Parastichopus regalis — The main host of *Carapus acus* in temperate waters of the Mediterranean Sea and northeastern Atlantic Ocean

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Abstract

Pearlfish, *Carapus acus*, live in association with several species of sea cucumbers. Its occurrence in hosts is largely dependent on host availability and its distribution from potential larval areas. The occurrence of *Carapus acus* in six sea cucumbers species from the Mediterranean Sea and northeastern Atlantic Ocean was assessed. The sea cucumber species *Parastichopus regalis* was the only host detected. Pearlfish from southeastern Spain (21 individuals) ranged in length from 7.0 cm to 21.5 cm. Two sea cucumbers from the area around Valencia harboured two adult fish each. These pairs of pearlfish, which were sampled during the summer, were able to breed inside of *P. regalis*, an event already noted by other authors. Pearlfish do not seem to choose their host according its size, as the correlation between fish length and host weight was not significant.

Introduction

Symbiosis, the close relationship between organisms of different species, can occur in the marine environment and, in relation to the species involved, can take place in various forms, such as mutualism, commensalism or parasitism (Eeckhaut 2003). More than 800 species have been found in association with different members of the echinoderm phylum (Lyskin and Britaev 2005), and the class Holothuroidea has the higher number of associations (Eeckhaut et al. 2004).

A variety of fish, most commonly pearlfish, are able to live in association with different invertebrates, including holothurians. Pearlfish belong to the family Carapidae, which comprises two subfamilies, Pyramodontinae and the Carapinae, and further divided into two tribes: Echiodontini and Carapini (two genera: *Carapus* and *Encheliophis*) (Parmentier et al. 2000). Members of the Carapini tribe are small, eel-like fish that have developed a symbiotic relationship with sea cucumbers in which the fish lives inside the sea cucumber respiratory trees, or coelomic cavity, using it for protection from predation, as a source of food, and to develop into its adult stage of life (Trott 1981). Slight injuries to the echinoderm are caused by the pearlfish, which pierce the holothurian digestive wall and the respiratory trees when they enter or leave their hosts.

Carapus acus (Brünnich, 1768) is a species recorded throughout the Mediterranean Sea and the west coast of North Africa in depths of 1–150 m (Nielsen et al. 1999). It is common in the western Mediterranean Sea, mainly around Italy, Spain and France, and also occurs in the Adriatic and Aegean seas. It is usually found in the sea cucumbers *Parastichopus regalis* and *Holothuria tubulosa* (Gmelin 1790). It is an elongated, slender, scaleless fish with long dorsal and anal fins meeting at the tip of the long pointed tail (Fig. 1). Members of this species are characterised by a rounded snout, strong dentition and a wide mouth opening. They possess a row of large external, incurved conical teeth and several smaller internal teeth (Parmentier et al. 1998, 2000). The eel-like body is translucent with about 15 silver-gold iridescent spots appearing laterally on the operculum and thoracic regions. As with other pearlfish, they are about 20 cm or less in length. *C. acus* reproduce during July–September (Trott and Olney 1986). Members of *C. acus* are able to produce sounds that are not used by the fish to identify the presence of another fish in the holothurian before penetration, but are produced in the presence of congeneric individuals already inside the host (Eeckhaut et al. 2004).

In this article we report on the presence of *C. acus* (Brünnich, 1768) in *Parastichopus regalis* (Cuvier, 1817) from the western Mediterranean Sea and show

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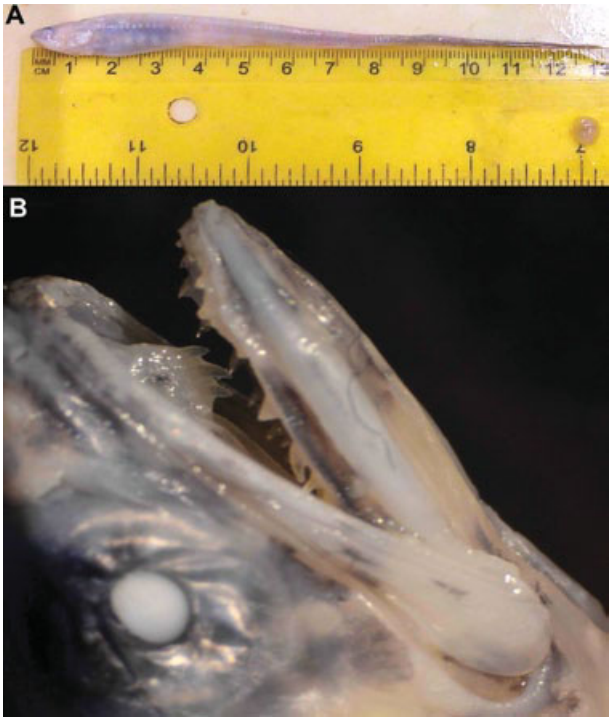

Figure 1. A) *Carapus acus* individual from southeastern Spain conserved in ethanol; B) Detail of *Carapus acus* dentition.

some anatomical, morphological and behavioural features of *C. acus* that could help gaining a better understanding of this species.

Materials and methods

Study sites

Sea cucumber sampling to test the occurrence frequency of Carapus acus individuals was carried out with the support of the CUMFISH project[4]. During the sampling, individuals belonging to different sea cucumber species were caught: *Holothuria poli* (Delle-Chaije, 1823), *H. mammata* (Grube, 1840), *H. tubulosa*, *H. sanctori* (Delle Chiaje, 1823), *H. arguinensis* (Koehler and Vaney, 1906) and *Parastichopus regalis*. The geographic range included the Mediterranean Sea and Atlantic Ocean according the species' geographic distribution (González-Wangüemert et al. 2013). Most of the samples were caught by scuba diving except for *P. regalis*, which lives in deep water. *P. regalis* individuals were collected in May–July 2013 by the Spanish Oceanographic Institute (IEO, http://www.ieo.es) during MEDITS (international trawl) surveys conducted along the continental shelf and slope off the southeastern Spanish coast. Individuals from Sicily were sampled in 2012–2013 using trawls, thanks to the collaboration with Istituto per l'Ambiente Marino Costiero del Consiglio Nazionale delle Ricerche (IAMC-CNR, http://www.iamc.cnr.it/IAMC/).

Analysis

The occurrence of *Carapus acus* inside of sea cucumbers was recorded. Each pearlfish was photographed inside its host, and its total length and total weight were measured. Specimens were then stored in 100% ethanol. A small (3–5 mg) section of tissue was removed from the dorsal muscle of each fish and placed in absolute ethanol for further genetic analysis. The correlation between host weight and fish size was calculated.

Total length and count of vertebrae of *C. acus* specimens found in *P. regalis* were chosen as meristic parameters (i.e. measurements that indicate the count of body parts related to body segmentation). Information regarding the axial skeleton was obtained using the Kodak DXS (digital x-ray specimen) 400 System, which offers the highest resolution available for digital specimen radiography.

Results and discussion

During the CUMFISH project, 1,880 individual sea cucumbers of different species were caught: *Holothuria tubulosa* (n = 390), *H. mammata* (n = 427), *H. poli* (n = 397), *H. sanctori* (n = 100), *H. arguinensis* (n = 269) and *Parastichopus regalis* (n = 297). The occurrence of *Carapus acus* was very variable, depending on the host species and their geographic origin. Of the six sea cucumber species studied belonging to two different genera, only *Parastichopus regalis* had *C. acus* as a commensalist. In Sicily, 51 individuals of this sea cucumber species were sampled, and 41.17% (21 individuals) had *C. acus* inside of them. In total, 241 individuals of *P. regalis* were collected along the eastern Spanish coast: 39 from Catalonia, 74 from Valencia, 37 from Alicante, 9 from Cabo de Gata, 9 from Castell de Ferro and 73 from Alboran Sea. Twenty-one individuals of *C. acus* were found inside holothurian specimens (*P. regalis*) from three localities: Alicante, Valencia and Cabo de Gata (Table 1); six were found in Alicante, fourteen in Valencia and only one in Cabo de Gata (TL = 12.1 cm; TW = 1.94 g). The occurrence frequencies in each locality were 16.2% for Alicante, 18.9% for Valencia and 11.1%, for Cabo de Gata.

The pearlfish from southeastern Spain ranged in length from 7.0 cm to 21.5 cm and in weight from 0.28 g to 16.78 g (Table 1). As already noted by other authors (e.g. Trott and Olney 1986; Eeckhaut et al. 2004), two fish have been observed inside the same

[4] The new resource for a hungry fishery, PTDC/MAR/119363/2010; http://www.ccmar.ualg.pt/cumfish/

Table 1. Morphological characteristics of *Carapus acus* found in *Parastichopus regalis* from southeastern Spain. Minimum and maximum values for each locality are in bold.

Locality	Sample code	Total length (cm)	Total weight (g)
Alicante (AC)	RAC7	13.10	2.23
	RAC20	14.80	3.33
	RAC21	**17.00**	**8.08**
	RAC22	15.00	3.62
	RAC34	14.10	3.12
	RAC37	**8.10**	**0.41**
Mean ± STD		13.70 ± 3.02	3.47 ± 2.54
Valencia (VL)	RVL9	14.00	2.79
	RVL15	7.50	**0.28**
	RVL25	12.00	5.20
	RVL27	13.00	2.24
	RVL33	**7.00**	0.68
	RVL42	**21.50**	11.35
	RVL48a	18.00	7.71
	RVL48b	18.50	11.46
	RVL50	17.00	**16.78**
	RVL52	17.50	9.68
	RVL54	14.50	3.40
	RVL55a	18.00	6.60
	RVL55b	20.00	8.45
	RVL58	16.00	4.59
Mean ± STD		15.32 ± 4.31	6.52 ± 4.71

STD = standard deviation

Figure 2. A) *Parastichopus regalis* hosting two *Carapus acus* individuals; B) *Carapus acus* attached to the sea cucumber's respiratory trees.

host (Fig. 2), in two sea cucumbers from around Valencia (sample codes RVL48 & RVL55). The size and weight of each member of the pair were compared: one pair measured 18 cm and 18.5 cm, and 7.71 g and 11.46 g, and the other pair measured 18 cm and 20 cm, and 6.6 g and 8.45 g. Therefore, both couples could be considered adults, although their sex determination was not possible due to the preservation status of their gonads. The reproductive behaviour among the Carapini tribe has never been described in detail but different elements suggest that coupling could occur inside sea cucumbers. In the wild, several individuals per host have been found, and these were sometimes adult carapids of the opposite sex. The presence of sexual pairs suggests that sea cucumbers could serve also as breeding sites (Eeckhaut et al. 2004); reproduction occurring in the respiratory trees would greatly favour the gametes meeting and the adults and their eggs could be temporarily sheltered from predators during fecundation (Parmentier and Vandewalle 2005). The *C. acus* reproduction has been recorded in July–September (Trott and Olney 1986). Maybe the couples sampled by us during the summer, were breeding inside the *P. regalis* individuals, considering that the fish were identified as adults according their length and weight, and were caught during their reproductive season.

Dimensional relationships were tested comparing host weight versus fish length (Fig. 3). Due to the natural contraction behaviour of sea cucumbers, weight was considered to be a better parameter for establishing size relationships among sea cucumbers and fish. The correlation between fish length and host weight was not high or significant and, therefore the fish probably does not choose its host in relation to its size.

Specimens of *C. acus* with similar dimensions were investigated by x-ray analysis in order to count vertebrae. Similar size fish were used in order to avoid mistakes

caused by different developmental stages. Usually, the calcification process is weaker in the distal part of the organisms, and therefore, vertebrae counts were less accurate in the tail region. The number of vertebrae ranged from 84 to 92 (Fig. 4). The body curvature displayed by fish differs remarkably among species, but some non-muscular features (e.g. number of vertebrae) are known to influence axial flexibility, especially in *Carapus* spp. fish (Schwarz et al. 2012).

In conclusion, *C. acus* was found only in individuals of *P. regalis* although more than 1,800 samples belonging to 6 different sea cucumber species were examined. Further studies using molecular markers will allow us to improve our knowledge of this species, particularly with regard to its genetic connectivity and diversity and possible links with its hosts.

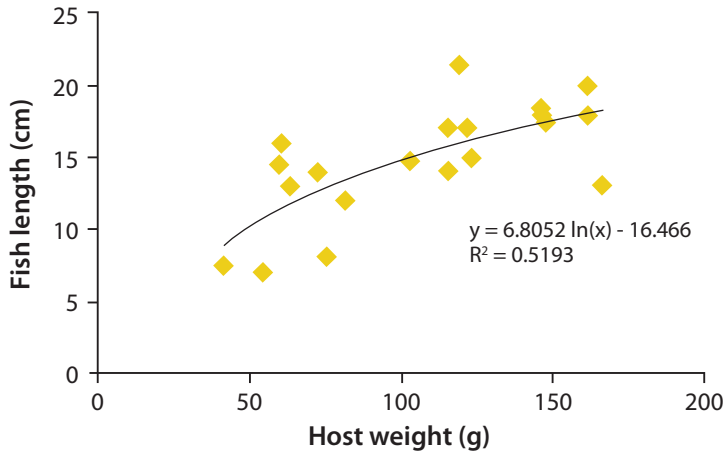

Figure 3. Regression among sea cucumber weight (g) and fish length (cm).

Figure 4. Skeleton of *Carapus acus* using Kodak DXS (digital x-ray).

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