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A multidisciplinary approach to identify priority areas for the conservation of a vulnerable family of fishes in Spanish Marine National Parks

Miquel Planas Oliver (mplanas@iim.csic.es)
Instituto de Investigaciones Marinas (CSIC) https://orcid.org/0000-0003-1217-5716
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

Research article

Keywords: National Park, syngnathids, habitat, stable isotopes, genetic identification, conservation

Posted Date: November 17th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-24731/v2

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Abstract

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

Results: Syngnathid diversity and abundance 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 National Parks (NP) differed isotopically, with much lower $\delta^{15}N$ in PNAC than in PNIA. The dominant species were *S. abaster* in PNAC and *S. acus* in PNIA. Syngnathids preferred less exposed sites in macroalgal assemblages in PNIA and *Cymodocea* meadows in PNAC. The occurrence of very large specimens, the absence of small-medium sizes and the isotopic comparison with a nearby population suggest that the population of *Syngnathus acus* (the dominant syngnathid in PNIA) mainly comprised breeders that migrate seasonally. Novel 16S rDNA haplotypes and sequence variants were detected for *H. 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 species misidentifications of genus *Syngnathus* in PNAC, and migratory events in PNIA. We propose several preferential sites in both NPs for future monitoring of syngnathid populations and some recommendations for their conservation.

Background

Syngnathidae is a singular fish family mostly inhabiting temperate and tropical sheltered, coastal marine waters (Foster and Vincent 2004; Kuiter 2009). Seahorses and pipefishes utilize rocky, muddy, sandy, and rubble bottom habitats, generally associated with macrophytes communities (Manning et al., 2019. Syngnathids are secondary consumers with specialized and opportunistic predatory strategies, ambushing small prey (mainly planktonic and nektonic crustaceans), showing a variety of diets, and foraging behaviours across genera and locations (Manning et al. 2019). Seaweeds and seagrass meadows promote the growth of most food sources and enhance the cryptic ability of syngnathids 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 and eutrophication) and disturbances through boating and shipping (IUCN 2019). More than half of syngnathid species (two seahorse and eleven pipefish species) inhabiting Spanish coasts are currently classified 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 (e.g. European genus *Syngnathus*) (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 for the global evaluation of syngnathid populations in Spanish coasts, particularly in marine National Parks (NP). Studies conducted in NPs would be highly 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 marine ecosystems to ensure species survival and success in biodiversity conservation. However, protection requires a deep knowledge and analysis of habitats, values and threats, particularly for exceptional species and populations.

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 , whereas the Western side is dominated by hard substrates covered by crusty, coralline and other turf-forming seaweeds (Piñeiro-Corbeira et al. 2020). That side is exposed to Atlantic open water and extreme sea currents and waves, mainly in winter. The Eastern side is less exposed due to its position facing the Ría de Vigo. That side is characterized by a high biodiversity and productivity, and therefore it is an area of special interest for fishing. Such high productivity 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 abundance in summer and seasonal changes in community structure. 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 (Cambie et al. 2012; Ouréns et al. 2015) and the use of some fishing gears impact negatively on syngnathids (by-catch and substrate degradation). Fishing ground is protected but not currently subject to special regulations (Ouréns et al. 2015). Increasing tourism and nature activities promote public awareness for the conservation of marine ecosystems (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. Algal beds, seagrass meadows and rocky bottoms dominate the subtidal zone. Three species of seagrass meadows are present: *Z. noltei* (<2m depth), *Cymodocea nodosa* (0- 25 m depth) and *Posidonia oceanica* (0-40 m depth).

Tourists visiting PNAC increases yearly, and recreational fishing and trawling in PNAC were banned in 1992. Small-scale fishing was regulated in 1995 but 80 small-scale boats from neighbouring towns 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 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/abundance 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 and a prevalence of a single mode (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. The presence of two or three mode samples in TR2, TR4 and TR3 reflected a mixture of particle sizes, including bioclastic gravel (bivalves and gastropods shells) and maerl elements. Different sedimentary environments (wide variability of textural characteristics) were present along some transects (e.g. TR3). 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 analysed considering data of 55 species with medium-high abundance (Additional files 2, 3). Diversity (H') and species richness (S) were particularly low in TR1, TR2, TR7 and TR10, especially in spring (Additional file 2). Seaweed cover increased in summer, especially 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 transects (P <0.05) and seasons (P <0.01). Those differences are reflected in the two-dimensional PCOs plot (Fig. 1). Spring (left) and summer (right) samples followed a gradient along axis 1 (20.4 % of total variation). Abundance 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, TR8 and TR9 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 six 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 abundance (0.06 - 0.13 syngnathids 100 m^{-2}) were recorded in mixed (sand-rock) or rocky substrates on transects TR3 and TR10 (32 and 43% of total specimens, respectively). Syngnathids were missing in the more exposed transects TR1, TR7, TR8 and TR9 (northern and southern areas with rocky substrate and coarse sand patches). TR1 was facing SW waves (prevalent component during storm winter conditions), while TR7, TR8 and TR9 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.

Most collected fishes were large adults, with *S. acus* averaging 31.8 ± 10.0 cm SL (range: 14.8 - 49.7 cm) and *H. guttulatus*, 22.6 ± 2.0 cm (range: 18.7 - 22.7 cm). Mean weights were 21.9 ± 5.2 g (range: 1.3 – 67.6 g) in *S. acus* and 22.6 ± 2.0 (range: 14.8 – 25.8 g) in *H. guttulatus*.

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). The species 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). Abundance 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 *Syngnathus acus*, two *Nerophis maculatus* Rafinesque, 1810) were sighted on 37 visual censuses and 15 specimens (10 *Syngnathus abaster* Risso, 1827, two *Syngnathus typhle* Linnaeus, 1758, two *Syngnathus acus* and, one *Nerophis maculatus*) were captured in seven fishing sets (Table 2). All specimens were captured at 11 - 21 m depth, except for two *N. maculatus* (< 8m 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}).

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; GenBank Accession Number: MW080699) identical to the reference for this species (AF356040; Wilson et al. 2001). Haplotypes Cytb_SA01 (MW080694) and Cytb_SA02 (MW080695) were the most abundant (nine and six individuals, respectively), whereas the rest were only found in one individual (Cytb_SA07: MW080696; Cytb_SA10: MW080697; Cytb_SA11: MW080698; Cytb_SA14: MW080700; Cytb_SA16: MW080701; Cytb_SA17: MW080702).

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: MW080705) 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: MW080703 and Cytb_SAb02: MW080704) in four and two fish, respectively; showing 139 variable sites respect to the *S. acus* sequence (Additional file 2). These two Cytb_Sab haplotypes showed a higher sequence identity with *S. typhle* haplotype reference (JX228148; identities > 98%) than with *S. abaster* (JX228141; identities ≤ 95%) available at GenBank database. Phylogenetic analysis grouped the two Cytb_SAb haplotypes from PNAC in a monophyletic cluster, clearly differentiated from available GenBank sequences of *S. abaster* (Mwale et al. 2013) and other pipefish species distributed in Mediterranean areas (*S. acus*, *S. typhle* and *S. rostellatus*) (Fig. 2).

Stable isotope signatures in syngnathids

In PNIA, *H. guttulatus* and *S. acus* (Table 3) differed significantly for $\delta^{13}C$ (ANOVA, P=0.026) but not for $\delta^{15}N$ (P=0.491). Isotopic values in *S. acus* ranged from -16.6 to -14.7 ‰ for $\delta^{13}C$ (-16.1 ± 0.4) and from 9.1 to 11.9 ‰ for $\delta^{15}N$ (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-sex comparisons showed inter-seasonal differences only for $\delta^{13}C$, 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). Sex-maturity comparisons showed similar $\delta^{13}C$ values for mature (-16.2 ± 0.3 ‰) and immature (15.9 ± 0.6 ‰) fishes (P=0.104) but lower $\delta^{15}N$ signals in the later (11.1 ± 0.5 ‰ for mature, 10.4 ± 0.8 ‰ for immature) (P=0.014).

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 $\delta^{13}C$ (-16.4 ± 1.8) and from 6.5 to 7.3 ‰ for $\delta^{15}N$ (6.9 ± 0.3).

Discussion

Diversity, distribution and habitat of syngnathids

PNIA and PNAC differed in habitat characteristics and syngnathids occurrence. Sixteen syngnathid species are known in Europe (Dawson 1986) but only five were identified in our study. Two species were sighted in PNIA: the seahorse *H. guttulatus* (very low abundance) and the pipefish *S. acus* (Highly dominant). Most specimens sampled from PNIA were very large, lacking young or small sized fishes. In PNAC, seahorses were absent and four pipefish species (*S. abaster, S. acus, S. typhle, N. maculatus*) were recorded, comprising mostly small specimens. Syngnathids were considered uncommon in PNAC, though occurrences of *S. acus*, *S. typhle*, *H. guttulatus* and *H. hippocampus* were known (Riera et al. 1993). Our results indicate low pipefish occurrences, with higher abundance in Es Burri Bay, particularly for *S. abaster*. This species is also the most common in other nearby areas (Mar Menor, SW Spain) (De Maya et al. 2004). *H. guttulatus* and *N. maculatus* are classified as *Data Deficient*, whereas the others are considered *Least 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).

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 (Wilson et al. 2001; Woodall et al. 2015). Available genetic sampling in PNAC allowed detecting novel haplotypes for a small number of pipefish morphologically identified as *N. maculatus* (1) and *S. abaster* (2) (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*). In the absence of available Cytb data, other possible species like *S. schmidti* and *S. phlegon* 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 north-western Pacific messmate pipefish (Sogabe and Takagi, 2013). Sample misidentifications cannot be ruled out, according to confuse discriminations reported for European *Syngnathus* species (Hablützel and Wilson 2011).

Differences in diversity, distribution and abundance of syngnathids are related to habitat characteristics (Vincent et al. 1995; Otero-Ferrer et al. 2015; Woodall et al. 2015). Many species are algae 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). In PNIA, seaweed communities are structurally complex and patchily distributed on mixed or rocky substrates (Peña and Bárbara 2006; Piñeiro-Corbeira et al. 2020. Most syngnathids in PNIA were located in semi-exposed or sheltered habitats on transects that showed the highest similarity regarding seaweed communities. Transect TR10 was particularly interesting since it was located in the most sheltered area and the unique site with seahorse occurrences. As for *S. acus* in PNIA, dominant 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 (García-Redondo et al. 2017, 2019) 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). 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). Its absence in PNIA could rely on the lack of seagrass meadows, even though this species may adapt to different types of 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 unexpected low pipefish abundance, which agrees with previous observations in similar habitats (Vincent et al. 1995). The highest abundance was recorded in *C. nodosa* meadows in Es Burri Bay (1.2-1.3 syngnathids 100 m^{-2}). Visual censuses of syngnathids in dense meadows are difficult due to fish crypsis. Captures with the first visual censuses from 2.8 to 21.5 m depth resulted substantially improved with *gánguil* gear operating at 11 – 16.5 m depth. However, global species richness and abundance in PNAC could have been underestimated. European syngnathids usually inhabit brackish areas (<10 m depth) but *C. nodosa* meadows are present at deeper depths (11-13 m depth) in Es Burri Bay. 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 above the depths imposed by gear, site and fishing permissions in *gánguil* sampling.

Changes in macroalgal assemblages in PNIA are occurring since 2012. Abundance of *Treptacantha baccata, T. usneoides* and *Saccorhiza polyschides* decrease, while turf (*Halopteris scoparia*, *Chondria coerulescens* or *Corallina* spp.), and non-native (*Codium fragile*, *Asparagopsis armata*) species increase

(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 but areas with high bottom mobility (sand waves and megarriples 3D) were avoided (TR2 and TR4). Some syngnathid species appear to be generalist considering distribution patterns and algal community characteristics whereas others prefer certain seaweed forms and feed on specific sources (Masonjones et al. 2010; Planas et al. 2020). 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 shallow and rocky areas (TR3) near the shore wave-breaking zone submitted to a certain degree of water agitation.

Due to the high dominance of *S. acus* in PNIA, the species deserves special consideration. The length–weight relationship was similar to that in the western Black Sea (Yildiz et al. 2015). The large specimens (31.8 ± 10.0 cm SL) 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 Archipelago raises 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 representative of specimens from nearby areas would clarify that dilemma.

Breeding season

Syngnathids may change habitat and prey preferences as they grow (Franzoi et al. 1993; 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 anything on this topic.

Isotopic signatures in syngnathids

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 those in other Mediterranean areas (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.

The pelagic food web from coastal areas (e.g. Arcade cove) in Galicia are typically enriched in $\delta^{13}C$ and $\delta^{15}N$ 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 but receiving freshwater inputs (Álvarez-Iglesias et al. 2006). The population of *S. acus* inhabiting Arcade cove markedly differ in size and isotopic signals from that in Cíes (Fig. 3). Habitat and trophic web characteristics in both areas also differ considerably (Filgueira and Castro 2011). The former receives anthropogenic wastewater inputs and it is characterized by a complex trophic web, and a locally important microphytobenthos production available to primary consumers through resuspension. The cove is a community with a high diversity of organic matter sources but terrestrial particulate organic matter does not seem to contribute significantly to consumers' most plausible diets (Filgueira and Castro 2011). Wastewater discharges would increase $\delta^{15}N$ 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). Both trends were reflected in isotopic signals of *S. acus* in Arcade cove (Fig. 4) but not in those from Cíes, with higher oceanic influence and more stable conditions. The higher $\delta^{15}N$ 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. 2020) (Fig. 4). Considering a trophic enrichment factor of 4.1 for $\delta^{15}N$ (Planas et al. 2020) and $\delta^{15}N$ values of filter-feeders in Arcade cove (8.98 ‰ in *Mytilus galloprovincialis*) (Filgueira and Castro 2011) and in Cíes (5.13 ‰ in *Musculus costulatus*) (Jiménez, 2019) as isotopic baselines (Post 2002), the resulting trophic levels for *S. acus* in Arcade and Cíes were 3.16 and 3.83, respectively. Such trophic level dissimilarities imply differences in resources exploitation as the result of disparities in trophic web composition and structure.

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 provided on potential migrations in *S. acus* on Cíes Archipelago. Isotopic signals in fin

tissues of syngnathids reflect the isotopic profile of the diet ingested 2-3 months earlier (M. Planas, unpublished observations). 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. However, that assumption is not supported by actual differences in $\delta^{15}N$ 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. 4). 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).

A lack of information on syngnathid populations has prevented conservation actions from being conducted in Spanish NPs. Further studies should provide more knowledge on those populations in order to undertake specific conservation actions 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 Bay has the greatest interest value for further conservation actions and monitoring. 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 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 protect sensible areas against habitat 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 (TR10 in PNIA), which supports a high density of vessel traffic during the touristic seasons. Seagrass meadows appear as essential communities to maintain syngnathid populations in PNAC as all fishes were captured there. Damage to seagrass meadows by anchoring of recreational boats in Es Burri Bay might compromise syngnathid populations in PNAC.

Conclusions

This is the first multidisciplinary study of syngnathid populations in Spanish coasts, specifically in the two marine Spanish NPs. It will contribute to the knowledge of syngnathid populations, leading to more informed and efficient management of both NPs. Species diversity, abundance, habitat preference, and isotopic signatures differed in both NPs, depending on habitat characteristics. Syngnathids preferred sheltered macroalgal assemblages in PNIA and *Cymodocea* meadows in PNAC. Our results seem to indicate 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 genetic and morphological sampling surveys to clarify the taxonomic status of the genus in PNAC and the consequences for species conservation. 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 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. 5), and (b) Cabrera Archipelago National Park (PNAC) (39°08'N, 2°56'W), in the western Mediterranean (Balearic Islands) (Fig. 6).

The study in PNIA was conducted in Cíes Archipelago, comprising three islands and various islets. PNIA was declared Nature Reserve in 1980 and Spanish National Park in 2002. The NP 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). Cíes Archipelago was declared Natural Park, Special Protection Area (SPA), Site of Community Importance (SCI), OSPAR area, and UNESCO World Heritage candidate (UNESCO 2019). 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).

In PNAC, 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). 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 due to low concentrations of dissolved inorganic nutrients and chlorophyll (Vives 1993) and light attenuation coefficient is extremely low. Depth and hydrodynamics are the dominant abiotic factors that affect habitat distribution and vary among sites throughout the archipelago (Ballesteros and Zabala 1993).

Swept sites

Based on previous knowledge (seaweeds cover, substrate characteristics and exposure level to open water), ten subtidal transects (TR1 to TR10) were selected along the western coast of Cíes Archipelago in PNIA (Fig. 5; Additional file 1), and visited in spring and summer 2016 (two visual censuses per site and season) to obtain an overview of habitat characteristics and spatial distribution of syngnathids. Transects were positioned parallel or perpendicular to the coastline (150 to 700 m length; 3 - 20 m depth) on rocky bottoms often interrupted by sandy patches. Two pairs of divers conducted 40 diurnal standard underwater visual census (UVC) (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. Through and crest zones were also sampled when bedforms were present. 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. Eleven subtidal sites (TR1 to TR11) including the main shallow benthic habitats present in PNAC were visited from 21st April to 1st December 2016 (17 – 26 °C) throughout the coast of Cabrera and Conillera islands (Fig. 6) for an overview of syngnathids distribution. We conducted 37 surveys using UVC (50 m length × 5 m wide; 60- 80 min per dive; 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. UVC 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. 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 UVC in PNAC, a small trawl net called *gánguil* (traditional gear for small crustaceans catching) was assayed on *C. nodosa* meadows. The 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 damage of fan mussel, *Pinna nobilis*, populations while sampling, PNAC authority only allowed the use of *gánguil* in *C. nodosa* meadows in Es Burri Bay (Fig. 6; 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. 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.

Fish collection

In UVC, syngnathids were hand-caught collected or manually extracted from the fishing gear, 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), sex, sexual status, meristics (fin rays, body rings) and body coloration were also annotated. The sexual status 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 analysed 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. Previously to grain size analysis, the organic matter was removed using 30% H_2O_2 for several days and salts were removed with further washings with distilled water. 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 (Pääbo 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, the 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, sex, 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 analysed 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 approved by the Regional Government Xunta de Galicia (Reference number: REGA ES360570202001/16/FUN/BIOL.AN/MPO02), and conducted in compliance with all bioethics standards on animal experimentation of the Spanish Government (R.D. 1201/2005, 10th October) and All the procedures were approved by the Bioethics Committee of CSIC. The corresponding author declares that all listed co-authors agreed to participate in the study and consent to being involved in the publication.

- Consent to publish: All listed co-authors consent to publish this manuscript 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.

- Funding: Study funded by Proyecto Hippoparques (1541S/2015 and 1580S/2015; Organismo Autónomo de Parques Nacionales - OAPN, Ministerio de Agricultura, Alimentación y Medio Ambiente, Spain). Project 1580S/2015 was in charge of genetics.

- Authors' contributions:

All authors have read and approved the manuscript.

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.

Acknowledgements

We acknowledge I. Ferreiro, D. Costas, R. Chamorro, L. Pereda, L. Royo, I. Zabarte, P. Arechavala, L. Ínsua, J. Pintado, the staff and keepers of PNIA and PNAC, and the Regional Government Xunta de Galicia for support in the project. Special thanks to Ledicia Prieto (University of the Basque Country, Spain) for excellent illustrations on seaweed assemblages in Cíes Archipelago. We acknowledge support of the publication fee by the CSIC Open Access Publication Support Initiative through its Unit of Information Resources for Research (URICI).

References

Álvarez I, de Castro M, Gómez-Gesteira M, Prego R. Inter- and intra-annual analysis of the salinity and temperature evolution in the Galician Rías Baixas-ocean boundary (northwest Spain). J Geophys Res. 2005; doi.org/10.1029/2004JC002504.

Álvarez-Iglesias P, Rubio B, Pérez-Arlucea M. Reliability of subtidal sediments as geochemical recorders of pollution inputs: San Simón Bay (Ría de Vigo, NW Spain). Estuar Coast Shelf Sci. 2006;70:507–21.

Álvarez-Salgado X, Gago J, Míguez BM, Gil-Coto, M, Pérez FF. Surface waters of the NW Iberian margin: Upwelling on the shelf versus outwelling of upwelled waters from the Rías Baixas. Estuar Coast Shelf Sci. 2000;51:821-37.

Amengual-Ramis JF, Vázquez-Archdale M, Cánovas-Pérez C, Morales-Nin B. The artisanal fishery of the spiny lobster (*Palinurus elephas*) in Cabrera National Park, Spain: Comparative study on traditional and modern traps with trammel nets. Fish Res. 2016;179:23-32.

Anonymous. Uova, larve e stadi giovanili di teleostei. In: Fauna e flora del Golfo di Napoli. Monografia 38, Puntata 3. Stazione Zoologica di Napoli; 1956. p. 385-1064.

Alvarado JJ, Barraza E, Sancho-Mejías TI. Central America echinoderms: Diversity, ecology and future perspectives. In: Alvarado JJ, Solís-Marín FA, editors. Echinoderm research and diversity in Latin America. Berlin: Springer-Verlag; 2013. p. 67–106.

Ballesteros E, Zabala M. El bentos: El marc físic. In: Moll-CSIC, editors. Història Natural del Arxipèlag de Cabrera. Palma de Mallorca: Societat d'Història Natural de le Balears; 1993. p. 663–86.

Blott, SJ, Pye K. Gradistat: A grain size distribution and statistics package for the analysis of unconsolidated sediments. Earth Surf Proc Land. 2001;26:1237-48.

Bode A, Álvarez-Osorio MT, Varela M. Phytoplankton and macrophyte contributions to littoral food webs in the Galician up welling estimated from stable isotopes. Mar Ecol Prog Ser. 2006;318:89–102.

Bode A, Álvarez-Osorio MT, Cabanas JM, Miranda A, Varela M. Recent trends in plankton and upwelling intensity off Galicia (NW Spain). Prog Ocenography. 2009;83:342-50.

Buttay L, Miranda A, Casas G, González-Quirós R, Nogueira E. Long-term and seasonal zooplankton dynamics in the northwest Iberian shelf and its relationship with meteo-climatic and hydrographic variability. J Plankton Res. 2015;38:106–21.

Cambiè G, Ouréns R, Vidal D, Carabel S, Freire J. Economic performance of coastal fisheries in Galicia (NW Spain): Case study of the Cíes Islands. Aquatic Living Res. 2012;25:195-204.

Carrizosa B. Cambios subacuáticos a corto plazo en el Parque Nacional Marítimo Terrestre de las Islas Atlánticas de Galicia: consecuencias para el uso recreativo del paisaje submarino. Algas. Bol Inf Soc Esp Ficol. 2016;84-8.

Casey SP, Hall HJ, Stanley HF, Vincent ACJ. The origin and evolution of seahorses (genus *Hippocampus*): a phylogenetic study using the cytochrome b gene of mitochondrial DNA. Mol Phylo Evol. 2004;30:261-72.

Crec'hriou R, Alemany F, Roussel E, Chassanite A, Marinaro JY, Mader J, Rochel E, Planes S. Fisheries replenishment of early life taxa: potential export of fish eggs and larvae from a temperate marine protected area. Fish Oceanogr. 2010;9:135-50.

Catalán I, Dunand A, Álvarez I, Alós J, Colinas N, Nash RDM. An evaluation of sampling methodology for assessing settlement of temperate fish in seagrass meadows. Mediterr Mar Sci. 2014;15:1–21.

Curtis JMR, Vincent ACJ. Life history of an unusual marine fish: survival, growth and movement patterns of *Hippocampus guttulatus*. J Fish Biol. 2006;68:707–33.

Dawson CE. Syngnathidae. In: Whitehead PJP, Bauchot ML, Hereau JC, Nielsen J, Tortonese E, editors. Fishes of the North-eastern Atlantic and the Mediterranean. Paris: UNESCO; 1986. p. 628-39.

De Maya, J.A., A. Andreu, P.A. Miñano, D. Verdiell Cubedo, A. Egea, F.J. Oliva Paterna, M. Torralva. Dinámica espacio-temporal de la familia Syngnathidae en las áreas someras del Mar Menor (SE, Murcia). Actas del III Congreso de la Naturaleza de la Región de Murcia; 2004. p. 125-31.

Faleiro F, Baptista M, Santos C, Aurélio ML, et al. Seahorses under a changing ocean: the impact of warming and acidification on the behaviour and physiology of a poor-swimming bony-armoured fish. Conserv Physiol. 2015. dx.plos.org/10.1093/conphys/cov009.

Filgueira R, Castro BG. Study of the trophic web of San Simon Bay (Ria de Vigo) by using stable isotopes. Cont Shelf Res. 2011;31:476-87.

Folk RL, Ward WC. Brazos River bar: A study in the significance of grain size parameters. J Sediment Petrol. 1957;27:3-26.

Foster SJ, Vincent ACJ. Life history and ecology of seahorses: implications for conservation and management. J Fish Bio. 2004;65:1-61.

Franzoi P, Maccagnani R, Rossi R, Ceccherelli VU. Life cycles and feeding habits of *Syngnathus taenionotus* and *S. abaster* (Pisces, Syngnathidae) in a brackish bay of the Po River Delta (Adriatic Sea). Mar Ecol Prog Ser, 1993;97:71-81.

Fraga, F. Upwelling off the Galician coast, Northwest Spain. In: Richards FA, editor. Upwelling Ecosystems. Washington: American Geophysical Union; 1981. p. 176–82.

García-Redondo V, Bárbara I, Díaz-Tapia P. Las praderas de *Zostera marina* L. del Parque Nacional Marítimo Terrestre de las Islas Atlánticas de Galicia y territorios adyacentes: Distribución, abundancia y flora asociada. NACC-Bioloxía. 2017;24:1-12.

García-Redondo V, Bárbara I, Díaz-Tapia P. *Zostera marina* meadows in the northwestern Spain: distribution, characteristics and anthropogenic pressures. Biodivers Conserv. 2019;28:1743-57.

Grau AM, Mayol J, Oliver J, Riera F, Riera MI. Llibre vermell dels peixos de les Illes Balears. Conselleria de Medi Ambient, Agricultura i Pesca. Govern Illes Balears; 2015.

Guerra A, Hernández-Urcera J, Garci, ME, Sestelo M, et al. Dwellers in dens on sandy bottoms: Ecological and behavioural traits of Octopus vulgaris. Sci Mar. 2014;78:405-14.

Guerra A, Hernández-Urcera J, Garci ME, Sestelo M, et al. Spawning habitat selection by *Octopus vulgaris*: New insights for a more effective management of this resource. Fish Res. 2015;167:313-22.

Guiry MD, Guiry GM. *AlgaeBase.* World-wide electronic publication, National University of Ireland. 2020. https://www.algaebase.org. Accessed 26 Feb 2020.

Gurkan S, Taskavak E. Length-weight relationships for syngnathid fishes of the Aegean Sea, Turkey. Belgian J Zool. 2007;137:219-22.

Gurkan S, Taskavak E, Hossucu B. The reproductive biology of the great pipefish *Syngnathus acus* (Family: Syngnathidae) in the Aegean Sea. North-West J Zool. 2009;5:179-90.

Hablützel P, Wilson A. Notes on the occurrence of *Syngnathus rostellatus* (Teleostei: Syngnathidae) in the Mediterranean. Mar Biodivers Rec. 2011;4:1-4.

Hamilton H, Saarman N, Short G, Sellas AB, et al. Molecular phylogeny and patterns of diversification in syngnathid fishes. Mol Phylogenet Evol. 2017;107:388-403.

Haver SM, Fournet MEH, Dziak RP, Gabriele C, et al. Comparing the underwater soundscapes of four U.S. National Parks and marine sanctuaries. Front Mar Sci. 2019; doi.org/10.3389/fmars.2019.00500.

Hobson KA, Barnett-Johnson R, Cerling T. Using isoscapes to track animal migration. In: West JB, Bowen GJ, Dawson TE, Tu KP, editors. Isoscapes: Understanding movement, pattern, and process on earth through isotope mapping. Springer Science+Business Media B.V.; 2010. p. 273-98.

IUCN. The IUCN Red List of seahorses and pipefishes in the Mediterranean Sea. 2017. https://www.iucn.org/sites/dev/files/content/documents/2017/ficha_seahorses_baja.pdf. Accessed 6 Nov 2019.

IUCN. The IUCN red list of threatened species. Version 2019-1. 2019. http://www.iucnredlist.org. 2019. Accessed 16 Oct 2019.

Jiménez A. Evolución anual de poblaciones de signátidos del archipiélago de las Islas Cíes (Parque Nacional de las Islas Atlánticas, NO España), Master Thesis Dissertation, University of Vigo; 2019.

Kendrick AJ, Hyndes GA. Patterns in the abundance and size-distribution of syngnathid fishes among habitats in a seagrass-dominated marine environment. Estuar Coast Shelf Sci. 2003;57:631–40.

Kuiter RH. Seahorses and their relatives. Seaford: Aquatic Photographics, Australia; 2009.

Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis v7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870-74.

Lazzari MA, Able KW. Northern pipefish, *Syngnathus fuscus*, occurrences over the mid-Atlantic bight continental shelf: evidence of seasonal migration. Environ Biol Fish. 1990;27:177-85.

López A, Vera M, Otero-Ferrer F, et al. Species identification and genetic structure of threatened seahorses in Gran Canaria Island (Spain) using mitochondrial and microsatellite markers. Conserv Genet. 2010. doi.org/10.1007/s10592-010-0116-6.

Lourie S. Measuring seahorses. Project Seahorse Technical Report No.4, Version 1.0. Project Seahorse, Fisheries Centre, University of British Columbia; 2003.

Manning CG, Foster SJ. Vincent ACJ. A review of the diets and feeding behaviours of a family of biologically diverse marine fishes (Family Syngnathidae). Rev Fish Biol Fisheries 2019;29:197–221.

Masonjones HD, Rose E, McRae LB, Dixson DL. An examination of the population dynamics of syngnathid fishes within Tampa Bay, Florida, USA. Curr Zool. 2010;56:118-33.

McKenna MF, Gabriele C, Kipple B. Effects of marine vessel management on the underwater acoustic environment of Glacier Bay National Park, AK. Ocean Coast Manag. 2017;139:102–12.

Monteiro NM, Almada VC, Santos AM, Vieira MN. The breeding ecology of the pipefish *Nerophis lumbriciformis* and its relation to latitude and water temperature. J Mar Biol Assoc UK. 2001;81:1031-3.

Morales-Nin B, Grau AM, Palmer M. Managing coastal zone fisheries: A Mediterranean case study. Ocean Coast Manage. 2010;53:99–106.

Mwale M, Kaiser H, Barker NP, Wilson AB, Teske PR. Identification of a uniquely southern African clade of coastal pipefishes *Syngnathus* spp. J Fish Biol. 2013;82:2045-62.

Nogueira E, Pérez FF, Ríos AF. Seasonal patterns and long-term trends in an estuarine upwelling ecosystem (Ría de Vigo, NW Spain). Estuar Coast Shelf Sci. 1997;44:285–300.

Oliveira F, Erzini K, Gonçalves JMS. Feeding habits of the deep-snouted pipefish *Syngnathus typhle* in a temperate coastal lagoon. Estuar Coast Shelf Sci. 2007;72:337-47.

Otero-Ferrer F, Herrera R, Tuset VM, Socorro J, Molina L. Spatial and seasonal patterns of European short-snouted seahorse *Hippocampus hippocampus* distribution in island coastal environments. Afri J Mar Sci. 2015;37:395-404.

Ouréns R, Cambié G, Freire J. Characterizing the complexity of the fleet dynamics for an effective fisheries management: The case of the Cíes Islands (NW Spain). Sci Mar. 2015;79:453-64.

Pääbo S, Thomas WK, Whitfield KM, Kumazawa Y, Wilson AC. Rearrangements of mitochondrial transfer-RNA genes in marsupials. J Mol Evol. 1991;33:426-30.

Palumbi SR, Martin AP, Romano SL, McMillan WO, Stice L, Grabowski G. The simple fool's guide to PCR. Department of Zoology, University of Hawaii; 1991.

Peña V, Bárbara I. Los fondos marinos de maërl del Parque Nacional de las Islas Atlánticas (Galicia, España): Distribución, abundancia y flora asociada. NACC (Bioloxía). 2016;15:7-25.

Picado A, Álvarez I, Vaz N, Varela R, Gómez-Gesteira M, Dias JM. Assessment of chlorophyll variability along the northwestern coast of Iberian Peninsula. J Sea Res 2014;93:2-11.

Piñeiro-Corbeira C, Barreiro R, Olmedo M, De la Cruz-Modino R. Recreational snorkeling activities to enhance seascape enjoyment and environmental education in the Islas Atlánticas de Galicia National Park (Spain). J Environ Manage 272:111065.

Planas M, Chamorro A, Quintas P, Vilar A. Establishment and maintenance of threatened long-snouted seahorse, *Hippocampus guttulatus*, broodstock in captivity. Aquaculture. 2008;283:19-28.

Planas M, Chamorro A, Paltrinieri A, Campos S, Nedelec K, Hernández-Urcera J. Effect of diet on breeders and inheritance in Syngnathids: Application of isotopic experimentally derived data to field studies. Mar Ecol Prog Ser. 2020; 650:107-123.

Post DM. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology. 2002;83:703-18.

Post DM, Craig A, Layman D, Albrey Arrington D, Takimoto G, Quatrocchi J, Montaña CG. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia. 2007;152:179-89.

Puertos del Estado. http://www.puertos.es/es-es/oceanografia/Paginas/portus.aspx (2017). Accessed 30 Dec 2019.

Riera F, Pou S, Grau AM. La Ictiofauna. In: Alcover JJ, Ballesteros E, Fornós JJ, editors. Història Natural de l'Arxipèlag de Cabrera, Editorial Moll-CSIC;1993. p. 623-44.

Rodil IF, Lastra M, López J. Spatial variability of benthic macrofauna in the Ría of Vigo (NW Spain): Effect of sediment type and food availability. Mar Biol Res. 2009;5:572-84.

Román M, Rendal S, Fernández E, Méndez G. Seasonal variability of the carbon and nitrogen isotopic signature in a *Zostera noltei* meadow at the NW Iberian Peninsula. Wetlands. 2018;38:739–53.

Román M, Fernández E, Méndez G. Anthropogenic nutrient inputs in the NW Iberian Peninsula estuaries determined by nitrogen and carbon isotopic signatures of *Zostera noltei* seagrass meadows. Mar Environ Res. 2019;143:30-38.

Rumolo P, Bonnano A, Barra M, Fanelli E, et al. Spatial variations in feeding habits and trophic levels of two small pelagic fish species in the central Mediterranean Sea. Mar Environ Res. 2016;115:65-77.

Scilipoti D. Studio della comunità ittica residente all'interno dello Stagnone di Marsala (Sicilia occidentale): Distribuzione delle specie e ripartizione delle risorse in dipendenza di habitat a diversa complessità strutturale. PhD Thesis Dissertation, University of Messina; 1998.

Shokri MR, Gladstone W, Jelbart J. The effectiveness of seahorses and pipefish (Pisces: Syngnathidae) as a flagship group to evaluate the conservation value of estuarine seagrass beds. Aquatic Conserv mar freshw Ecosyst. 2009;19:588–95.

Skóra KE. The broad-nosed pipefish. In: Głowaciński Z, editor. Polish Red Data Book of Animals. Vertebrates. Warszawa: PWRiL; 2001. p. 316-18.

Smith TM, Hindell JS, Jenkins GP, Connolly RM. Edge effects on fish associated with seagrass and sand patches. Mar Ecol Prog Ser. 2008;359:203–13.

Sogabe A, Takagi M. Population genetic structure of the messmate pipefish *Corythoichthys haematopterus* in the northwest pacific: evidence for a cryptic species. SpringerPlus. 2013. doi.org/10.1186/2193-1801-2-408.

Spjotvoll E, Stoline MR. An Extension of the T-Method of multiple comparison to include the cases with unequal sample sizes. J Am Stat Assoc. 1973;68:975-8.

Tarnowska K, Sapota MR. Presence of the broad-nosed pipefish (*Syngnathus typhle*) in coastal waters of the Gulf of Gdańsk. Int J Oceanogr Hydrobiology. 2007;36:39-48.

Thayer GW, Murphey PL, LaCroix MW. Responses of plant communities in Western Florida Bay to the die-off of seagrasses. Bull Mar Sci. 1994;54:718-26.

Thayer GW, Powell AB, Hoss DE. Composition of larval, juvenile, and small adult fishes relative to changes in environmental conditions in Florida bay. Estuaries, 1999;22:518–33.

Valdés JL, Román MR, Álvarez-Ossorio MT, Gauzens AL, Miranda A. Zooplankton composition and distribution off the coast of Galicia, Spain. J Plankton Res. 1990;12:629–43.

Valladares S, Planas M. Non-lethal dorsal fin sampling for stable isotope analysis in seahorses. Aquatic Ecol. 2012 ;46:363–370.

Vives F. Aspectes hidrgràfiques i plantónics dekl voltants de l'Arxipèlag de Cabrera. In: Alcover JJ, Ballesteros E, Fornós JJ, editors. Història Natural de l'Arxipèlag de Cabrera. Editorial Moll-CSIC; 1993. p. 487-502.

Varvara L. Study on the biology of the syngnathidae family in Greece. PhD Thesis Dissertation, University of Ioannina; 2015.

Villegas-Ríos D, Alós J, March D, Palmer M, Mucientes G, Saborido-Rey F. Home range and diel behavior of the ballan wrasse, *Labrus bergylta*, determined by acoustic telemetry. J Sea Res. 2013;80:61-71.

Vincent ACJ, Berglund A, Ahnesjö I. Reproductive ecology of five pipefish species in one eelgrass meadow. Environ Biol Fish. 1995;44:347–61.

Vizzini S, Mazzola A. The trophic structure of the pipefish community (Pisces Syngnathidae) from a Western Mediterranean seagrass meadow based on stable isotope analysis. Estuaries. 2004;27:325-33.

UNESCO. World Heritage candidate. https://whc.unesco.org/en/tentativelists/6286/ (2019). Accessed 13 Dec 2019.

Wilson AB, Vincent A, Ahnesjo I, Meyer A. Male pregnancy in seahorses and pipefishes (Family Syngnathidae): Rapid diversification of paternal brood pouch morphology inferred from a molecular phylogeny. J Hered. 2001;92:159-66.

Woodall LC, Koldewey HJ, Boehm JT, Shaw W. Past and present drivers of population structure in a small coastal fish, the European long snouted seahorse *Hippocampus guttulatus*. Conserv Genet. 2015. doi.org/10.1007/s10592-015-0728-y.

Woodall LC, Otero-Ferrer F, Correia M, Curtis J, et al. A synthesis of European seahorse taxonomy, population structure, and habitat use as a basis for assessment, monitoring and conservation. Mar Biol. 2018;165(1):19.

WoRMS Editorial Board. World Register of Marine Species. http://www.marinespecies.org (2020). Accessed 22 Dec 2019.

Wunder MB. Using isoscapes to model probability surfaces for determining geographic origins. In: West JB, Bowen GJ, Dawson TE, Tu KP, editors. Isoscapes: Understanding movement, pattern, and process on earth through isotope mapping. Springer Science+Business Media B.V.; 2010. p. 251-70.

Yildiz T, Uzer U, Karakulak, FS. Preliminary report of a biometric analysis of greater pipefish *Syngnathus acus* Linnaeus, 1758 for the western Black Sea. Turk J Zool. 2015;39:917-24.

Figures

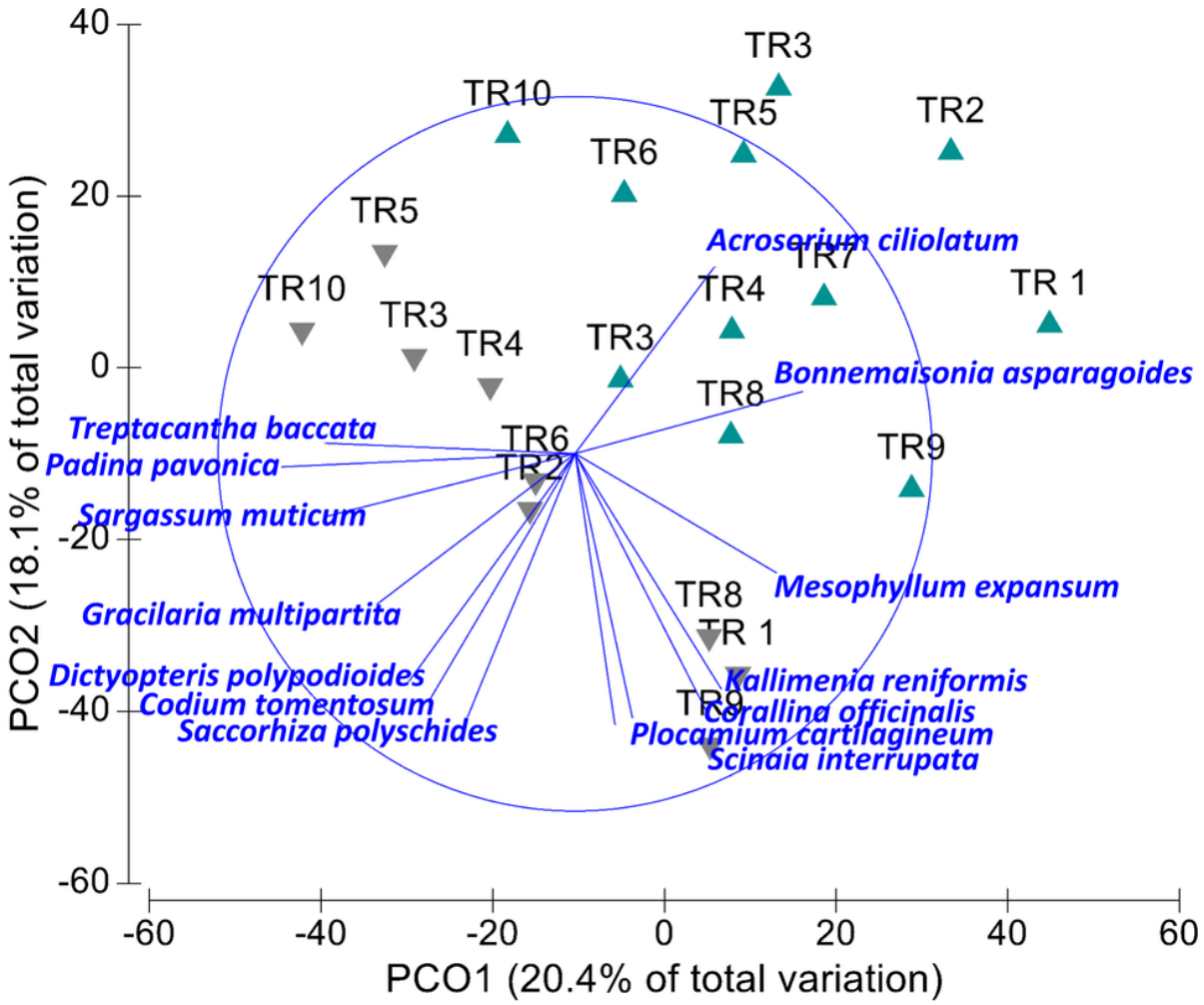
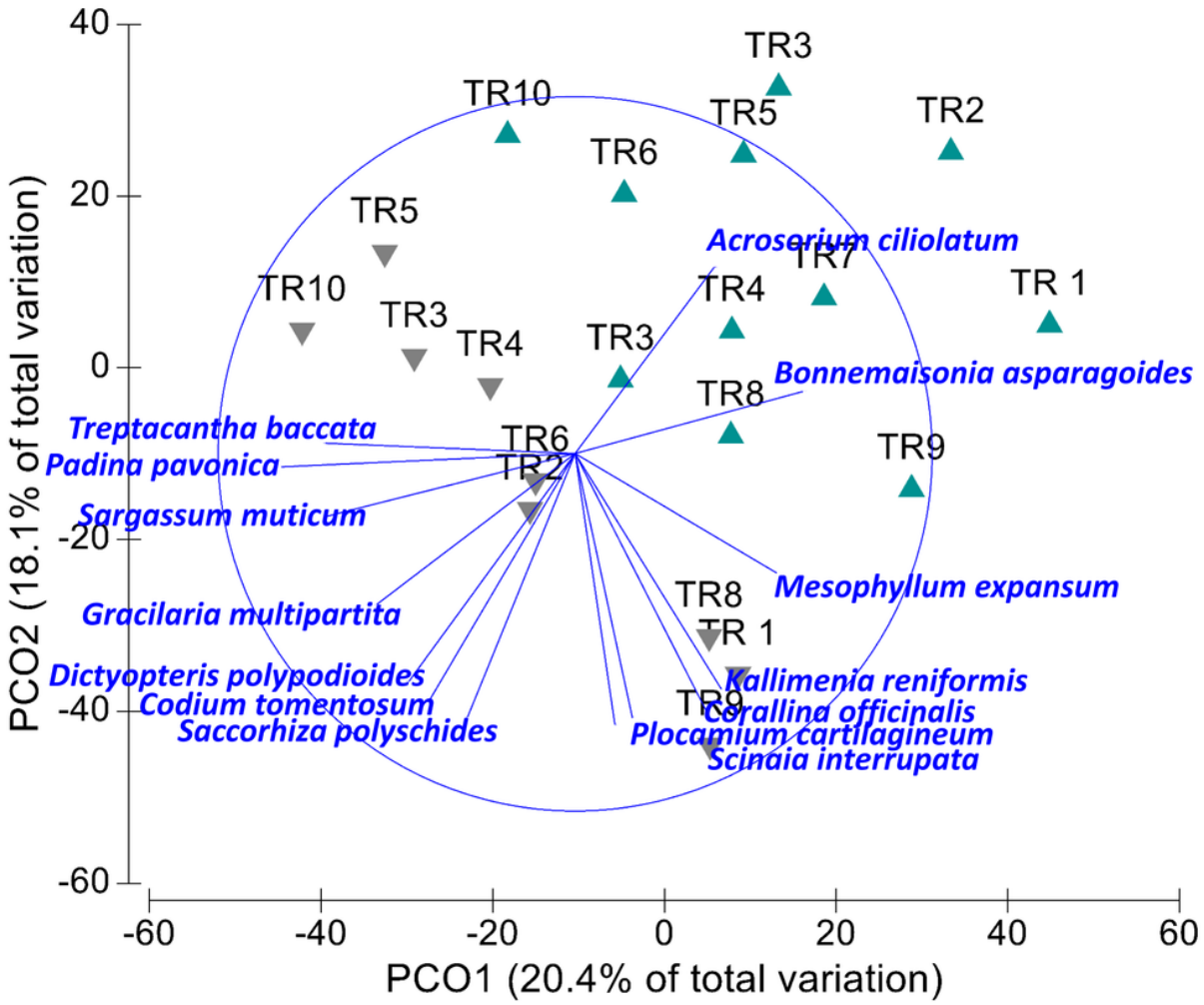


Figure 1

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.



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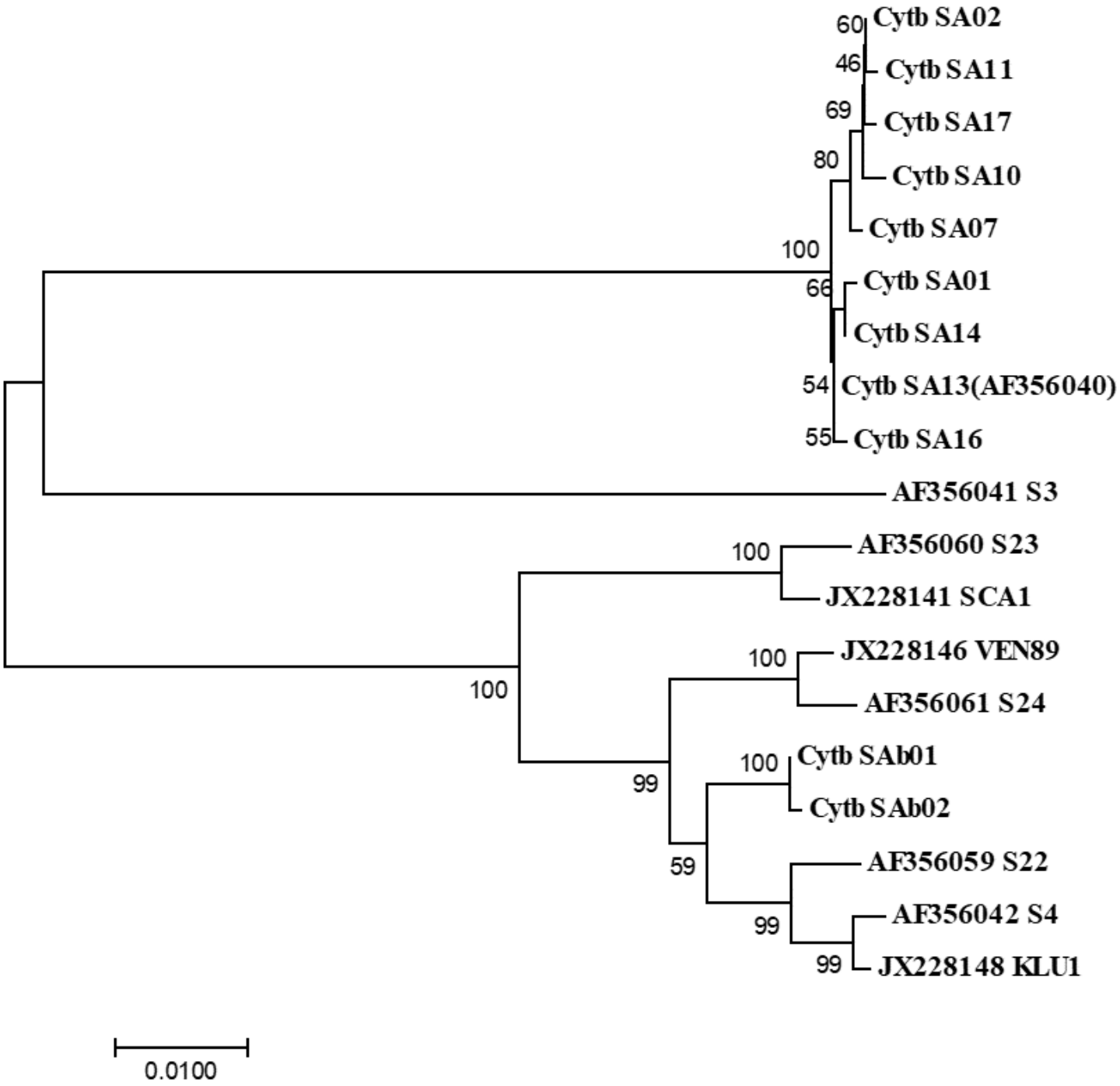
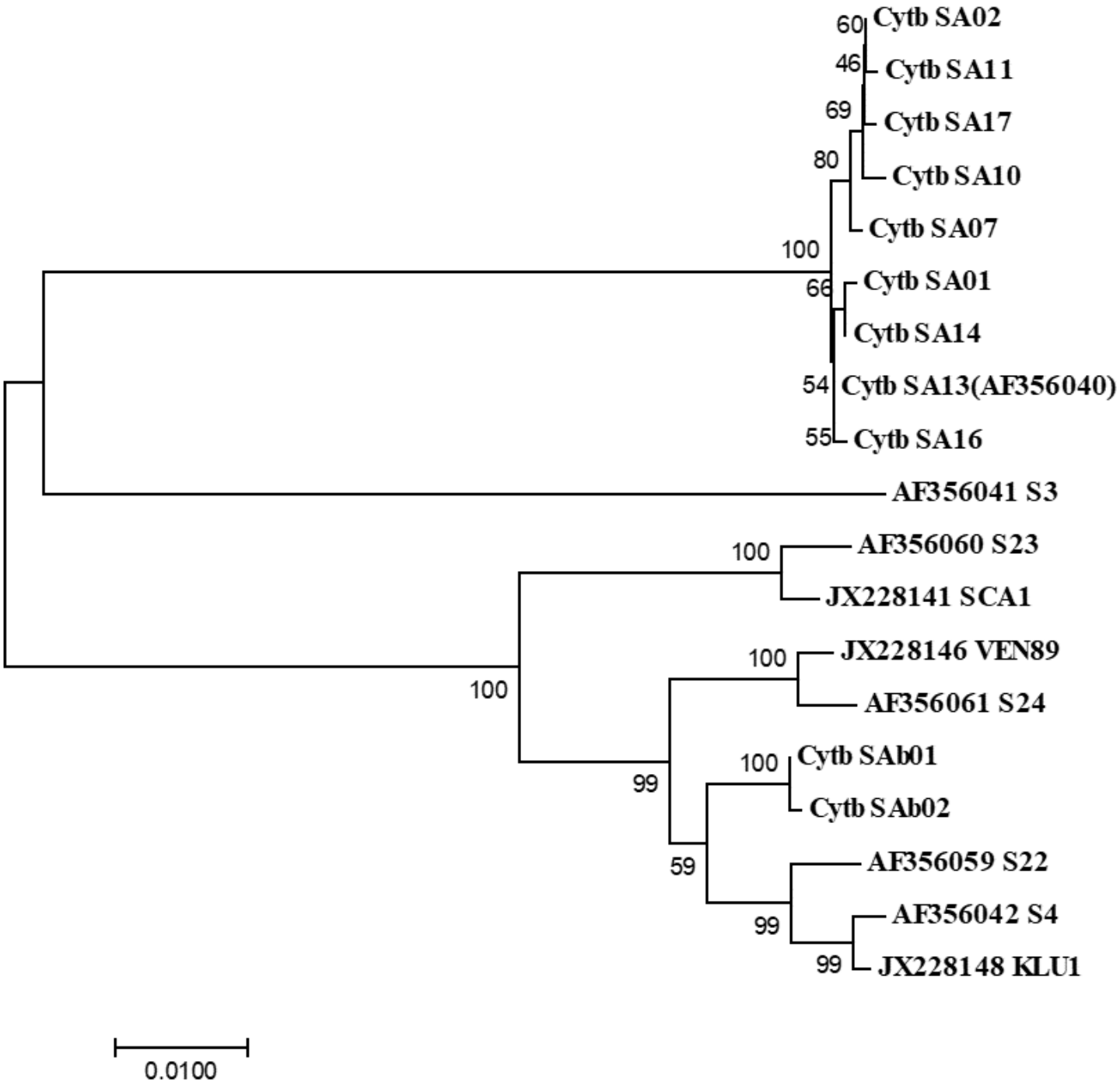


Figure 2

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).



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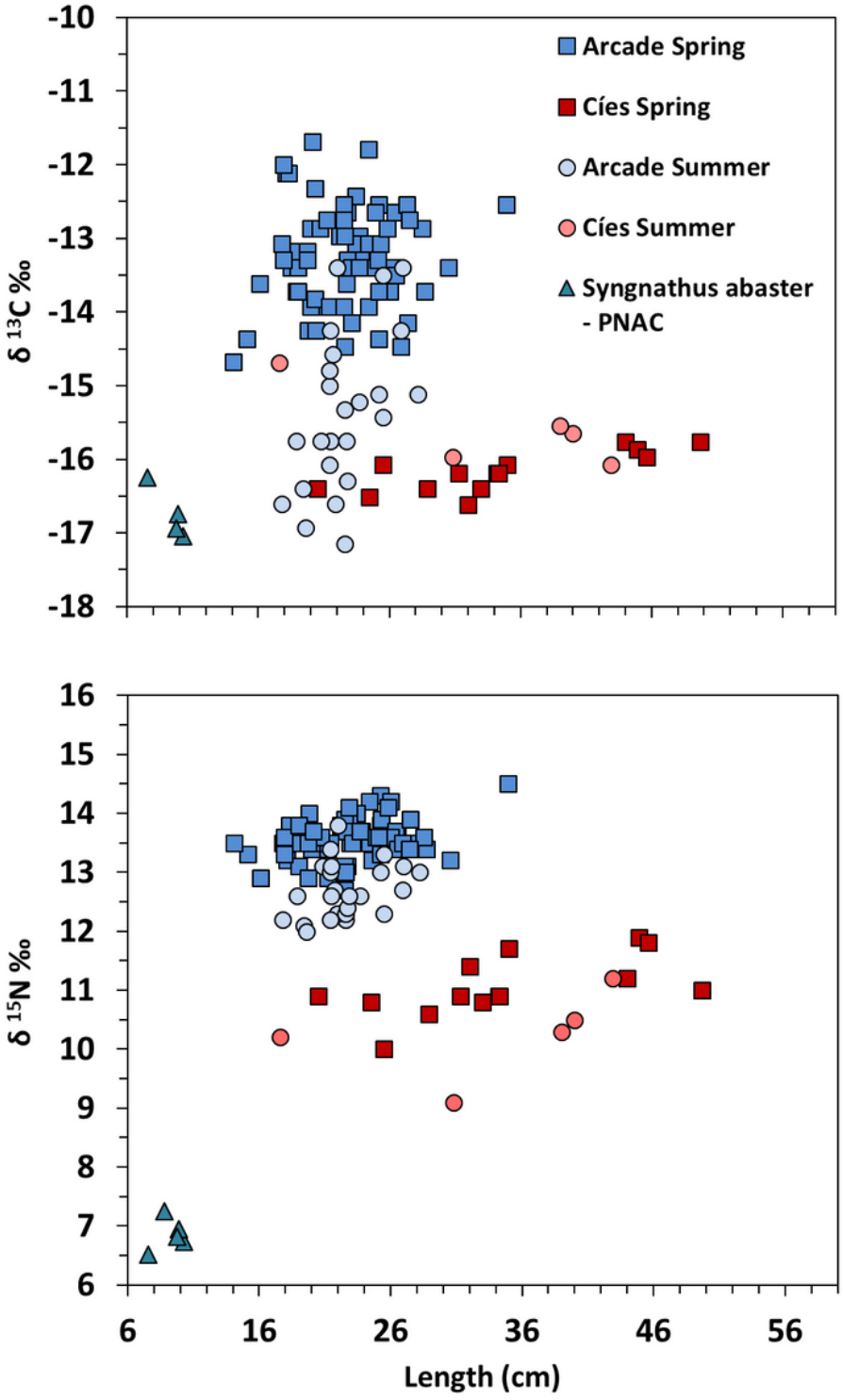
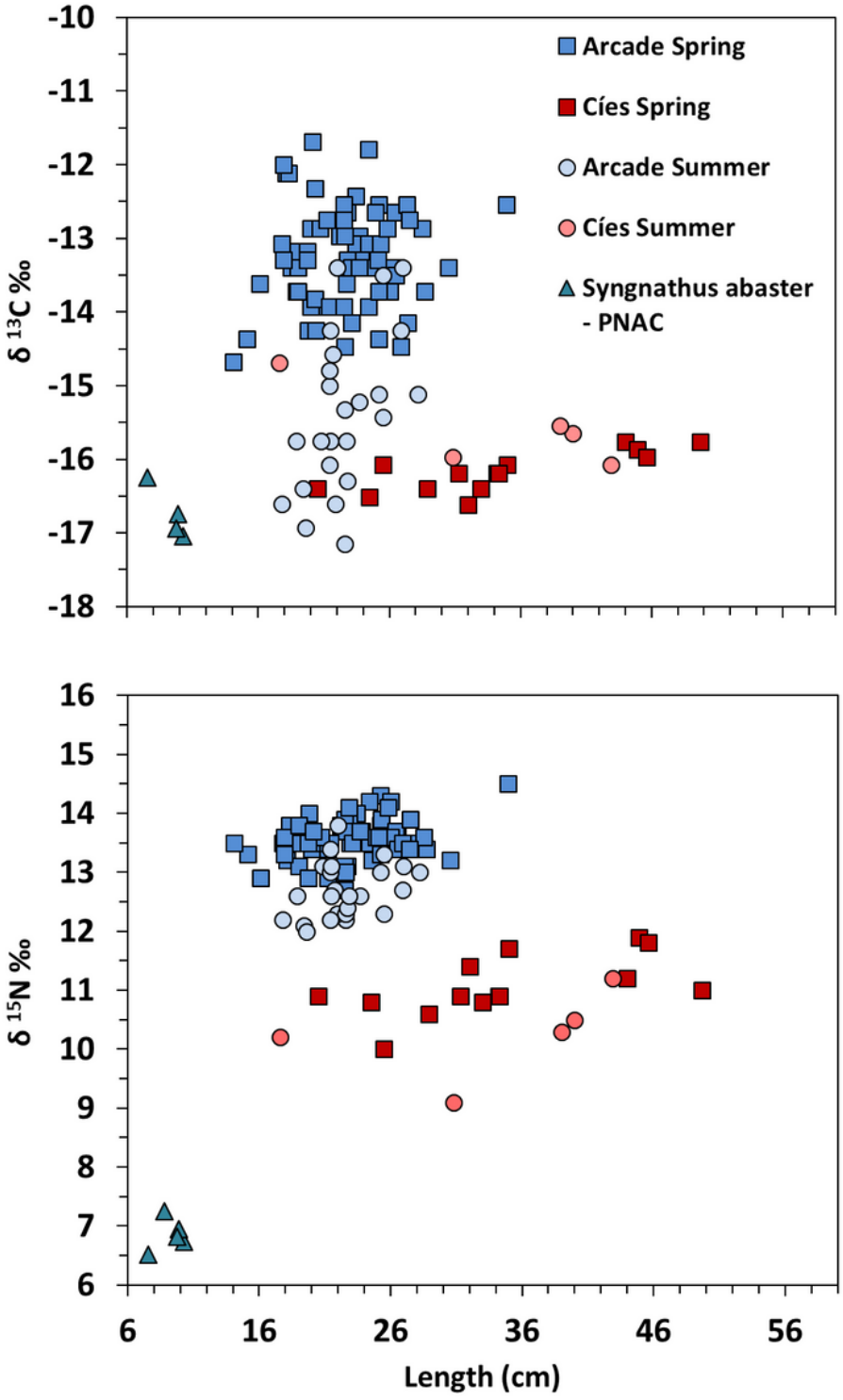


Figure 3

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.



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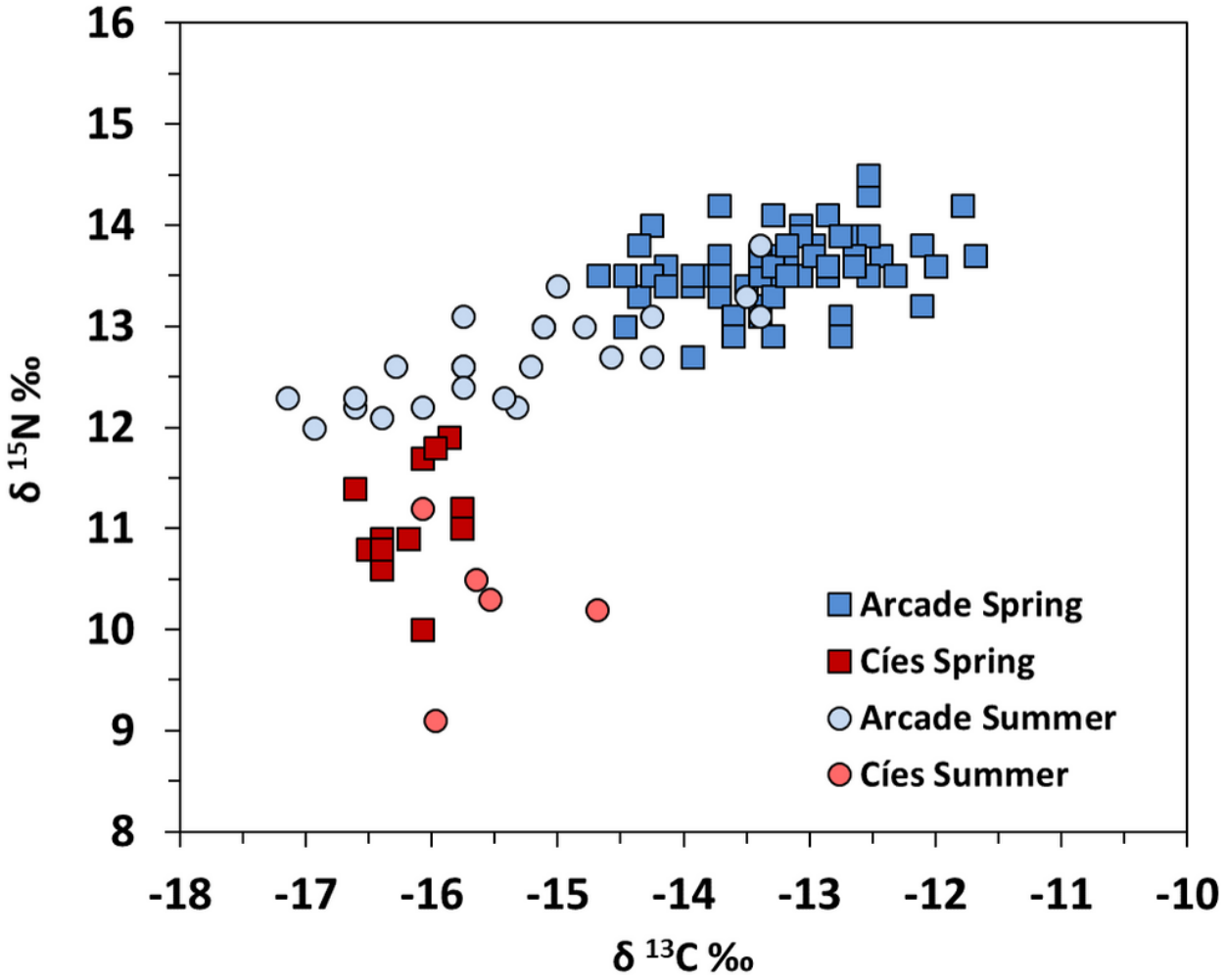
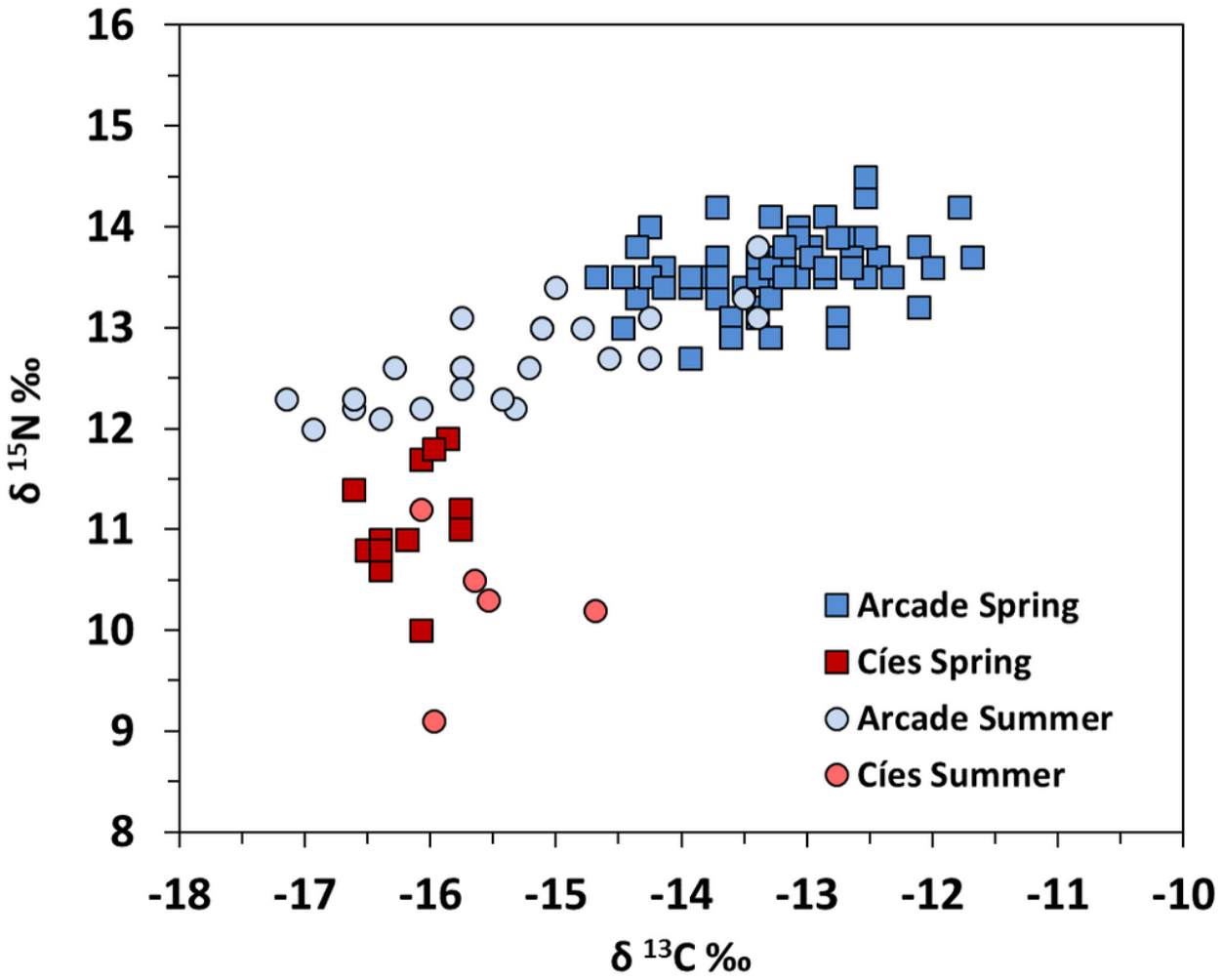


Figure 4

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.



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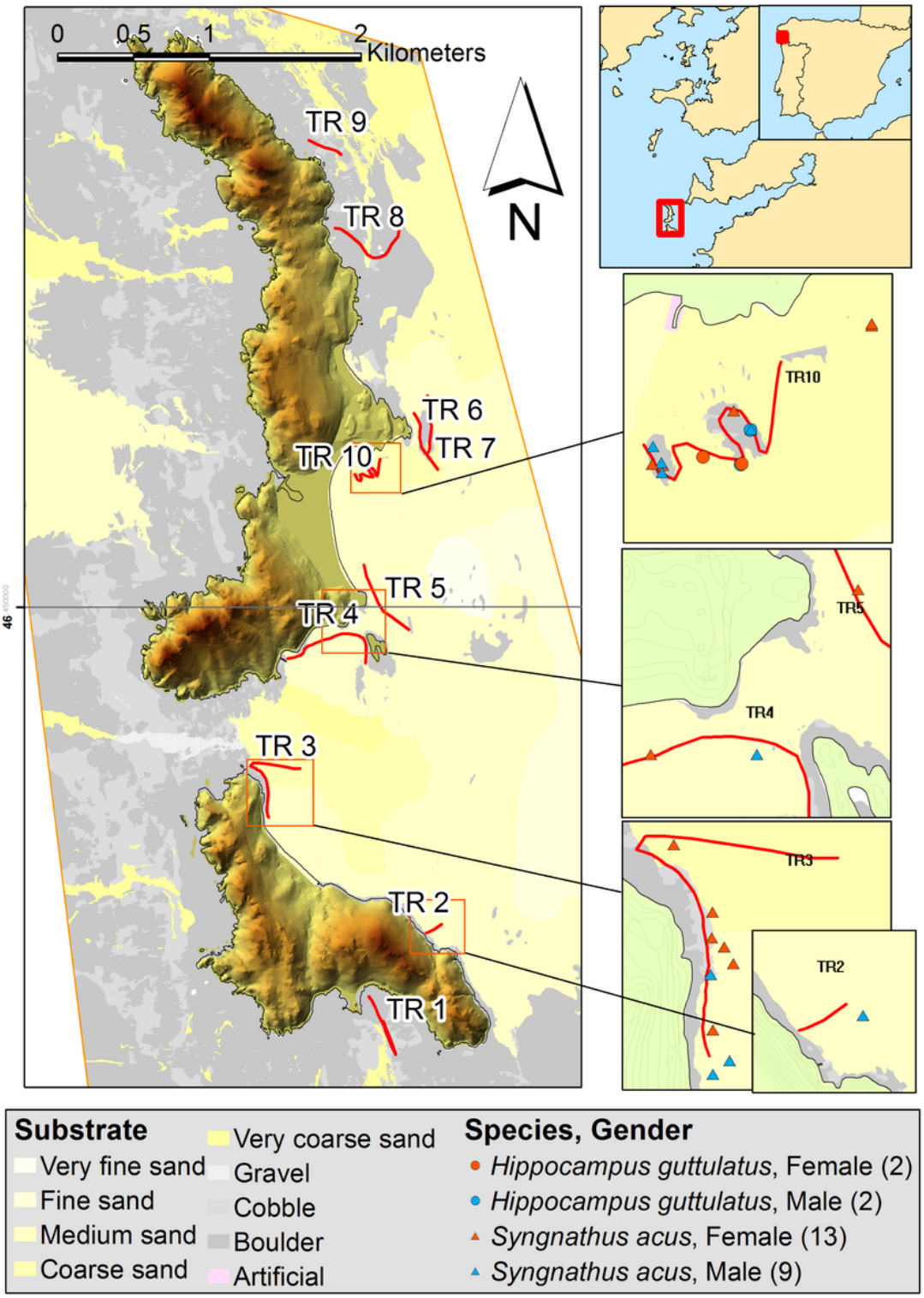
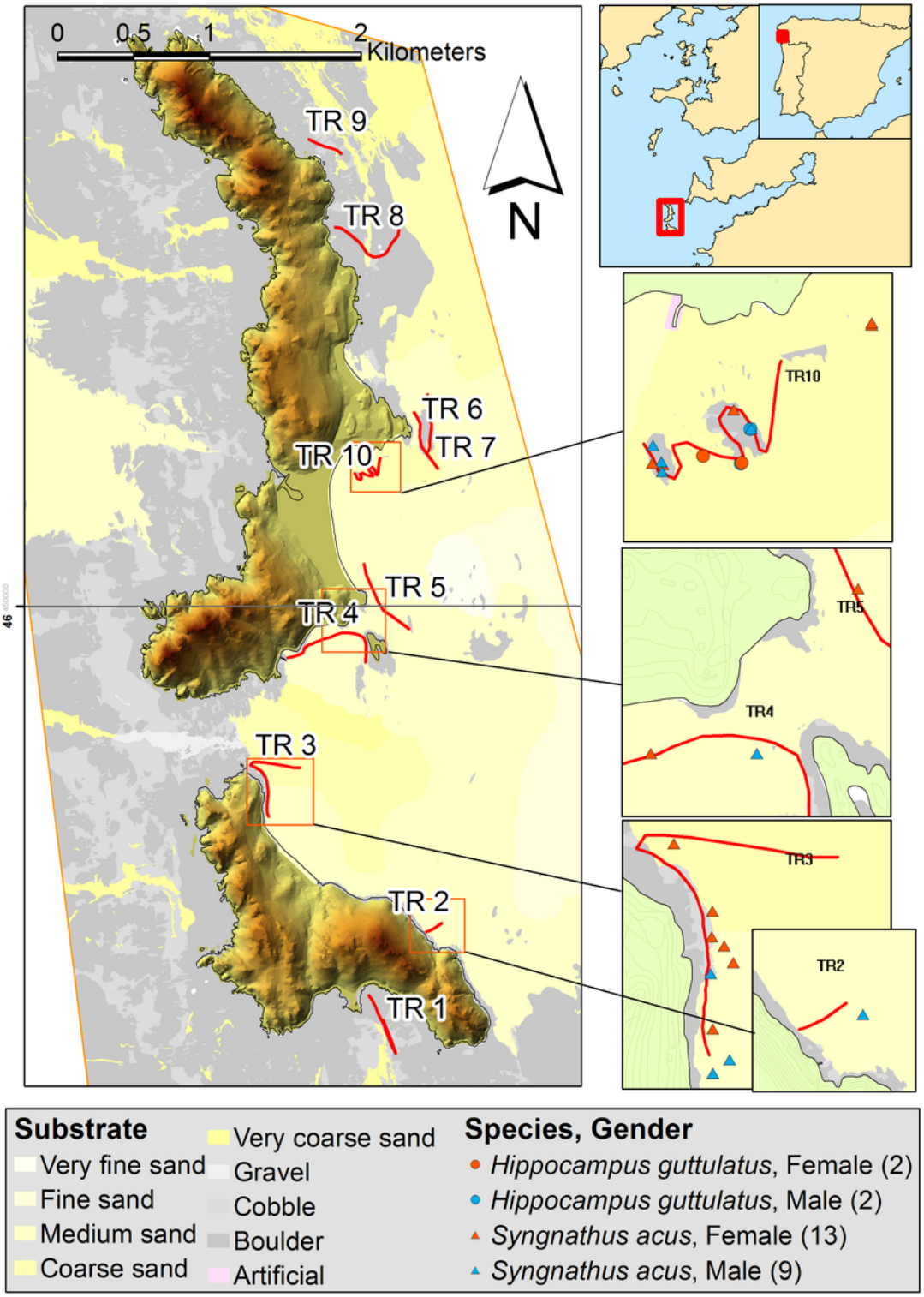


Figure 5

PNIA – Study area and transects (red lines; TR1 to TR10) surveyed for syngnathids in Cíes Archipelago (Galicia, NW Iberian Peninsula). Transects TR2-TR5 included rocky outcrops and sandy substrates, but resolution in the map at the presented scale do not show the rocky outcrops. We acknowledge the information provided by OAPN on GIS layer.



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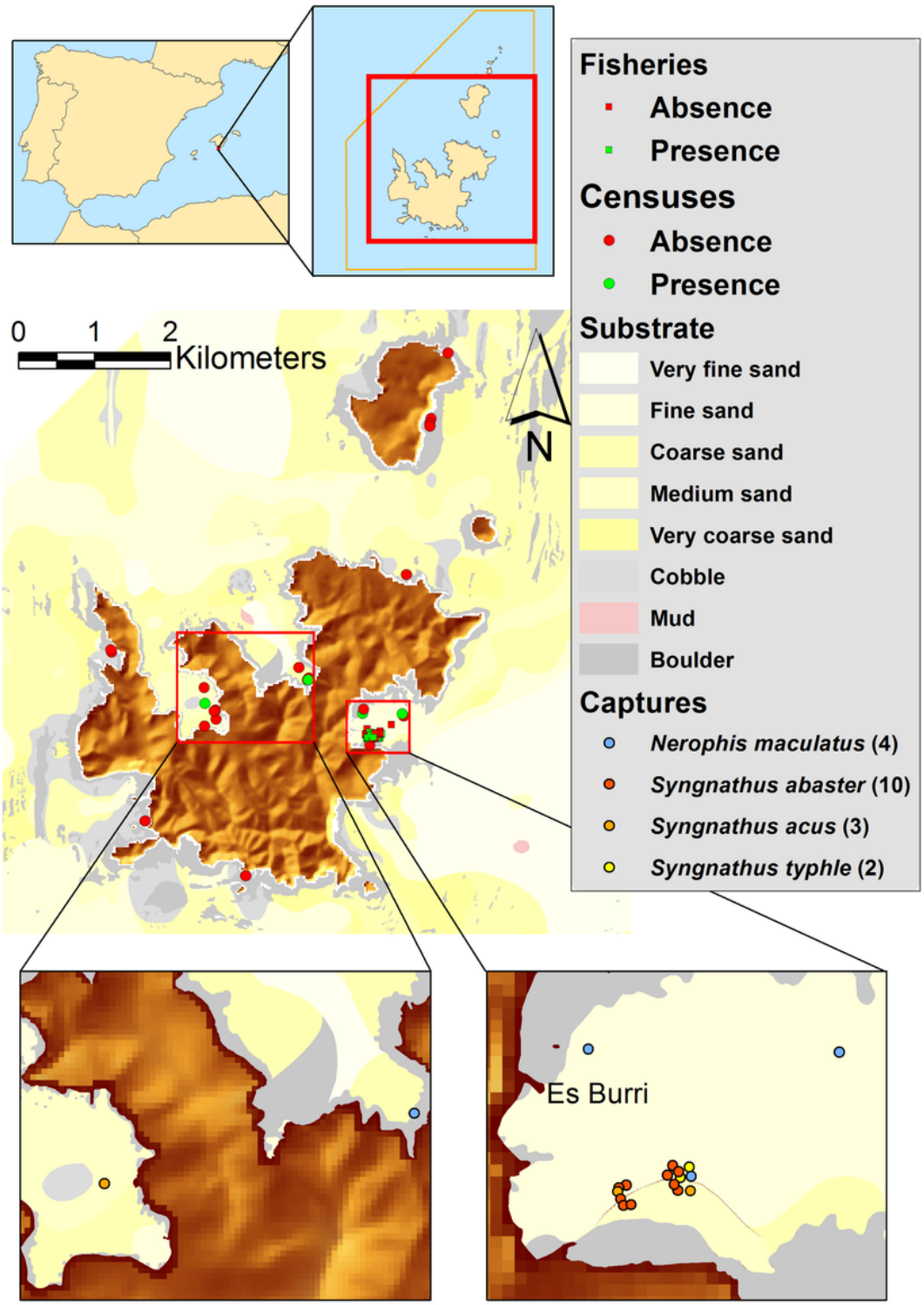
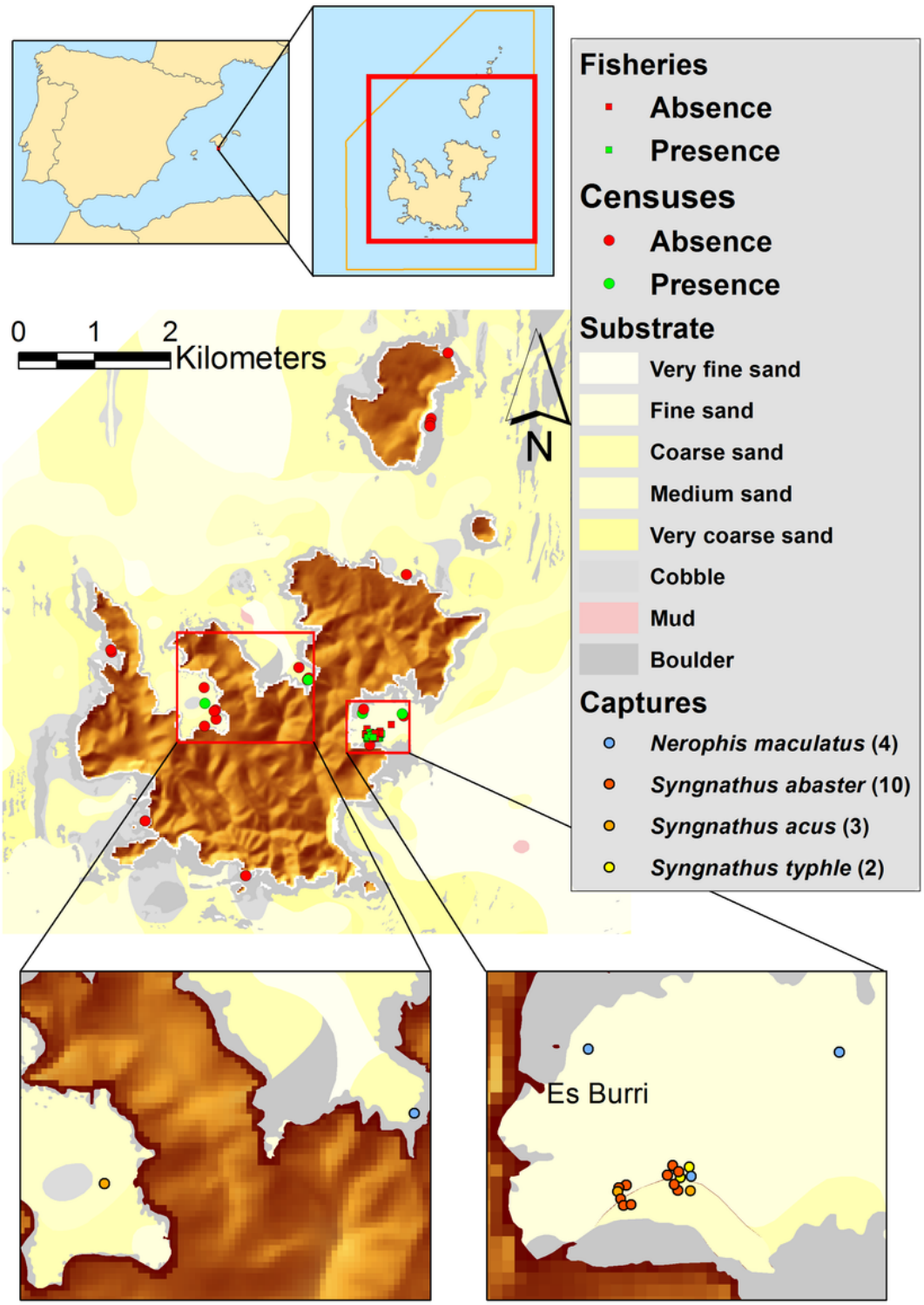


Figure 6

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. We acknowledge the information provided by OAPN on GIS layer.



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