Spanish Marine National Parks: Priority areas for the conservation of a vulnerable family of fishes

Miquel Planas Oliver

Instituto de Investigaciones Marinas (CSIC)

Cristina Piñeiro-Corbeira

Universidade da Coruna

Carmen Bouza

Universidade de Santiago de Compostela

Inés Castejón-Silvo

Institut Mediterrani d'Estudis Avancats

Manuel Vera

Universidade de Santiago de Compostela

Marcos Regueira

Instituto de Investigacions Marinas

Verónica Ochoa

Instituto de Investigacions Marinas

Ignacio Bárbara

Universidade da Coruna

Jorge Terrados

Institut Mediterrani d'Estudis Avancats

Alexandro Chamorro

Instituto de Investigaciones Marinas

Rodolfo Barreiro

Universidade da Coruna

Jorge Hernández-Urcera

Instituto de Investigaciones Marinas

Irene Alejo

Universidade de Vigo

Miguel Nombela

Universidade de Vigo

Manuel Enrique García

Instituto de Investigaciones Marinas

Belén G. Pardo

Universidade de Santiago de Compostela

Viviana Peña

Universidade da Coruna

Pilar Díaz
Universidade da Coruna

Javier Cremades
Universidade da Coruna

Beatriz Morales-Nin
Institut Mediterrani d'Estudis Avancats

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Abstract

Background: Syngnathid fishes (Actinopterygii, Syngnathidae) are flagship species highly associated to seaweed and seagrass habitats of marine ecosystems biodiversity. Seahorses and pipefish are highly vulnerable to anthropogenic and environmental disturbances, but most species are currently Data Defficient by IUCN (IUCN, 2019), requiring more biological and ecological research. This study provides the first insights on syngnathid populations in two Spanish National Parks (PNIA –Atlantic- and PNAC – Mediterranean-). Fish were collected periodically, marked, morphologically identified, analyzed for size, weight, gender and sexual maturity, and sampled for further stable isotope and genetic identification. Due the scarcity of previous information, habitat characteristics were also assessed in PNIA.

Results: Syngnathid diversity and abundances were low, with two species identified in PNIA (Hippocampus guttulatus and Syngnathus acus) and four in PNAC (S. abaster , S. acus , S. typhle and Nerophis maculatus). Syngnathids from both NPs differed isotopically, with much lower δ15 N in PNAC. The dominant species were S. abaster in PNAC and particularly S. acus in PNIA. Syngnathids preferred less exposed sites in macroalgal assemblages in PNIA and Cymodocea meadows in PNAC. In S. acus from PNIA, the occurrence of very large specimens, the absence of small-medium sizes and the isotopic comparison with a nearby population suggest that the population is mainly founded by breeders that migrate seasonally. Novel 16S rDNA haplotypes and sequence variants were detected for Hippocampus guttulatus , N. maculatus , S. acus, and S. abaster . Our data suggest the presence of a cryptic Syngnathus species in PNAC,

Conclusions: This is the first multidisciplinary approach to the study of syngnathids in Spanish marine NPs. Habitat preferences and population characteristics in both NPs differed. Further studies are needed to assess potential misidentifications of Syngnathus genus in PNAC, and migratory events in PNIA. We propose several preferential sites in both NPs for future monitoring of syngnathid populations and some recommendations to undertake.

Background

Syngnathidae is a singular fish family mostly inhabiting temperate and tropical sheltered, coastal marine waters (Foster and Vincent 2004; Kuiter 2009; Planas et al. 2017). Seahorses and pipefish utilize rocky, muddy, sandy, and rubble bottom habitats, generally associated to macrophytes communities. Commonly, they have elongated bodies, a tiny mouth at the end of a tubular snout and semi-flexible bodies encased in thick bony plates and rings. As a result, they are relatively slow motion compared with other fishes. Different types of mating patterns (mate choice and mating competition) and brood structures (from simple ventral gluing areas to fully enclosed pouches) are present in these ovoviviparous fishes, with egg production synchronized with brooding in males. Syngnathids are secondary consumers with specialized and opportunistic predatory strategies, ambushing small prey (mainly planktonic and nektonic crustaceans), and showing a variety of diets and foraging behaviors across genera and

locations. Seaweeds and meadows promote the growth of most food sources. In some species, several appendages enhance their cryptic ability to avoid predators.

Syngnathids are valuable flagship species for conservation programs that will simultaneously benefit other fish (Shokri et al. 2009). Many species are vulnerable and threatened by habitat loss (pollution, sedimentation, eutrophication) and degradation caused by commercial, residential, and habitat disturbance through boating and shipping (IUCN 2019). More than half of syngnathid species (two seahorse and eleven pipefish species) inhabiting Spanish coasts are currently considered as Data Deficient, and further research is needed to understand their biology and ecology (IUCN 2019).

Confuse identifications have been reported due to cryptic morphology and unclear diagnostic traits among species, stressed by historical reference labelling errors in particular cases such as European *Syngnathus* genus (Hablützel and Wilson 2011; Woodall et al. 2018). Genetic data are useful to solve taxonomic issues and complement morphological information, as a basic step towards the characterization and conservation of species and associated habitats (Woodalll et al. 2018). Different mitochondrial markers have shown strong molecular support for species identification of seahorse and pipefish to clarify population and conservation studies (e.g., López et al. 2010; Sogabe and Takagi 2013; Woodall et al. 2018).

Studies on syngnathids in the Iberian Peninsula are scarce, and the present study is the first approach to globally evaluate syngnathid populations in Spanish coasts, particularly in marine National Parks (NP). Studies conducted in NPs would be very valuable considering their protection status and the supposed reduced impacts of most potential disturbances. Currently, there are two marine National Parks (NPs) in Spain, differing in their characteristics and biodiversity: Atlantic Islands National Park (PNIA) (Atlantic Ocean, NW Spain) and Cabrera Archipelago National Park (PNAC) (Balearic Islands, Mediterranean Sea). NPs are the best areas for marine ecosystems to survive and succeed in biodiversity conservation. However, protection requires a deep knowledge and analysis of habitats, values and threats, particularly for exceptional species and populations.

PNIA was declared Nature Reserve in 1980 and Spanish National Park in 2002. Cíes Archipelago is located in PNIA and was declared Natural Park, Special Protection Area (SPA), Site of Community Importance (SCI), OSPAR area, and UNESCO World Heritage candidate (UNESCO, 2019). PNIA has a total area of 8,480 ha and the marine area (86% of total surface) is characterized by cold waters with a slight warm influence by the Atlantic Gulf Stream (Prego et al. 2001). Tidal regime is semi-diurnal with an average range of about 3 m, a minimum range (neap tides) of 1.2 m and a maximum (spring tides) exceeding 3.5 m (Rey Salgado 1993). Seawater temperature is homogeneous in winter (13–16 °C) and stratified in summer (12–18 °C) due to the warming of upper layers. Surface water temperature typically ranges from 13.4 °C to 18.7 °C in the southern coast and from 13.4 °C to 18.0 °C in the northern coast (Puertos del Estado 2017). Marine ecosystems in PNIA host complex habitats and numerous ecological niches due to the extraordinary rich biota inhabiting soft and rocky floors typical of protected, semi exposed and exposed environments. Rocky shores are covered by seaweeds ranging from kelp forests,

fucacean algae to algal turfs (Piñeiro et al. 2014; Fernández et al. 2020). The Western side is exposed to Atlantic open water, and extreme sea currents and waves, particularly in winter. The subtidal zone of that side is dominated by hard substrates covered by crusty, coralline and other turf-forming seaweeds, and fauna such as sponges, bryozoans or ascidians. The Eastern side is less exposed due to its position facing the Ría de Vigo. The area is also characterized by high species richness and production creating an extraordinary commercial interest for fisheries and aquaculture (Picado et al. 2014). It is promoted by important seasonal phytoplankton blooms (Álvarez-Salgado et al. 2000; Rodil et al. 2009), and secondary production (Valdés et al. 1990; Buttay et al. 2015), with high abundances in summer and changes in community structure throughout the year. Copepods are largely predominant in winter, being accompanied in summer by other groups of fauna (Buttay et al. 2015).

The fishery system in PNIA is complex, comprising 565 boats (mostly artisanal) that use 19 fishing gears in an area of 26.6 km^2 (Ouréns et al. 2015), targeting common octopus, cuttlefish, shrimps, sardine, crabs, sea urchins, clams, razor shells or goose barnacles (Cambie et al. 2012). Some of those gears, especially active fishing ones, have negative impacts on syngnathids (by-catch and substrate degradation). Fishing ground is protected but not currently subject to a special regulation. The pressure exerted on the resources is unknown and effective regulations cannot be implemented (Ouréns et al. 2015). Tourism has sharply increased in last decade, and nature activities (snorkel or scuba diving) are increasing, promoting the awareness for the conservation of marine ecosystems (Piñeiro et al. 2014; Piñeiro-Corbeira et al. 2020).

Cabrera Archipelago National Park (PNAC) is an IUCN category II Marine Protected Area (MPA) located 10 km southeast of Majorca (Balearic Islands, Mediterranean Sea), declared Spanish National Park in 1991, and has expanded up to 90.800 ha in 2019. Oceanographic data indicate stratification of summer water column, horizontal distribution of water masses and hydrodynamic features linked with Mediterranean seasonality (Crec'hriou et al. 2010). Coastal and deep marine ecosystems (< 2,000 m depth), including seagrass meadows and deep corals reefs, are protected. Waters up to 100−150 m depth are highly influenced by Atlantic Ocean water entering the Mediterranean through the Gibraltar Strait. Sea surface temperature ranges between 14.6 °C in winter and 27.5 °C in summer (Ballesteros and Zabala, 1993). Coastal waters are oligotrophic (0.04−0.08 µmol phosphate l^{-1} and 0.25−0.8 µmol nitrate l^{-1}) and light attenuation coefficient is extremely low (0.063 m^{-1}). Depth and hydrodynamics are the dominant abiotic factors that affect habitat distribution and vary among sites throughout the archipelago (Ballesteros and Zabala 1993). The water is oligotrophic (Vives 1993) due to low concentrations of dissolved inorganic nutrients and chlorophyll (about 0.2 mg m^{-3}). The subtidal zone is dominated by algal beds, seagrass meadows and rocky bottoms. Photophilic algae beds (0−10 m depth) are dominated by canopy-forming species such as *Cystoseira* spp., *Dictyopteris membranacea*, *Sargassum vulgare*, *Halopteris scoparia*, *Padina pavonica* and *Dictyota dichotoma with* epiphytic algae such as *Jania* spp. Algal beds at deeper (10−35 m) well illuminated sites are dominated *D. membranacea*, *H. scoparia* (photophilic) and *D. dichotoma* or by *Flabellia petiolata*, *Peyssonnelia spp., Rhodymenia ardissonei, Plocamium cartilagineum* when light availability decreases (Ballesteros et al. 1993). Three

species of seagrass meadows are present: *Posidonia oceanica* (0–40 m depth), *Cymodocea nodosa* (0–25 m depth) and *Z. noltei* (< 2 m depth). Recreational fishing and trawling in PNAC were banned by the Park Management Plan in 1992, while small-scale fishing was regulated in 1995 and further revised in 2001. A total of 80 small-scale boats from four neighboring towns, which have been traditionally fishing in Cabrera waters, continue fishing in some areas (Morales-Nin et al. 2010). Fishing gears are regulated albeit overexploitation signs on the lobster trammel net fishery are evident (Amengual-Ramis et al. 2016). The number of tourists visiting PNAC increases yearly and official statistics accounted for 100,000 visitors and almost 900 yachts in 2017.

The aims of this study were threefold. First, to assess distribution and habitat use of syngnathids in PNIA and PNAC, each with highly distinctive environmental characteristics and vegetal assemblages. Second, to characterize syngnathid populations, which include the assessment of genetics identification and stable isotopes signals. Finally, the unavailability of historical data for syngnathids in the Iberian Peninsula prevents the assessment of population trends. Hence, the third aim of this study was the selection of specific sites for further monitoring of distribution/abundances and temporal-seasonal patterns on important biological and ecological features (e.g. diet composition, animal migration). The results achieved would be valuable for the development of further conservation actions in both NPs.

Results

Habitat characterization in Cíes Archipelago (PNIA)

Soft bottom sediments were mostly coarse sandy (569 μm), with 90% sand, 9% bioclastic gravel and < 1% mud (Additional file 2). Muddy sands, with > 20% mud (< 63 μm), were only located in the deepest (17.6 m to 21 m) and distal areas of TR5, in the immediate vicinity of the muddy bottoms characteristics of the central part of Ría de Vigo. Particle selection ranged between moderate and poor, with a prevalence of sediments with a single mode (65% of samples). Two and three mode samples in TR2, 4 and 3 (these last two near rocky outcrops) reflected a mixture of particle sizes, corresponding with greater content of bioclastic gravel (bivalves and gastropods shells) as well as maerl substrate. Skewness/asymmetry indicated a tendency towards the coarser fractions (bioclastic component).

Different sedimentary environments (wide variability of textural characteristics) were present along some transects. An example was TR3, with sectors exposed to W waves, characterized by coarse sediments and bedforms (megarripples 3D), and others protected by rocky outcrops close to the shoreline. Syngnathids were mostly sighted in sheltered sectors, preferring habitats with medium sands, better sorted and lacking mud (Additional file 2).

Similarity of seaweed assemblages was analyzed in PNIA considering data of 55 species with medium-high abundance (Additional files 2, 3). Diversity (H') and species richness (S) were particularly low in TR1, 2, 7 and 10, especially in spring (Additional file 2). Seaweed cover increased in summer, particularly in TR8 (633.8) and TR9 (861.0), but it was noticeable low in TR10 (42 in spring; 107 in summer) (Additional

file 2). PERMANOVA results showed significant differences in assemblage structures for both transects ($P < 0.05$) and seasons ($P < 0.01$). Those differences were evident in the two-dimensional PCOs plot (Fig. 3). Spring (left) and summer (right) samples followed a gradient along axis 1 (20.4% of total variation). Abundances increased in summer for most species, especially for *Treptacantha baccata, Padina pavonica, Corallina officinalis* or *Codium tomentosum* (strong negative correlation with PCO1; Spearman correlation > 0.65). Differences between transects were explained by axis 2 (18.1% of total variation), reflecting wave exposure. Transects TR1, 8 and 9 were clearly separated from the others, especially from TR10 and TR3. These results explained spatial differences between transects, with TR9, TR8 and TR7 as the most northern sites of Cíes Archipelago, and TR1 located in the west side of the southern island. The remaining transects (especially TR10) were located in areas less exposed to wave impact and current actions. Vectors overlay in PCO plot indicated that species such as *T. baccata, P. pavonica* or *C. tomentosum* were more abundant on less exposed areas, while *Mesophyllum expansum, C. officinalis, Plocamium cartilagineum* and *Kallymenia reniformis* preferred more wave-exposed sites (Spearman correlation > 0.65).

Syngnathids In Pnia And Pnac

In PNIA, two species of syngnathids were identified morphologically and genetically: the long-snouted seahorse *Hippocampus guttulatus* Cuvier, 1829, and the greater pipefish *Syngnathus acus*, Linnaeus 1758. A total of 28 specimens were sighted in PNIA from 4 to 15 m depth (mostly at < 8 m), with 6 transects providing at least one fish (Table 1). None of the individuals marked in spring were recaptured in summer. All PCO showed a positive correlation of syngnathids with seaweed assemblages on transects TR3, TR4, TR5 and especially TR10 (Spearman correlation > 0.65) in summer (Additional file 2). The highest abundances (0.06–0.13 syngnathids 100 m^{-2}) were recorded in mixed (sand-rock) or rocky substrates on transects TR3 and 10 (32 and 43% of total specimens, respectively). Syngnathids were missing in the mostly exposed transects TR1, 7, 8 and 9 (northern and southern areas with rocky substrate and coarse sand patches). TR1 was facing SW waves (prevalent component during storm winter conditions), while TR7, 8 and 9 were facing N waves (prevalent component during storm summer conditions). The most common species was *S. acus* (n = 24), which comprised 86% of total fish sighted.

Table 1

PNIA - Syngnathids (*Syngnathus acus* and *Hippocampus guttulatus*) captured in spring and summer 2016 surveys. TR − Transect. SL − Standard length. W − Wet weight. * Not captured.

Species	TR	Date	Depth (m)	SL (cm)	W (g)	Gender	Sexual state	Substrate
Spring 2016								
S. acus	2	4-may	15	23.8	8.4	Male	Pregnant	Gravel
	5	5-may	6	14.8	1.3	Female		Sandy
	6	5-may	5.5	44.0	64.7	Female	Ovigerous	Sandy
	6	5-may	6	34.2	27.2	Female	Ovigerous	Sandy
	10	20-may	4	32.0	23.2	Male	Pregnant	Rocky
	3	7-jun	5.5	44.9	66.7	Male	Pregnant	Sandy-Rocky
	3	7-jun	5.5	28.9	14.6	Female	Ovigerous	Sandy-Rocky
	3	7-jun	6	35.0	25.1	Female	Ovigerous	Sandy-Rocky
	3	7-jun	5	31.3	28.6	Male	Pregnant	Sandy-Rocky
	3	7-jun	5.5	24.5	6.2	Male	Pregnant	Sandy-Rocky
	3	7-jun	5.5	25.2	10.6	Female	Ovigerous	Sandy-Rocky
	3	7-jun	6	34.3	25.9	Female	Ovigerous	Sandy-Rocky
	3	7-jun	6	15.0	-	Female		Sandy-Rocky
	10	8-jun	4	49.7	62.5	Female	Ovigerous	Rocky
	10	8-jun	4	25.5	16.6	Male	Pregnant	Rocky
	10	9-jun	7	20.5	4.1	Female		Sandy
	10	9-jun	7.5	33.0	21.4	Male	Pregnant	Sandy

Species	TR	Date	Depth (m)	SL (cm)	W (g)	Gender	Sexual state	Substrate
	10	9-jun	5	45.6	67.6	Male	Pregnant	Sandy-Rocky
H. guttulatus	10	9-jun	8.5	22.7	25.8	Female		Rocky
	10	9-jun	8.5	21.8	25.6	Male		Rocky
Summer 2016								
S. acus	2*	1-sep	-	-	-	-		
	3	6-sep	6	40.0	50.7	Female		Sandy
	4	6-sep	6.5	30.8	21.6	Male		Rocky
	4	6-sep	4	17.6	3.1	Female		Sandy
	10	7-sep	7.5	39.0	40.4	Female		Sandy-Rocky
	10	7-sep	7.5	42.9	58.6	Male		Sandy-Rocky
H. guttulatus	10	7-sep	8	19.5	21.3	Male		Rocky
	10	7-sep	8	18.3	14.8	Female		Rocky

Most collected fishes were large adults, with average 31.8 ± 10.0 cm SL (range: 14.8–49.7 cm) in *S. acus* and 22.6 ± 2.0 cm (range: 18.7–22.7 cm) in *H. guttulatus*. Mean weights were 21.9 ± 5.2 g (range: 1.3–67.6 g) and 22.6 ± 2.0 (range: 14.8–25.8 g), respectively. In *S. acus*, meristic features were: 20 trunk rings (range: 19–20), 42 tail rings (41–44), 12 pectoral fin rays (9–12), 38 dorsal fin rays (37–41), 3 anal fin rays and 10 caudal fin rays. Only four seahorses were observed (TR10; 8.0-8.5 m depth). *S. acus* showed positive allometry (b = 3.32) (Additional file 2), and lengths and weights in spring and summer did not differ significantly (Tukey HSD, P = 0.519 for length, P = 0.471 for weight). Pregnant males and ovigerous females did not differ neither in length (P = 0.464) nor weight (P = 0.983). Abundances declined in summer (25%), when mature individuals were not observed. Contrarily, 90% of males and 70% of females collected in spring were pregnant (pouch carrying fertilized eggs/embryos) or ovigerous (full gonads with hydrated eggs), respectively. The minimum length recorded was 23.8 cm (8.4 g) in pregnant males and 25.2 cm (10.7 g) in ovigerous females.

In PNAC, four pipefish species were morphologically identified but seahorses were lacking. Only three specimens (one *S. acus*, two *N. maculatus* Rafinesque, 1810) were sighted on 37 visual censuses and 15 specimens (ten *S. abaster* Risso, 1827, two *S. typhle* Linnaeus, 1758, two *S. acus*, one *N. maculatus*) were

captured in 7 fishing sets (Table 2). Except for two *N. maculatus* (< 8 m depth), all specimens were captured at 11−21 m depth. Occurrences in *C. nodosa* meadows (Es Burri) by fishing sampling and visual census were similar (1.3 and 1.2 syngnathids 100 m^{-2}, respectively), but two-folds higher than by visual censuses in *P. oceanica* meadows and macroalgal beds in rocky substrates (0.03 individuals per 100 m^{-2}).

Table 2

PNAC: Syngnathids captured in 2016 surveys and sampling information. VC - Visual census, MC - Manual capture, TN -Trawl net ("gánguil"). SL − Standard length, nm - Not measured.

Species	Site	Date	Depth (m)	SL (cm)	Benthic community	Sampling method
Syngnathus acus	Es Port	21 April	13.5–15	27.0	*Cymodocea nodosa*	TN, MC
	Es Burri	6 Sept	11−13	11.5	*Cymodocea nodosa*	TN
	Es Burri	8 Sept	13−15	4.4	*Cymodocea nodosa*	TN
Syngnathus abaster	Es Burri	6 Sept	11−13	7.6	*Cymodocea nodosa*	TN
	Es Burri	6 Sept	11−13	7.0	*Cymodocea nodosa*	TN
	Es Burri	8 Sept	13−15	9.0	*Cymodocea nodosa*	TN
	Es Burri	8 Sept	13−15	4.1	*Cymodocea nodosa*	TN
	Es Burri	1 Dec	11−13	10.3	*Cymodocea nodosa*	TN
	Es Burri	1 Dec	11−13	8.1	*Cymodocea nodosa*	TN
	Es Burri	1 Dec	11−13	9.9	*Cymodocea nodosa*	TN
	Es Burri	2 Dec	13−15	8.8	*Cymodocea nodosa*	TN
	Es Burri	2 Dec	13−15	9.8	*Cymodocea nodosa*	TN
	Es Burri	2 Dec	13−15	7.6	*Cymodocea nodosa*	TN
Syngnatus typhle	Es Burri	8 Sept	13−15	6.4	*Cymodocea nodosa*	TN
	Es Burri	8 Sept	13−15	6.4	*Cymodocea nodosa*	TN
Nerophis maculatus	Es Burri	23 April	6−8	nm	*Posidonia oceanica*	TN, MC
	Es Burri	8 Sept	13−15	10.0	*Cymodocea nodosa*	TN

Species	Site	Date	Depth (m)	SL (cm)	Benthic community	Sampling method
	Es Burri	9 Sept	19–20	12.5	*Cymodocea nodosa*	TN, MC
	Santa María	1 Dec	4.4-6	nm	*Posidonia oceanica*	MC (outside TN)

Genetic Identification In Syngnathids

Length for Cytb sequences was 1149 base pairs (bp) in *S. acus*. In PNIA, nine Cytb haplotypes (12 variable sites) were detected (Additional file 2) and identified as *S. acus* (identity > 99.5% and e-value = 0.0), one of them (Cytb_SA13) identical to the reference for this species (AF356040; Wilson et al. 2001). Haplotypes Cytb_SA01 and Cytb_SA02 were the most abundant (nine and six individuals, respectively), whereas the rest were only found in one individual (requested GenBank Accession Numbers).

Seahorses were identified as *H. guttulatus*. Three Cytb haplotypes (564 bp) were detected (Cytb_HG01-03), comprising two variable sites (five when the reference sequence was included) (Additional file 2). Cytb_HG01, Cytb_HG02 and Cytb_HG03 were identical to *H. guttulatus* sequences reported across European populations (Woodall et al. 2015): KM061961 (GB10), KM061963 (GB7) and KM061980 (GB23), respectively. The most abundant *H. guttulatus* haplotype was Cytb_HG03 (two individuals).

A novel 16S rDNA haplotype of 521 pb (16S_NM01; pending GenBank Acc. Number) was detected for one pipefish morphologically identified as *N. maculatus*, with 48 variable sites respect to a related reference species (*N. ophidion*; AF354994). For six specimens morphologically identified as *S. abaster*, two Cytb haplotypes were detected: Cytb_SAb01 and Cytb_SAb02 (Pending GenBank Acc. Numbers) in four and two fish, respectively; showing 139 variable sites respect to the *S. acus* sequence (Additional file 2). Higher sequence identity against *S. typhle* (JX228148; > 98%) than *S. abaster* (JX228141; ≤ 95%) references was observed. Phylogenetic analysis grouped in a monophyletic cluster the two Cytb_SAb haplotypes from PNAC, soundly differentiated from available sequences of *S. abaster* (Mwale et al. 2013) and other pipefish species distributed in Mediterranean areas (*S. acus*, *S. typhle* and *S. rostellatus*) (Fig. 4).

Stable Isotope Signatures In Syngnathids

In PNIA, *H. guttulatus* and *S. acus* (Table 3) differed significantly for δ^{13}C (ANOVA, P = 0.026) but not for δ^{15}N (P = 0.491). Isotopic values in *S. acus* ranged from − 16.6 to -14.7‰ for δ^{13}C (-16.1 ± 0.4) and from 9.1 to 11.9‰ for δ^{15}N (10.8 ± 0.7), being correlated with fish size (Outliers not included; Pearson correlation; n = 17, P = 0.001 and 0.014, respectively) (Additional file 2). Season-gender comparisons showed inter-seasonal differences only for δ^{13}C, with spring values (-16.2 ± 0.3‰) lower than in summer

(-15.6 ± 0.5‰) (MANOVA, P = 0.008), and values in males (-16.2 ± 0.2‰) lower than in females (-15.9 ± 0.6‰) (P = 0.021). Gender-maturity comparisons showed similar δ^{13}C values for mature (-16.2 ± 0.3‰) and immature (15.9 ± 0.6‰) fishes (P = 0.104) but lower δ^{15}N signals in the later (11.1 ± 0.5‰ for mature, 10.4 ± 0.8‰ for immature) (P = 0.014).

Table 3

PNIA - Mean (± sd) δ^{13}C and δ^{15}N values in *H. guttulatus* and *S. acus* sampled in spring and summer 2016 in Cíes Archipelago.

| Species | Season | δ^{13}C | | | | δ^{15}N | | | |
		Mean ± sd	Max	Min	n	Mean ± sd	Max	Min	n
H. guttulatus	Pooled	-16.6 ± 0.2	-16.3	-16.8	4	11.0 ± 0.4	11.6	10.6	4
	Spring	-16.5 ± 0.2	-16.3	-16.6	2	11.2 ± 0.5	11.6	10.9	2
	Summer	-16.7 ± 0.2	-16.5	-16.8	2	10.8 ± 0.4	11.1	10.6	2
	♀ Spring	-16.3 ± 0.0	-16.3	-16.3	1	11.6 ± 0.0	11.6	11.6	1
	♀ Summer	-16.8 ± 0.0	-16.8	-16.8	1	11.1 ± 0.0	11.1	11.1	1
	♂ Spring	-16.6 ± 0.0	-16.6	-16.6	1	10.9 ± 0.0	10.9	10.9	1
	♂ Summer	-16.5 ± 0.0	-16.5	-16.5	1	10.6 ± 0.0	10.6	10.6	1
S. acus	Pooled	-16.1 ± 0.4	-14.7	-16.6	21	10.8 ± 0.7	11.9	9.1	21
	Spring	-16.2 ± 0.3	-15.8	-16.6	16	11.0 ± 0.7	11.9	9.8	16
	Summer	-15.6 ± 0.5	-14.7	-16.1	5	10.3 ± 0.8	11.2	9.1	5
	♀ Spring	-16.2 ± 0.3	-15.9	-16.6	8	11.1 ± 0.6	11.9	10.0	8
	♀ Summer	-16.0 ± 0.1	-16.0	-16.1	2	10.1 ± 1.5	11.2	9.1	2
	♂ Spring	-16.2 ± 0.3	-15.8	-16.6	8	10.9 ± 0.5	11.7	9.8	8
	♂ Summer	-15.3 ± 0.5	-14.7	-15.7	3	10.3 ± 0.2	10.5	10.2	3

SIA in PNAC was only performed on a reduced number of *S. abaster* (n = 5; 7.6–10.3 cm length). Isotopic values were not correlated with fish size (Spearman correlation = -0.3 and 0.1 for N^{15} and C^{13} respectively), ranging from − 15.2 to -19.6‰ for δ^{13}C (-16.4 ± 1.8) and from 6.5 to 7.3‰ for δ^{15}N (6.9 ± 0.3).

Discussion

Diversity, distribution and habitat of syngnathids

Sixteen syngnathid species have been reported in Europe (Dawson 1986) but only five were identified in our study. *H. guttulatus* and *N. maculatus* are *Data Deficient*, whereas the others are "Less Concern" (IUCN, 2017). In Balearic Islands, *S. abaster* is *Vulnerable*, *S. typhle* is *Near Threatened*, and *S. acus* and *N. maculatus* are *Least Concern* (Grau et al. 2015). Two species were sighted in PNIA: the seahorse *H. guttulatus* (very low abundance) and pipefish *S. acus* (Highly dominant). Most specimens sampled from PNIA were very large, lacking young or small sized fishes. Four pipefish species (*S. abaster, S. acus, S. typhle, N. maculatus*) were recorded in PNAC, comprising mostly small specimens, but seahorses were absent. Syngnathids were considered uncommon in PNAC, with previous references to *S. acus*, *S. typhle*, *H. guttulatus* and *H. hippocampus* (Riera et al. 1993). Our results indicate low occurrences, although pipefish, particularly *S. abaster*, were more abundant in Es Burri Bay. This species is also the most common in other nearby areas (Mar Menor, SW Spain) (De Maya et al. 2004).

High congruence between genetic markers and morphological data for species identification was observed, except for *S. abaster* in PNAC. In PNIA, eight novel Cytb haplotypes were found for *S. acus*, and also common sequence variants respect to Northern and Southern European populations of *S. acus* (1) and *H. guttulatus (3)*, respectively, according to previous reports (Wilson et al. 2001; Woodall et al. 2015). Available genetic sampling in PNAC allowed detecting novel haplotypes for a small number of pipefish identified as *N. maculatus* (1) and *S. abaster* (2) based on morphology data (http://species-identification.org/index.php; Hablützel and Wilson 2011). The two new Cytb haplotypes detected in putative *S. abaster* samples from PNAC clustered in a single monophyletic group, supporting morphological identification, but separately from the few Cytb sequences available for voucher samples of the species (Mwale et al. 2013) and from other congeneric pipefish distributed in the Mediterranean (*S. acus*, *S. rostellatus*, *S. taenionotus*, *S. typhle*). Other possible species like *S. schmidti* and *S. phlegon* in absence of available Cytb data were morphologically discriminated using non-overlapping ranges for meristic traits. All these observations could suggest the presence of a cryptic *Syngnathus* species in PNAC, as reported for the northwestern Pacific messmate pipefish in absence of morphological differences (Sogabe and Takagi, 2013). Possible sample misidentifications cannot be ruled out, according to confuse discriminations reported for European *Syngnathus* species (Habltzel and Wilson 2011). Further genetic and morphological sampling surveys will be needed to clarify the taxonomic status of *Syngnathus* in PNAC and the consequences for species conservation.

PNIA and PNAC differed in habitat characteristics and syngnathids occurrence. Differences in diversity, distribution and abundance are related to habitat characteristics (Vincent et al. 1995; Otero-Ferrer et al. 2015; Woodall et al. 2015). Many species are commonly algal and seagrass residents closely associated with specific habitats that best enable camouflage (Scilipoti 1998; Kendrick and Hyndes 2003; Foster and Vincent 2004; Masonjones et al. 2010; Woodall et al. 2018). Seagrass meadows are currently lacking in Cíes (García-Redondo et al. 2017, 2019) and seaweed communities are structurally complex and patchy distributed on mixed or rocky substrates (Peña and Bárbara 2006; Piñeiro et al. 2014; Fernández et al. 2020). Most syngnathids in PNIA were located in semi-exposed or sheltered habitats on transects that showed the highest similarity regarding vegetal communities. Transect TR10 was particularly interesting since it was located in the mostly sheltered area of PNIA but it was also the only transect with seahorse

occurrence. As for *S. acus* in PNIA, most abundant pipefish species form monospecific populations (Vincent et al. 1995; Masonjones et al. 2010) but many European pipefish species may vary their habitat occupancy and overlap a great deal (Vincent et al. 1995), as shown in PNAC. Seagrass meadows are lacking in PNIA but PNAC seabed was partially covered by large extensions of seagrass meadows (*P. oceanica* and *C. nodosa*), which is a typical cover enhancing the occurrence of syngnathids in some Mediterranean areas (Vizzini and Mazzola 2004), and their ability to effectively mimic leaves. That is the case of *S. typhle*, a pipefish that preferentially display an upright position in seagrasses with narrow leaves (e.g *Zostera*) (Vincent et al. 1995; Skóra 2001). The absence of that species in PNIA and its occurrence in PNAC could rely on the lack of seagrass meadows in the former, even though this pipefish may adapt to habitats (Tarnowska and Sapota 2007). Appropriate habitats for syngnathids may not be determined simply by the presence or absence of vegetation but also by the prevalence of seaweed communities that best enable them to remain inconspicuous to predators (Kendrick and Hyndes 2003). All pipefish in PNAC were collected in *C. nodosa* and *P. oceanica* meadows, suggesting that macroalgal beds are less preferred than seagrass meadows.

In PNAC, the results showed extremely low abundances (Vincent et al. 1995). However, the highest abundances were recorded in *C. nodosa* meadows in Es Burri Bay (1.2–1.3 syngnathids 100 m^{-2}). The extremely low abundances of pipefish in PNAC was unexpected. Sampling syngnathids in dense meadows by means of visual censuses is difficult due to fish crypsis. Captures with the first visual censuses and fishing from 2.8 to 21.5 m depth resulted substantially improved with 'gánguil' gear operating at 11–16.5 m depth. However, actual species richness and abundances in PNAC could have been underestimated. European syngnathids usually inhabit brackish areas at < 10 m depth, but *C. nodosa* meadows are present at deeper depths in Es Burri Bay), where most syngnathids were captured (11–13 m depth). The dominant pipefish *S. abaster* in PNAC commonly inhabit at 0.5–5 m depth (Dawson 1986; De Maya et al. 2004), which is clearly lower than depths operationally imposed by gear and site in our samplings with "gánguil".

Changes in macroalgal assemblages in PNIA are occurring since 2012. The abundance of *Treptacantha baccata*, *T. usneoides* and *Saccorhiza polyschides* decreased, while turf (*Halopteris scoparia*, *Chondria coerulescens* or *Corallina* spp.), and non-native (*Codium fragile*, *Asparagopsis armata*) species have increased their frequency of occurrence (Carrizosa 2016). The progressive habitat loss and the increase in less optimal seaweed species can also cause dramatic changes in resident fauna and community composition (Thayer et al. 1994, 1999). Most syngnathids from PNIA were captured in shallow waters (< 10 m depth) on sandy substrates with low proportions of gravel, some mud and preferably nearby rocky outcrops that provides better refuge and protection (TR3, TR10). Coastal sheltered areas protected from SW (TR1) and N waves (TR7- TR9) were preferred by syngnathids but areas with high bottom mobility (sand waves and megarriples 3D) were avoided (TR2 and some areas of TR4). Some syngnathid species appear to be generalist considering distribution patterns and algal community characteristics but others prefer certain seaweed forms and feed on specific sources (Masonjones et al. 2010; Planas et al. in press). Distribution patterns can be partially explained by the exposure to waves and open sea (Smith et

al. 2008; Masonjones et al. 2010), which has a great impact on seaweed cover. However, *S. acus* was also able to inhabit sites with a certain degree of water agitation such as shallow and rocky areas near the shore wave-breaking zone in PNIA (TR3).

Due to the high dominance in PNIA, *S. acus* deserves special consideration. The length–weight relationship in PNIA was similar to that in the western Black Sea (Yildiz et al. 2015). The large average SL (31.8 ± 10.0 cm) in PNIA was noteworthy compared to PNAC) and other Mediterranean populations but did not differ from others in eelgrass meadows from Northern Europe (Dawson 1986; Vincent et al. 1995; Gurkan and Taskavak 2007; Gurkan et al. 2009; Yildiz et al. 2015). However, the absence of small-medium sized specimens in Cíes Islands was unexpected, which begs the questions of whether there is a resident population of adults (with dispersal of small individuals towards other areas) and/or whether the fishes migrate seasonally to Cíes from nearby areas only for breeding. None of the specimens marked in spring were recaptured in summer, suggesting that they might not be so site faithful as reported (Vincent et al. 1995). A comparative isotopic study and further genetic analysis with informative markers respect to specimens from nearby areas would clarify that dilemma.

Breeding Season

Syngnathids may change habitat and prey preferences as they grow (Franzoi et al. 1993; Perante et al. 1998; Kendrick and Hyndes 2005; Curtis and Vincent 2006; Oliveira et al. 2007). The absence of small and medium-sized immature specimens in Cíes suggests that young fishes prefer less exposed nearby sites, and/or that small juveniles are dispersed by currents to other areas. Sexual maturity in females and males was reached at 6.9 and 6.1 cm size, respectively. These sizes are smaller than reported in the Aegean Sea (7.7 cm in females; 8.1 cm in males) (Gurkan et al. 2009). In our study, mature specimens of *S. acus* were present in early May - June but not in early September. These findings agree with the reported breeding season for the species (January to August), with peaks of hydrated-oocyte carrying females and pregnant males in March-July depending on latitude and temperature (Anonymous 1956; Vincent et al. 1995; Gurkan et al. 2009). In Cíes Archipelago, temperatures raised from 14.1 in May to 18.1 °C in June, and dropped to 16.3 °C in September. Hence, the breeding season in syngnathids from PNIA seems to be limited by water temperature (Monteiro et al. 2001). In PNAC, the small number of pipefish and their small size prevents from concluding remarks.

Isotopic Signatures In Syngnathids

The pelagic food web from coastal areas (e.g. Arcade cove) in Galicia are typically enriched in δ^{13}C and δ^{15}N compared to more oceanic areas (e.g. Cíes Archipelago) (Bode et al. 2003). Arcade cove is located in San Simón Bay on the inner part of Ría de Vigo (30 km from Cíes Archipelago). The cove is a shallow mesotidal *Zostera* meadow with low hydrodynamic conditions, receiving freshwater inputs from Verdugo River (Álvarez-Iglesias et al. 2006). The population of *S. acus* inhabiting Arcade cove markedly differ from

that in Cíes, particularly for fish size and isotopic signals (Fig- 5). Also, habitat characteristics and trophic web structure in Arcade and Cíes differ considerably (Filgueira and Castro 2011, Fernández et al. 2020). The former receives anthropogenic wastewater inputs and is characterized by a complex trophic web, and an important local microphytobenthos production available to primary consumers through resuspension forced by tidal hydrodynamics. The cove is a community with a high diversity of organic matter sources; however, terrestrial POM below 60 mm does not seem to contribute significantly to consumers' most plausible diets (Filgueira and Castro 2011). Wastewater discharges would contribute to the increase in $\delta^{15}N$ values in Arcade cove as shown in other similar areas (Bode et al. 2006). Surface dissolved nitrogen concentrations (DIN) and isotopic discrimination for $\delta^{15}N$ in Ría de Vigo are typically higher from October to April, decreasing from May to September (Nogueira et al. 1997). Hence, higher $\delta^{15}N$ signatures would be expected in spring-summer (Román et al. 2018, 2019). The opposite trend would occur in $\delta^{13}C$ signatures (Román et al. 2019). Those trends were reproduced in isotopic signals of *S. acus* in Arcade cove (Fig. 6) but not in those from Cíes, with higher oceanic influence and more stable conditions. The enriched $\delta^{15}N$ signatures in Arcade cove were reflected in isotopic signatures of the whole web trophic chain (Filgueira and Castro 2011), including *S. acus* specimens (13.3 ± 0.5‰; range: 12.0–14.3‰) (Planas et al. unpublished observations). Lower trophic levels in *S. acus* from Cíes would be expected from Fig. 6. However, the expected trophic positions were masked by the large differences in isotopic signals between Cíes and Arcade, as reflected in $\delta^{15}N$ values of filter-feeders, with values of -17.14‰ for $\delta^{13}C$ and 8.98‰ for $\delta^{15}N$ in *Mytilus galloprovincialis* from Arcade cove (Filgueira and Castro 2011) and – 17.66‰ for $\delta^{13}C$ and 5.13‰ for $\delta^{15}N$ in *Musculus costulatus* (Jiménez, 2019). We calculated the trophic position of *S. acus* in both sites considering bivalve species as isotopic baselines (Post 2002) and trophic enrichment factors of 4.1 (Planas et al. in press), resulting in estimated trophic levels of 3.16 in Arcade and 3.83 in Cíes. Hence, these values suggest dissimilarities for resource exploitation in both sites, resulting from differential characteristics on biotope and trophic web composition and structure.

The extreme scarcity of pipefish in PNAC prevents from concluding remarks on isotopic patterns and trophic characteristics of pipefish. Pipefish from PNAC showed lower isotopic signals (particularly for $\delta^{15}N$) than in PNIA, which agrees with some isotopic values in Mediterranean zooplankton (Rumolo et al. 2016), but not with values from a semi-enclosed basin in the western Mediterranean (Vizzini and Mazzola 2004). Disagreements might be driven by differences in resource exploitation and resource partitioning (especially organic matter sources at the base of the food web) depending on the study site.

Hypothesizing Migratory Events In Pnia

Isotopic profiles in tissues has proven useful to build isoscapes and infer geographic origins and spatial connections (Hobson et al. 2010; Wunder 2010), which was not the scope of our study. However, some hypothesis can be given on potential migrations in *S. acus*. Assuming the existence of winter-spring migratory events from areas nearby Cíes, fin isotopic signals in pipefish captured in Cíes in summer would reflect those of the diet ingested on a nearby area in spring (M. Planas, unpublished observations).

However, that assumption is not supported by actual differences in δ^{15}N signatures (2.7‰) between specimens from Arcade in spring (13.8 ± 0.4‰; range: 13.2–14.5) and those from Cíes in summer (10.1 ± 1.5‰) (Fig. 5). The sharp drop in abundances from late summer (Jiménez 2019) and in the absence of more data, we hypothesize that spring-summer populations of _S. acus_ in Cíes Archipelago are mainly founded by large resident specimens, undergoing migration into adjacent habitats to avoid harsh autumn-winter conditions as reported in other syngnathids (Lazzari and Able 1990; Vincent et al. 1995; Monteiro et al. 2001; Foster and Vincent 2004; Masonjones et al. 2010; Vincent et al 1995). Subsequently, the species would return to Cíes in early spring for breeding when males begin developing their brood structures and after plant-cover recovery.

Directions For Research And Conservation

There is increasing public awareness of the challenges of marine biodiversity from habitat destruction, over-fishing and development. Efforts are needed to protect and value marine biodiversity, especially species and communities that require relatively large areas of undisturbed habitat. NPs are areas set aside for the preservation of the natural environment to protect natural biodiversity along with its underlying ecological structure and supporting environmental processes, and to promote education and recreation (IUCN). PNIA and PNAC differ in regulatory and environmental protections, and biota and abiotic components, which determine population characteristics of inhabiting syngnathids. Both high quality environments are tourist destinations supporting at least one jetty, bollards where ships can tie up under permission, and an internationally recognized wildlife. Due to the lack of previous studies, the trend of syngnathid populations is unknown and their future is uncertain. The main concern is the human and fishing pressure, particularly in PNIA. Current diversity and abundances of syngnathids in marine Spanish NPs are extremely scarce, with only five species identified, and there is the need of protecting those limited populations. Our recommendations of potential management and research priorities are as follows:

- The present study provided first data on syngnathid populations in Spanish NPs. The availability of historical data and a continuous monitoring of syngnathid populations and temporal-seasonal variability are imperative for trends assessment. Hence, an objective of this study was the selection of specific sites for further monitoring (Additional file 1). The higher proportion of species/abundances in PNIA indicate that further monitoring should focus on Eastern-PNIA (TR2-TR5) and particularly in Rodas Bay (TR10). Those areas include rocky and sandy-gravel substrates, maerl beds as well as seaweed communities enhancing protection and habitat suitability for syngnathids. Rodas Bay is also interesting from a conservation point of view since it is also a preferential habitat for small _Octopus vulgaris_ (Guerra et al. 2014). In PNAC, considering the benthic communities and the higher abundance of pipefish compared to other locations, Es Burri has the greatest interest value for further conservation actions and monitoring. Seagrass meadows appear as essential communities to maintain syngnathid populations in PNAC as all fish were captured there. Damage to seagrass meadows by anchoring of recreational boats in Es Burri Bay might compromise syngnathid populations in PNAC. Studies not based on long-term monitoring may

lead to erroneous or incomplete assumptions. The study of temporal patterns of syngnathids diversity will provide valuable information on the assessment of species' sensitivity to habitat disturbances and climate warming (Faleiro et al. 2015), and on optimal conditions for captive breeding and further population reinforcement for the most endangered species if necessary.

- Some of the main unsolved questions arisen from the study is whether Cíes Archipelago should be considered a breeding sanctuary for *S. acus*. Understanding fish movement patterns and migrations from/to other nearby areas is another pivotal topic than needs addressing. For that, further isotopic and genetic information, and acoustic telemetric studies in specimens tagged with transmitters would provide valuable information to undertake further conservation actions (Villegas-Ríos 2013).

- The management of vessel transits to physically and ecologically protect sensible areas against substrate loss (marine flora communities) and to mitigate anthropogenic sound is necessary (McKenna et al. 2017). Long-term soundscape monitoring and more restricted vessel anchorage conditions for resource management (Haver et al. 2019) is especially essential in Rodas Bay (Transect 10 in PNIA), which support a high density of vessel traffic during the touristic seasons.

Conclusions

This is the first multidisciplinary approach to the study of syngnathids in Spanish coasts, specifically in two marine NPs. It will contribute to the knowledge of syngnathid populations, leading to more informed and efficient management of both NPs. Species diversity, abundances, habitat preferences, and isotopic signatures differed in both NPs, depending on habitat characteristics. Syngnathids preferred sheltered macroalgal assemblages in PNIA and *Cymodocea* meadows in PNAC. It is suggested that PNIA is a breeding sanctuary for *S. acus*, which migrate seasonally. Genetic markers agreed with meristic characteristics, except for *S. abaster* in PNAC, suggesting the presence of cryptic *Syngnathus* species, and the need of further analyses to assess potential misidentifications in the genus.

Preferential sites for future monitoring of syngnathid populations in both NPs, some actions to undertake for conservation purposes and further research priorities are proposed. Syngnathids, particularly seahorses, are flagship species attracting the attention of citizens. Efficient further actions will enhance public engagement with marine biodiversity, resulting also in social, economic and wellbeing profits.

Methods

Study and swept areas

The study was carried out in (a) Cíes Archipelago (42°13'N, 8°54'W), in Atlantic Islands National Park (PNIA), located on the outer area of the Ría de Vigo (NW Iberian Peninsula) (Fig. 1), and (b) Cabrera Archipelago National Park (PNAC) (39°08'N, 2°56'W), in the western Mediterranean (Balearic Islands) (Fig. 2).

PNIA is located at the northern limit of the eastern boundary upwelling system off NW Africa and SW Europe. Northerly winds induce coastal upwelling in this region during most of spring and summer (Fraga, 1981) and colder nutrient-rich subsurface water known as Eastern North Atlantic Central Water (ENACW) inside the estuaries (Nogueira et al. 1997; Álvarez et al. 2005). The study in PNIA was conducted in Cíes Archipelago, comprising three islands and various islets. It is a highly productive area supporting highly diverse ecosystems (Fernández et al. 2020).

Based on previous knowledge (seaweeds cover, substrate characteristics and exposure level to open water) (Fernández et al. 2019), ten subtidal transects (TR1 to TR10) were selected along the western coast of Cíes Archipelago (Fig. 1; Additional file 1), and visited in spring and summer 2016 (two visual censuses per site and season; from May to September 2016) to obtain an overview of habitat characteristics and the spatial distribution of syngnathid species. Transects were positioned parallel or perpendicular to the coastline (150 to 700 m length; 3–20 m depth) on rocky bottoms often interrupted by occasional sandy patches. Two pairs of divers conducted 40 diurnal underwater visual surveys (50 min per survey; 160 diving hours) along the East coast, covering a total surface of 8.22 ha (10 transects, 5 m wide). All syngnathids sighted were recorded and captured by the divers searching adjacent (belt transects) and separated by the maximum distance allowed for horizontal visibility (commonly 2.5 m). One pair of divers also recorded the characteristics (species, seaweeds cover) of seaweeds communities, and the other pair sampled the sediment.

Average temperatures were calculated using data of the Galician Oceanographic Network (MeteoGalicia database; www.meteogalicia.gal) from a buoy located in the southern area of Cíes Islands (42°10.691'N, 8°53'589W), recording average daily temperatures at 6 m depth. Survey water temperatures were calculated as the average temperature for the period comprising one week before and after the sampling day.

Soft bottom substrates were found among rocky outcrops or in the edge of rocky reefs. To characterize sediments of the swept area in PNIA, the uppermost 2 cm of sediment were underwater manually collected using plastic pots along each transect and considering changes in bottom characteristics. When bedforms were present, both through and crest zone were sampled. A total of 76 sediment samples was collected in spring (52) and summer (24), and conserved at 4 °C for further textural and compositional analysis.

PNAC includes a main island (Cabrera) and a group of four minor islands and several islets, totaling a land area of 13.2 km^2 and a coast line length of 53.8 km (Servera 1993). Eleven subtidal sites (TR1 to TR11) including the main shallow benthic habitats present in PNAC were visited from 21st April to 1st December 2016 throughout the coast of Cabrera and Conillera islands (Fig. 2) to acquire a general overview on the potential distribution of syngnathids. We conducted 37 surveys using standard underwater visual census (50 m length × 5 m wide; 60–80 min per dive, 17–26 °C) at least two visual censuses per site). A total surface of 0.925 ha was surveyed covering a depth gradient from 2.8 to 21.5 m. Visual censuses were performed on *Posidonia oceanica* meadows, *Cymodocea nodosa*

meadows, photophilic macroalgal beds on rocky substratum and mixed habitats formed by these communities. Two pairs of divers participated in each survey recording and capturing all syngnathids sighted. The depth, water temperature, position and habitat type (substrate, benthic community) were annotated for each fish captured.

Due to the low number of syngnathids encountered with visual censuses in PNAC, a small trawl net called 'gambera' or 'gánguil' (traditional gear for small crustaceans catching) was assayed on *C. nodosa* meadows. This gear has a rolling stainless steel cylinder incorporated in the bottom of the mouth for protecting *P. oceanica* and *C. nodosa* leaves from snagging and tearing while operating. The beam trawl was 3 m long and it had a 0.8 m mouth aperture with 1.2 cm^2 mesh size (Catalán et al. 2014). To avoid damaging of fan mussel, *Pinna nobilis*, populations while sampling, PNAC authority only allowed the use of 'gánguil' in the *C. nodosa* meadows in Es Burri Bay (Fig. 3; 39°8.604'N 2°57.524'E). Seven fishing sets were carried out from September to December 2016, covering a total area of 0.114 ha from 11 m to 16.5 m depth.

The depth, position and habitat type (also substrate and seaweed assemblages in PNIA) were annotated for each fish captured in both study areas. Flora and fauna nomenclature followed codes of Guiry and Guiry (2020) and WoRMS Editorial Board (2020). Swept areas were calculated according to Guerra et al. (2015), considering the effective sampling time, the net sampling distance, the distance between divers and the number of divers who participated in each census.

Fish Collection

In visual census, syngnathids were hand-caught collected or manually extracted from the fishing set capture, introduced in numbered plastic bags and transferred to a support boat. In PNIA, once on land, the fish were morphologically identified, anesthetized with Ethyl 3-aminobenzoate methane sulfonate (MS-222; 0.1 g L^{-1}; Sigma-Aldrich Co., USA) and marked subcutaneously using visible implant fluorescent elastomers (VIFE; Northwest Marine Technology Inc., USA) on the ventral surface of the trunk (pipefish) or laterally (seahorses). All anaesthetized fish were weighted (W, g) and sized for standard length (SL, cm). In PNAC, the fish were morphologically identified on board, anesthetized, sized as reported above but not weighted because it was not possible to stabilize the balance in boat conditions. A fraction of the fish collected by fishing in PNAC were sacrificed for sampling (stable isotopes and genetic analysis) due to their small size (with permission of NP authority).

Dorsal fin samples were taken by fin-clipping (Planas et al. 2008), transferred to screw-capped tubes containing 95% ethanol and conserved at 4 °C for further genetic and stable isotope analysis (SIA). The presence of previous marks (recapture events), gender, sexual state, meristics (fin rays, body rings) and body coloration were also annotated. The sexual state was recorded considering pregnancy in males and trunk shape (holding of hydrated eggs) in females. Species identification was evaluated genetically using

DNA extracted from dorsal fin samples available from PNIA and PNAC surveys. In PNIA, all fishes from visual censuses were released at the capture site within 2–3 hours after sampling.

For SL measurement, the fishes were placed on a plate including a measurement scale and photographed laterally (seahorses) or measured directly (pipefish). Seahorse images were analyzed in the laboratory to determine length using image-processing software (NIS Elements Nikon and ImageJ2). Seahorses were measured as head + trunk + tail length (curved measurement in seahorses) (Lourie 2003).

Sediment Analysis

The analysis of sediments was only carried out in PNIA. For compositional analysis, the content of organic carbon and inorganic carbon (calcium carbonate content is equivalent to bioclastic component for this regional setting) was determined by a LECO CNS-2000 Macro Elemental Analyser at CACTI (University of Vigo). Those analyses were performed on the fractions < 2 mm, in order to avoid distortional results due to gravel components (> 2 mm, maerl, bivalve and gastropod shells).

For textural analysis, the bulk grain size distribution was performed by dry sieving at the sedimentology laboratory of the Department of Marine Geosciences and Territorial Planning (University of Vigo). Previously to grain size analysis, the organic matter was removed, using 30% hydrogen peroxide for several days and further washing with distilled water for the elimination of salts. A conductivity meter was used to check chloride washout. Afterwards, the samples were dried at 50 °C and dry sieved between 4 mm and 63 μm (sieve size intervals of 1/2 ø). The resulting grain size distribution was treated with the GRADISTAT program (Blott and Pye 2001). For statistical parameters (mean, selection, asymmetry and kurtosis or pointing of the grain size curve), the nomenclature of Folk and Ward (1957) classification was used.

Dna Sequence Analysis

DNA was extracted from dorsal fin tissue collected from the following morphologically identified specimens: i) twenty-two wild greater pipefish (*Syngnathus acus*) and four long-snouted seahorses (*Hippocampus guttulatus*) from PNIA; and ii) six black-striped pipefish (*S. abaster*) and one spotted pipefish (*Nerophis maculatus*) from PNAC. Genomic DNA was isolated using NucleoSpin Tissue XS kit (Macherey-Nagel Inc., Germany) and for extremely small tissue samples further amplified using GenomiPhi V2 kit (Healthcare, USA).

Two mitochondrial markers (i.e. cytochrome b (Cytb) and 16S rDNA) were assayed for the molecular identification of sampled specimens from two divergent phylogenetic groups (Syngnathinae and Nerophinae subfamilies, respectively; Hamilton et al. 2017). Universal primers L14275F (Päabo et al. 1991) and H15926R (Wilson et al. 2001) were used to amplify Cytb in *Syngnathus* pipefish, while the specific primers SHORSE5.3L (Casey et al. 2004) and GUTCYTBR (Woodall 2015) in seahorses. To

overcome low Cytb amplification success in *N. maculatus*, the universal primers 16Sa-L2510 and 16Sb-H3080 (Palumbi et al. 1991) were used to amplify 16S rDNA in this species. PCR reactions in 50 µL included 100 ng of template DNA, 1X PCR Gold Buffer (Applied Biosystems), 2.5 mM of $MgCl_2$, 400 µM of dNTPs, 0.2 µM of each primer and 1 and 1.25 units of Amplitaq Gold™ DNA polymerase (Applied Biosystems) for pipefish and seahorse, respectively. Specific PCR programs were used for pipefish (95 °C for 10 min, 33 cycles of 93 °C for 1 min, 50 °C for 1 min and 72 °C for 3 min, plus final extension at 72 °C for 10 min) and seahorses (94 °C for 10 min, 35 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min, plus final extension at 72 °C for 2 min). Sequences were obtained using the ABI PRISM BigDye™ Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM® 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Variable sites were checked with SEQSCAPE 2.5 (Applied Biosystems), using Genbank sequences AF356040, AF354994 (from Sweden; Wilson et al. 2001) and AF192664 (from UK; Casey et al. 2004) as reference for *S. acus*, *N. ophidion* and *H. guttulatus*, respectively. Variable positions and haplotypes were obtained using MEGA 7.0 (Kumar et al. 2016). Species identification of sampled haplotypes was performed using BLASTn tool with default parameters within NCBI database. Evolutionary relationships among *S. abaster* haplotypes from PNAC and GenBank sequences of Mediterranean-distributed *Syngnathus* species were inferred using the Neighbor-Joining method based on p-distance implemented in MEGA, and clustering support evaluated using bootstrap test (1000 replicates).

Stable Isotopes Analysis (sia)

For $\delta^{13}C$ and $\delta^{15}N$ analysis in syngnathids, fin samples were rinsed with distilled water, transferred to tin capsules, dried in oven at 60 °C for 24 h and weighted (± 1 µg). Due to the low lipid content in fin samples conserved in ethanol (< 5% lipids, C/N < 3.56) (Post et al. 2007), further full defatting was not necessary (Valladares and Planas 2012). Samples were analysed at SAI (University of A Coruña) by continuous flow isotope ratio mass spectrometry using a FlashEA1112 elemental analyser (Thermo Finnigan, Italy) coupled to a Delta Plus mass spectrometer (FinniganMat, Germany) through a Conflo II interface. Isotopic values are expressed as permil (‰) in conventional delta relative to VPDB (Vienna Pee Dee Belemnite) and Atmospheric Air. The precision (standard deviation) for SIA of the laboratory standard (acetanilide) was ± 0.15‰ (1-sigma, n = 10).

Geographic Information

GIS was managed with ArcGIS v.10.5 software to represent the maps. Layers of bionomic maps for both NPs (OAPN, unpublished observations) were incorporated. Sampled sites/transects and syngnathid capture locations were recorded and added to a geodatabase. Biological information of the specimens (species, sex, size, weight and sexual stage) was joined to each register. Available abiotic information (topographic and bathymetric layers), as well as bionomic information, were also added to geodatabase.

Cartographic data were projected in UTM 29N/UTM 31N reference system (for PNIA and PNAC, respectively) using ETRS89 Datum.

Data analysis

All means are reported with standard deviation. The data were checked for normality and homogeneity of variances (Shapiro–Wilk and Levene's tests). Analyses of variance (ANOVA/MANOVA) were used to examine the effects of season, gender, reproductive status, length, weight and isotopic values in syngnathids. Tukey's HSD test adjusted for unequal sample sizes were performed for post hoc comparisons (Spjotvoll and Stoline, 1973). Statistical analyses were performed using R packages, with significance set at $P = 0.05$.

Diversity, species richness and total number of species were estimated for seaweeds in PNIA. Differences between transects and seasons were analyzed using PERMANOVA for each univariate variable. *P*-values were estimated with an asymptotic permutation distribution generated by the Monte Carlo method. PERMANOVA was also used for seaweed assemblage comparisons across transects and seasons using Bray-Curtis pairwise similarities. Patterns in the structure of assemblages were visualized with principal coordinates (PCO) plots of samples and centroids of each combination of Transect x Time in the Bray-Curtis space. Data and statistical analysis were performed with R (Glht and Factoextra packages) and PRIMER-e v6 and PERMANOVA + for PRIMER (Massey University, New Zealand).

Abbreviations

C: Total carbon

GIS: Geographic information system

OAPN: Organismo Autónomo de Parques Nacionales españoles

IUCN: International union for the conservation of nature and natural resources

MANOVA: Multivariate analysis of variance

N: Total nitrogen

NCBI: National Center for Biotechnology Information

NP: National Park

PCO: Principal coordinates analysis

PERMANOVA: Permutational analysis of variance

PNAC: Cabrera Archipelago National Park

PNIA: Atlantic Islands National Park

SCUBA: self-contained underwater breathing apparatus

SD: Standard deviation

SIA: Stable isotope analysis

SL: Standard length

TR: Transect

UTM: Universal Transverse Mercator system

W: Weight

WORMS: World register of marine species

Declarations

- Ethics approval and consent to participate: Fish capture, handling and sampling were conducted in compliance with all bioethics standards on animal experimentation of the Spanish Government (R.D. 1201/2005, 10th October) and the Regional Government Xunta de Galicia (REGA ES360570202001/16/FUN/BIOL.AN/MPO02). The corresponding author declares that all listed co-authors consent to being involved in the publication
- Consent to publish: All listed co-authors consent to publish this manuscxript and understand that the text and any pictures or videos published in the article will be freely available on the internet and may be seen by the general public. The pictures, videos and text may also appear on other websites or in print, may be translated into other languages or used for commercial purposes. All authors have been offered the opportunity to read the manuscript.
- Availability of data and material: As we are working on a long-term project, the datasets used and analysed during the current study are available from the corresponding author on reasonable request.
- Competing interests: The authors declare that they have no competing interests.
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- Authors' contributions:

MP: Project coordinator, site selection and field sampling, laboratory and data analysis, SIA, manuscript writing and review.

CPC: Site selection and field sampling, macroalgal assemblages, data analysis, writing contribution, manuscript review.

CB: Genetic analyses coordinator, data analysis, writing contribution, manuscript review.

IC: Field sampling, laboratory and data analysis, SIA, writing contribution, manuscript review.

MV: Mitochondrial DNA analyses, data analysis, writing contribution, manuscript review.

MR: GIS mapping, data analysis, supplementary materials (video).

VO: Macroalgal assemblages, data analysis.

IB: Site selection and field sampling, macroalgal assemblages, data analysis, writing contribution, manuscript review.

JT: Site selection, sampling design and field sampling, manuscript review.

AC: Sites selection, field sampling.

RB: Macroalgal assemblages, data analysis, manuscript review.

JHU: Field sampling, data analysis, writing contribution, manuscript review.

IA: Sediment analysis, writing contribution, manuscript review.

MN: Sediment analysis, writing contribution, manuscript review.

MEG: Sites selection, field sampling in PNIA, video.

BGP: DNA extraction and sequencing analysis, data analysis, writing contribution, manuscript review.

MEG: Sites selection, field sampling, supplementary materials (video).

VP: Field sampling, manuscript review.

PD: Field sampling, manuscript review.

JC: Field sampling design, manuscript review

BMN: Field sampling design, manuscript review.

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Figures
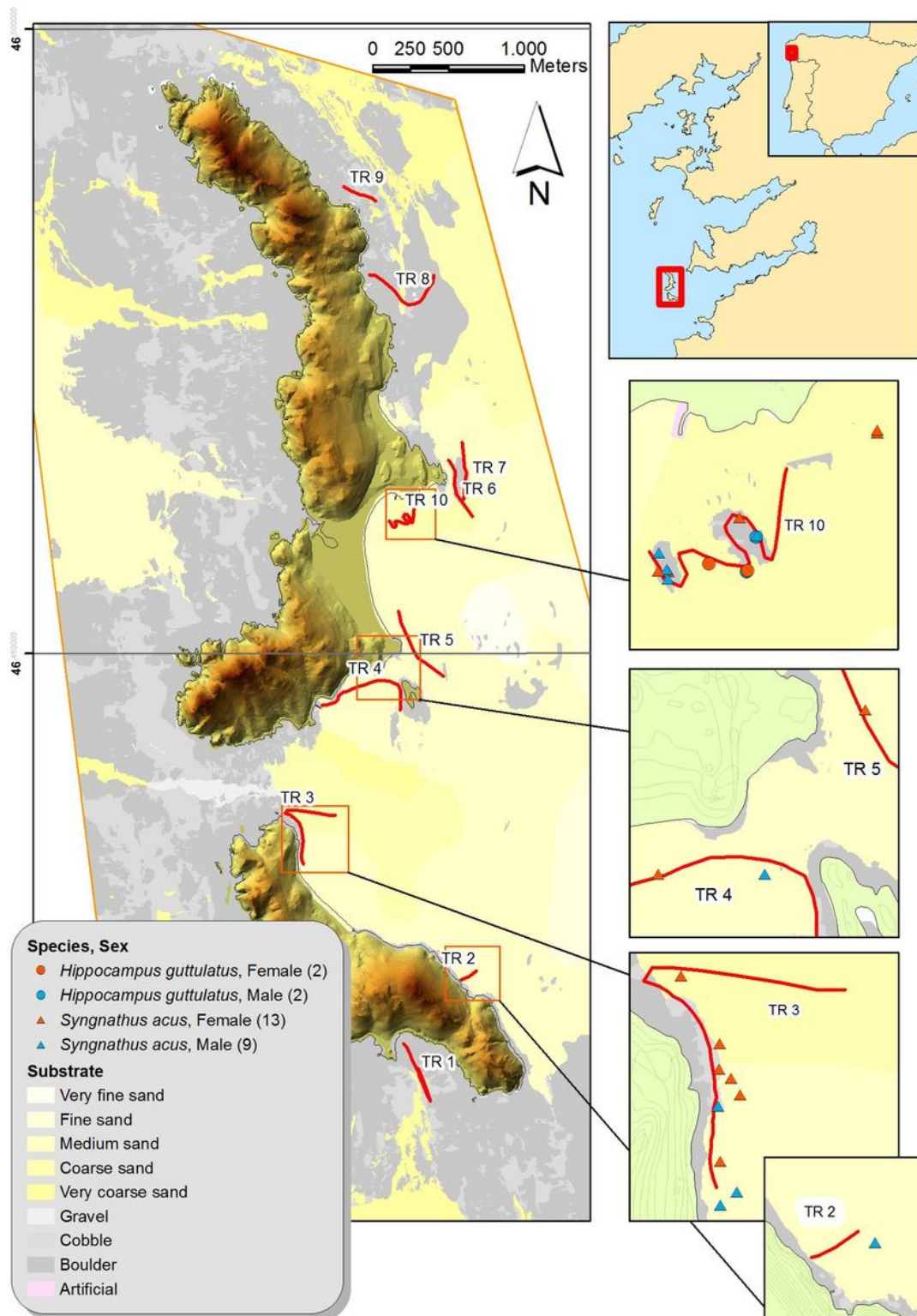
Figure 1

PNIA – Study area and transects (red lines; TR1 to TR10) surveyed for syngnathids in Cíes Archipelago (Galicia, NW Iberian Peninsula). Right: Capture sites. Species, sex, specimens sighted, and substrate type are shown. Transects TR2-5 included rocky outcrops and sandy substrates, but resolution in the map at the presented scale do not show the rocky outcrops.
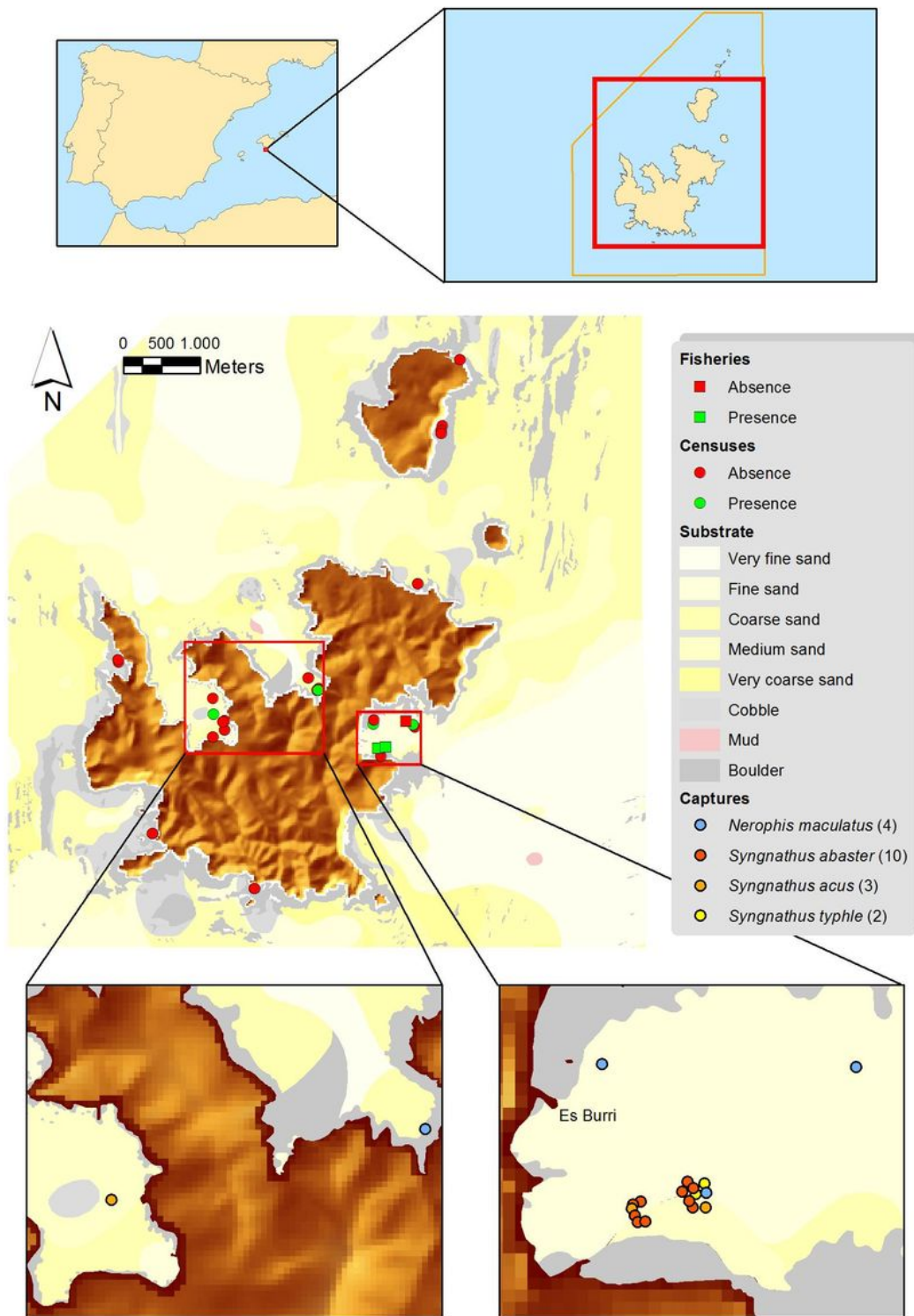
Figure 2

PNAC – Study area and surveyed sites for syngnathids in Cabrera Archipelago (Balearic Islands, West Mediterranean). Upper: Presences and absences of syngnathids. Below: Capture sites in Cabrera Island. Species, sex and specimens sighted, and substrate type are shown.
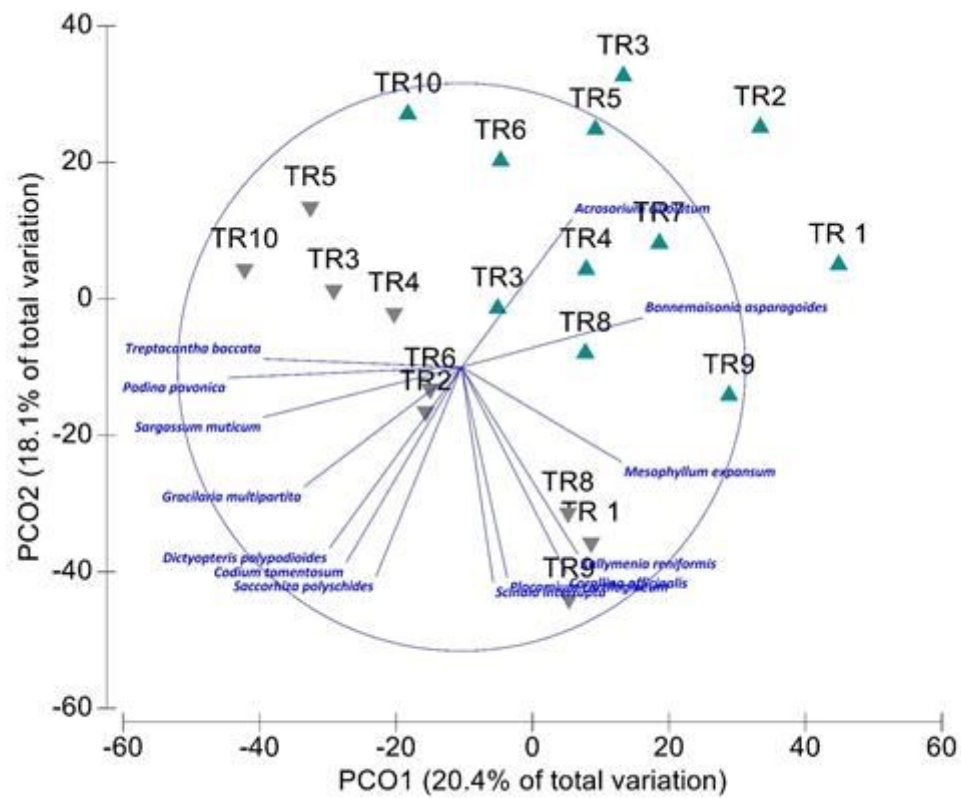
Figure 3

PNIA - Principal coordinates ordination of samples of Transect x Season pairwise combinations for seaweed assemblages on spring (green) and summer (grey). Overlay vectors are species whose cover has a Spearman correlation >0.65 with any axis.
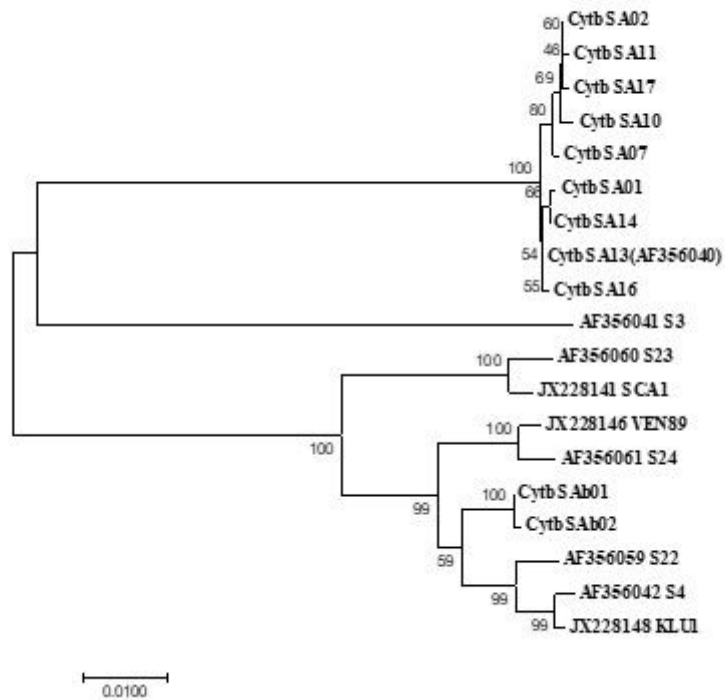
Figure 4

NJ tree (p-distance) for the Syngnathus genus. "SA" and "SAb" show S. acus and S. abaster haplotypes, respectively. Numbers on branches indicate the bootstrap value for their confidence (1,000 replicates). GenBank reference sequences for S. abaster (AF356060_S23; JX228141_SCA1), S. typhle (AF356042_S4; AF356059_S22; JX228148_KLU1), S. acus (AF356040), S. rostellatus (AF356041_S3) and S. taenionatus (AF356061_S24; JX228146_VEN89) are provided (see Mwale et al. 2013. JFB, 82, 2045-2062, doi:10.1111/jfb.12130).
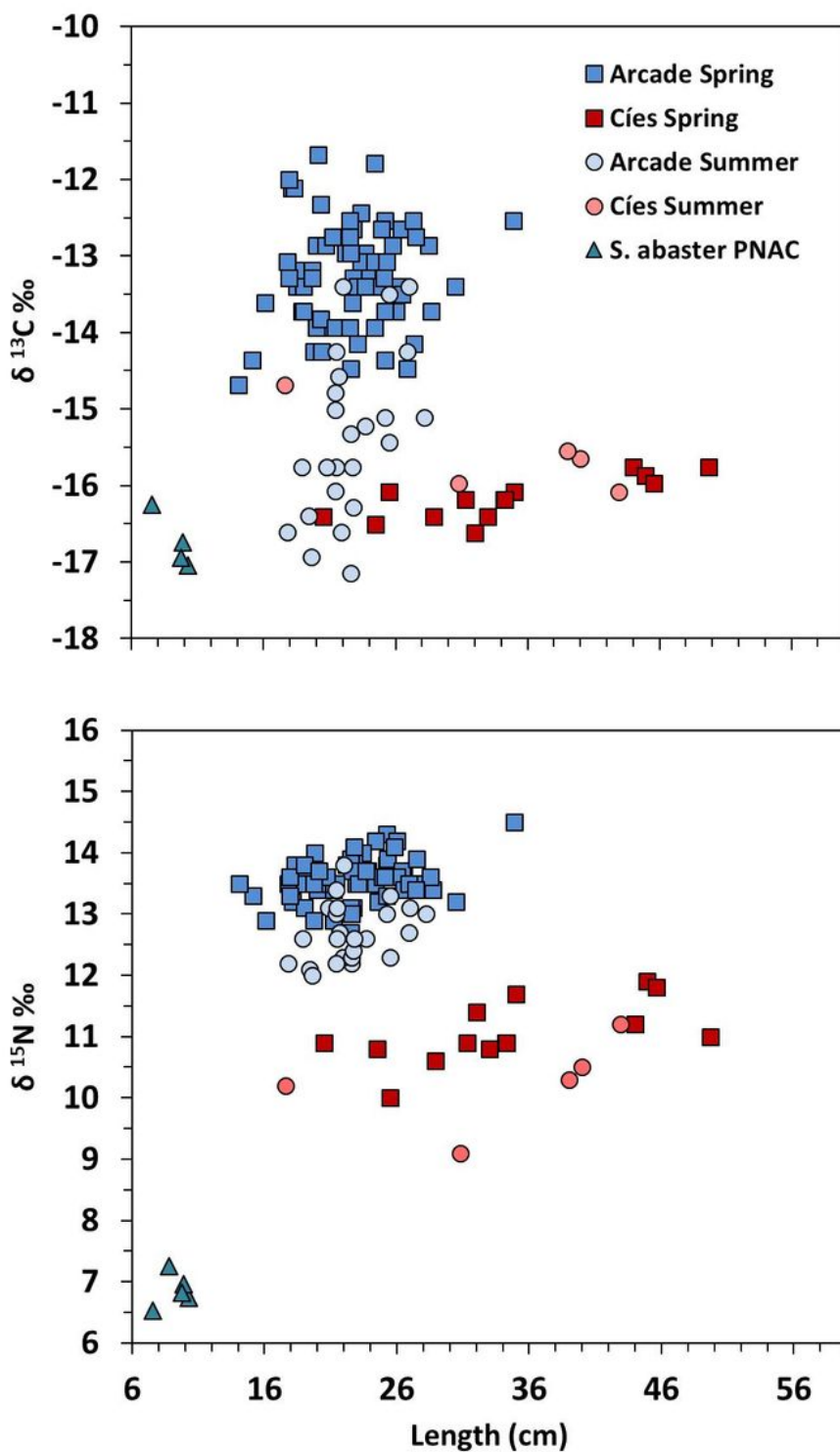
Figure 5

Scatter plot of stable isotopes-length relationships in Syngnathus acus captured in spring and summer 2016 in Cíes Archipelago (PNIA). Data for Arcade Cove pipefish (M. Planas, unpublished observations) and for S. abaster from PNAC (December 2016) are provided for comparative purposes. Arcade Cove specimens were collected on spring and summer 2016.
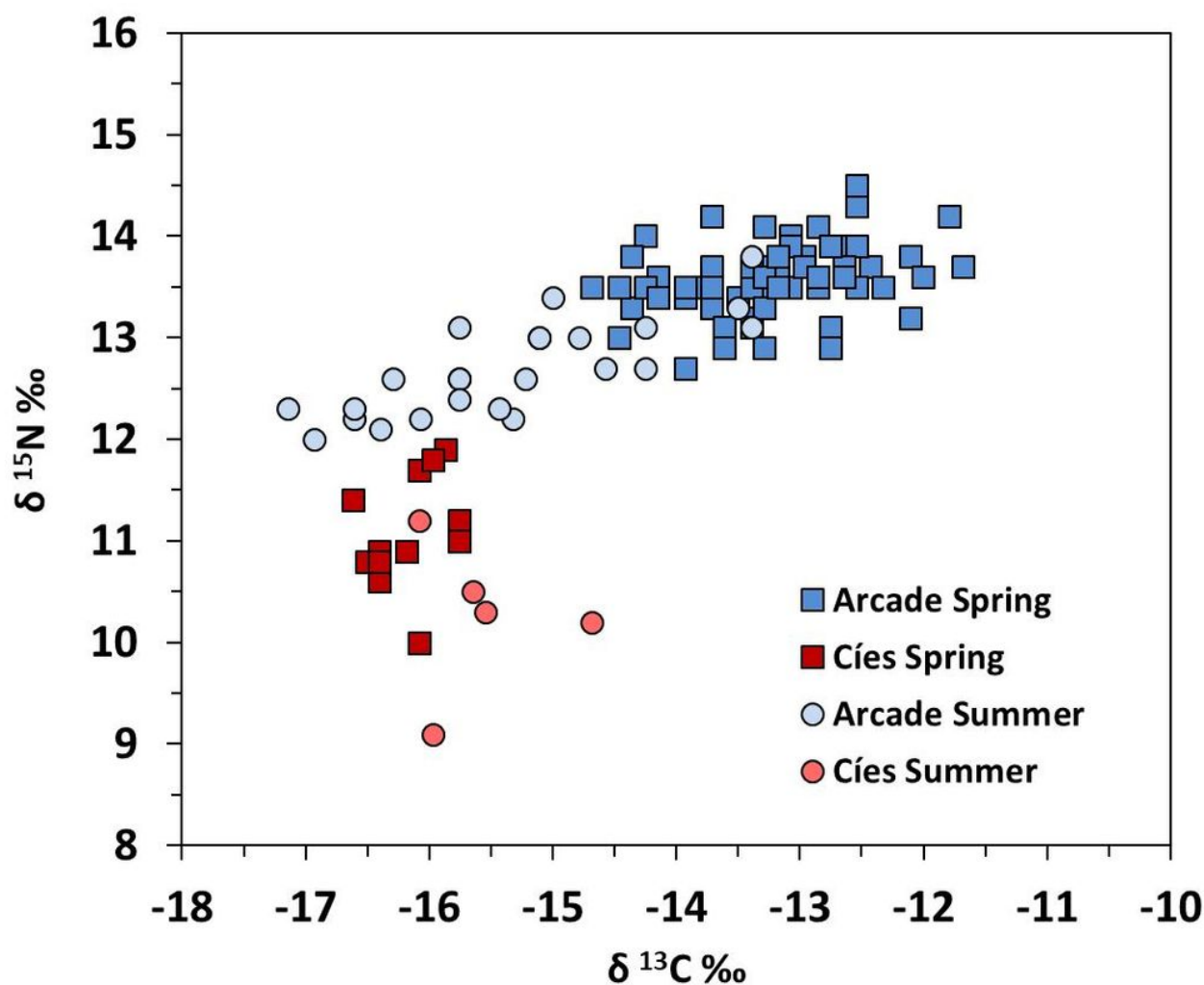
Figure 6

Schematic representation of the relationships between isotopic values (δ13C and δ15N and Syngnathus acus from Cíes Archipelago (present study) and Arcade Cove (M. Planas, unpublished observations). Samples collected in spring and summer 2016. Similarity groups (polygons) from hierarchical clustering (Ward's method) are shown.