructure created for ports and industry.

Despite a dataset biased toward one collecting method and three common species, the number of syngnathid species recorded in CS/OA reflects both high ecological richness and a high inventory completeness. In part, this is a consequence of the comprehensive dataset compiled using multiple methods over a long time. However, it is also a true measure of diversity in the region. Estimated species accumulation curves strongly asymptote and converge around the same estimate of species richness that we recorded. Only three additional species of syngnathids are known from nearby waters and CS/OA is unlikely to have suitable habitat/depth for two of these (Supplementary Material S2, [48]). Despite more than 1000 records being added since the year 2000, no additional species have been added to our inventory.

Noteworthy in our results is the contrast between cumulative historical richness (21 species over a century) and contemporary richness (15 species between 2021 and 2024). For conservation, these metrics serve distinct purposes. The cumulative inventory represents the ecosystem's maximum historical biodiversity value, while the contemporary data may reveal which species are actively persisting. We do not know if this contrast in diversity reflects a real difference in historical versus contemporary diversity or is a result of detectability and effort. If it indicates that species absent from contemporary surveys have faced local population declines or extirpation, it signals the need for targeted species and/or habitat protections. However, this disparity may reflect differences in sampling effort/method, as the historical inventory spans decades of accumulation using multiple methods, whereas the contemporary dataset captures a limited, short-term timeframe dominated by citizen science and large trawls. In this context, detectability is important to consider for rare or very small species. Of the six species not detected since 2000 (Table 3), all are rare in the broader coastal environments around CS/OA. Five of these species were only detected in low abundance in CS/OA by dedicated syngnathid sampling in the 1990s [2], of which three have not been reported after 2000 from the broader region outside CS/OA, demonstrating they are rarely recorded. The sixth species *U. guentheri* is the smallest species in the region and was not collected in CS/OA during dedicated syngnathid trawls but has been detected in larval fish collections. There are several recent adult records from the nearby Swan River estuary and it may be more common that current sampling suggests or CS/OA may provide less suitable habitat.

Supporting 21 of the 25 species in this portion of the southwestern Australian syngnathid "hotspot" [14,48], CS/OA is undeniably valuable for Australia's temperate syngnathid fishes. Although no species are solely reliant on CS/OA, it is clearly a stronghold for some (e.g., *H. subelongatus*). All syngnathid species from CS/OA are endemic to Australia. Most (17 species) are endemic to the southern half of Australia, some as far east as New South Wales or Queensland, others only 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 making them especially vulnerable to local impacts from human modifications and ocean warming.

After climate change [12], habitat loss, primarily through pollution, coastal infrastructure development and destructive fishing methods, remains one of the most significant pressures facing shallow marine communities that support syngnathids [11,18,21,64–68]. Syngnathid fishes are potentially highly susceptible to such disturbances due to a range of life-history characteristics [6,69]. Most syngnathid species tend to inhabit coastal neritic waters, which are most impacted by anthropogenic stressors. Furthermore, they are often restricted to habitats that are degraded by those stressors (e.g., seagrass). Some species have habitat specificity down to a single seagrass species. For example, *V. poecilolaemus* is found almost exclusively in meadows of *Posidonia sinuosa*, as supported by abundance comparisons using standardised effort across vegetated and unvegetated habitats in CS/OA [2]. In addition, most species have low population densities and, despite seemingly large distribution ranges, many species are patchily distributed. For example, despite a distribution encompassing most of the temperate west coast of Australia (Cape Leeuwin to Shark Bay), *H. subelongatus* is rarely found outside the three large turbid 'bays' with estuary-like conditions—Cockburn Sound, Swan River estuary, Shark Bay.

Cockburn Sound has undergone significant changes over the last 100+ years, including negative impacts from nutrient enrichment that resulted in nearly 80% of its seagrass area being lost, along with infill for port infrastructure [17]. Despite these impacts, the region contains a high syngnathid diversity. This partly reflects syngnathids using a range of habitats within CS/OA to varying degrees, including the pelagic environment, although each habitat type supports a slightly different suite of species and/or life stages. The greatest diversity of syngnathid fishes in CS/OA was recorded from seagrass meadows. While these habitats were probably also the best sampled, this conforms with the association of many syngnathid species with seagrass meadows across the globe, particularly pipefishes [4,70,71]. It also partly reflects the high diversity of seagrass species in the region [72], and the segregation of species or life history stages to particular seagrass habitats. For example, based on a targeted, quantitative assessment of habitat use by syngnathids using beam trawls, *S. argus* is more abundant in meadows of *Posidonia sinuosa* and *P. coriacae*, with an ontogenetic shift from the latter to former, while *S. nigra* occurs predominantly in *P. coriacea* [2]. However, our assessment based on an expansion of collection methods, shows that both *Stigmatopora* species, as well as a range of other species, also occur in other structured habitats, including sponges, rubble, reef and coastal infrastructure. Eighteen of the 21 species were recorded over reef, while 11 species were recorded around coastal infrastructure such as jetties and shipwrecks. It is therefore relevant that these structured habitats have expanded in Cockburn Sound over the period of seagrass loss [17]. We hypothesise that there would be winners and losers resulting from these changes. The large reduction in seagrass area would almost certainly result in decreased abundances of species such as *S. argus* that are strongly associated with seagrass in Cockburn Sound but is unlikely to have caused local extinctions of those species since seagrass meadows still occur in the sound and the fish can occupy other habitats. In comparison, we imagine that the addition of coastal infrastructure may have enhanced the amount of structured habitat in Cockburn Sound, a system that would have had very little structured, hard-surfaced habitats prior to European arrival [17]. Species such as *F. tigris* and *H. subelongatus* have relatively high occurrences on these human-made habitats, relative to nearby habitats. Both species are known to be sponge and rubble areas that are typically associated with human-made structures such as pylons of jetties [1,13,73] (also see Supplementary Material S2), suggesting that they may benefit from the expansion of these structures. In addition, syngnathid fishes appear to have a patchy spatial distribution across the whole

area, including the vast soft-sediment basin of central Cockburn Sound. This may relate to the reduced hydrodynamics of Cockburn Sound, conditions that positively influence syngnathid species elsewhere [4]. Thus, CS/OA appears to provide a refuge for a high diversity of Syngnathidae despite it being highly modified from port, industry and coastal developments, suggesting that other highly modified coastal systems could also play a key role in supporting high syngnathid diversity.

Six percent of syngnathids globally are threatened with extinction and, given that many species were assessed by IUCN as Data Deficient, that number is likely to be an underestimate [11]. For some species at least, our data provides additional insights to warrant revised assessments. Eighteen of the species recorded from CS/OA were considered Least Concern (Table 2), although four species (*P. eques*, *P. taeniolatus*, *S. argus* and *S. nigra*) had evidence of decreasing populations across their whole distribution [74–77]. Three species were Data Deficient (*H. subelongatus*, *I. australe*, *M. meraculus*; Table 2). We have shown that *H. subelongatus* is one of the most common syngnathids in the region and may benefit from infrastructure construction. Very few specimens of *M. meraculus* and *I. australe* were known at the time of the last IUCN assessments [78,79] and, although they remain relatively uncommon, we have shown that these species are increasingly being photographed by a growing cohort of citizen scientists.

Life history attributes may make living in a highly disturbed environment challenging for most species of syngnathids [66,80]. Species with low adult population density, short breeding season, complex mating system [10,50,51], low fecundity, short pelagic larval stage and limited dispersal [44] may be impacted by poorly timed maritime or construction activity, especially if it coincides with stochastic weather events or adverse environmental conditions (e.g., marine heatwave; [81]). In addition, some species have small home ranges and are short lived, 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 [5]. The diet of many species is dominated by only a few target prey items (see Supplementary Material S2) meaning impacts to prey populations may have a sizeable flow-on effect to syngnathids. Conversely, species with an extended breeding season, prolonged pelagic larval stage, a capacity to disperse, behavioural plasticity and a broad diet may be more adaptable and better able to avoid localised impacts [80], especially if they can also utilise the increasing amount of artificial structure [82,83].

Biodiversity data are often necessarily reduced to community level analyses with the taxonomic identity of species making up the community buried in statistics [84,85]. This is especially true for syngnathid fishes, which are of low interest to fisheries, uncommon and difficult to sample and identify. Furthermore, assessments of biodiversity at small regional scales such as CS/OA often remain as unpublished reports [22,60]. As compiled data from multiple sources using multiple methods, the dataset presented here is the most comprehensive and quality checked data available for CS/OA and highlights the need for similar assessments for other key areas within temperate southern Australia, and other hotspots of syngnathid diversity and endemism. From our assessment, it is evident that no single method of survey is likely to provide a comprehensive assessment of the biodiversity and spatio-temporal distribution of syngnathids [2,21–24,86]. However, as we have shown, the need for different methods to overcome the issues of habitat choice, catchability and detectability to assess biodiversity, with the presence of both very rare and highly abundant species, leads to biased datasets for community-wide ecological or temporal change analyses. The utility of citizen science initiatives in detecting species presence and distributions is increasingly recognised [26–28,69,87]. Importantly, involving non-professionals creates a sense of stewardship and engagement [88]. Citizen

scientists contributed 14% of the records for the current project in around two years. The iNaturalist records have publicly available images and data that are verifiable by experts and importantly, freely available for other researchers. One limitation of this source of data is that records are often concentrated around popular dive locations or easily accessible sites (see Figure 3). Although the successful identification of syngnathid taxa demonstrates the efficacy of eDNA analysis in detecting target species within aquatic ecosystems, there were relatively few syngnathid detections (Supplementary Material S1) despite several species being abundant in the region. This highlights the limitations with eDNA to assess diversity of syngnathids. Indeed, detecting syngnathid eDNA in aquatic environments has been shown to be inherently challenging because they are less prone to shedding detectable amounts of DNA into the environment due to their small body size, absence of scales, low population density/biomass and preference for cryptic habitats that might retain shed DNA [32].

Cockburn Sound and Owen Anchorage pose a common dilemma. It is one of Australia's crucial ports contributing substantially to the national GDP and naval defence, with infrastructure to expand in the near future [34,35]. At the same time, it is home to a remarkable diversity of potentially vulnerable fishes that are still relatively poorly understood. Indeed, the absence of six species from recent surveys warrants consideration in planning for future environmental pressures. Specific mitigation efforts to protect syngnathid fishes from development are rarely empirically tested. While the provision of artificial 'replacement' habitat (e.g., seahorse hotels, jetties) is beneficial for some species [82,89], it often provides only one type of habitat among several that might be under threat and is probably not suitable for most species. 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 [6,66].

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d18070437/s1, Supplementary Material S1. Environmental DNA (eDNA) sampling methods and results [additional references [90–95]]; Supplementary Material S2. Species accounts summarising taxonomic, life history and ecological data compiled for each syngnathid species in Cockburn Sound and Owen Anchorage (CS/OA), with abundance statement for CS/OA and IUCN assessments [additional references [79,96–174]]; Supplementary Material S3. Larval records compiled through the course of the current project.

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Data Availability Statement: The data that support the findings will be available through Data WA and the Shared Land Information Platform (SLIP) at https://www.data.wa.gov.au following the completion of the WAMSI Westport Marine Science Program.

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Abbreviation

The following abbreviation is used in this manuscript:

CS/OA Cockburn Sound/Owen Anchorage

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