Títol del treball: Study of the population of the symbiotic polychaete *Oxydromus humesi* hosted by the bivalve *Scrobicularia plana* in Cádiz Bay.

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Study of the population of the symbiotic polychaete *Oxydromus humesi* hosted by the bivalve *Scrobicularia plana* in Cádiz Bay

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Cover image. *Oxydromus humesi* (the symbiont) and *Scrobicularia plana* (the host). Entering into the host through the siphon. Extracted from Martin et al. (2015).

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Abstract *Oxydromus humesi* is a strict bivalve endobiont, probably a parasite, living in association with *Tellina nymphalis* in north of Pointe-Noire (Congo mangrove swamps, West Africa) and *Scrobicularia plana* (intertidal) and *Psammotreta cumana* (subtidal) in Cádiz Bay salt marshes (southern Atlantic coast of Iberian Peninsula). The symbiont shows a regular distribution (one, exceptionally two or three symbionts per host, being one male, one female and one small worm) with intraspecific competition and complex host-entering behaviour observed in experimental conditions. This work aims to study the population dynamics of the above-mentioned species hosted by *S. plana* in Cádiz Bay to increase knowledge on the biology of *O. humesi* and to contribute to the growing knowledge on symbiotic polychaetes. The population of the symbiont in the intertidal area of Rio San Pedro (Cádiz Bay) seems to reproduce actively through all the year, with a higher number of ripe females in spring and, particularly, in summer. Although *S. plana* is very abundant in the studied area, with a large amount of specimens having the size most preferred by the symbiont (i.e., from >26 mm to about 36 mm long), the prevalence tends to be very low (usually <5% and reaching up to ~20%). This fact, together with the presence of a highly infested population (>85% in the specimens longer than 20 mm) of *P. cumana* in Río San Pedro mouth, lead us to discuss on the environmental requirements and host-specificity of *O. humesi.* We suggest that the studied intertidal could be at the limit of the ecological distribution of this symbiont in the Cádiz Bay region. The symbiotic hesionid seems to show a seasonal pattern of prevalence, which may be related with its reproduction (the periods with more ripe females tended to coincide with those of lower prevalence). Taking into account these data, we propose that males undertake reproductive migrations probably to improve the possibilities of fertilization, whilst the females stay throughout the whole life inside the host. The life-cycle of *O. humesi* remains unknown since the species' discovery in the coasts of the Republic of Congo. However, our data allow us to suggest that its life span may be of one year, with the adults dying after reproducing.

Keywords Hesionidae · Symbiosis · Bivalvia · Population dynamics · Reproduction · Cádiz Bay · NE Atlantic.

Resumen *Oxydromus humesi* es un endobionte de bivalvos, probablemente un parásito, que vive en simbiosis con *Tellina nymphalis* en el norte de Pointe-Noire (manglares del Congo, Oeste de África) y *Scrobicularia plana* (intermareal) y *Psammotreta cumana* (submareal) en las marismas de la Bahía de Cádiz (costas Atlánticas del sur de la Península Ibérica). Los simbiontes muestran una distribución regular (uno, excepcionalmente dos o tres simbiontes por huésped, siendo uno macho, uno hembra y uno de talla pequeña). Presenta competencia intraespecífica y un complejo comportamiento de entrada al huésped observado en condiciones experimentales. Este trabajo pretende estudiar la dinámica poblacional de la anterior especie hospedada por *S. plana* en la Bahía de Cádiz para aumentar el conocimiento sobre la biología de *O. humesi* y contribuir a la creciente información existente sobre poliquetos simbiontes. La población del simbionte en el medio intermareal del Río San Pedro (Bahía de Cádiz) parece reproducirse durante todo el año, con un elevado número de hembras fértiles en primavera y, especialmente, en verano. Aunque *S. plana* es muy abundante en la zona, con una gran cantidad de especímenes presentando la talla preferida por los simbiontes (de 26 mm a 36 mm de largo), la prevalencia tiende a ser muy baja (usualmente <5% llegando a alcanzar ~20%). Este hecho, junto con la presencia de una población altamente infestada (>85% en individuos más largos de 20 mm) de *P. cumana* en la entrada del Río San Pedro, nos permite discutir sobre los requerimientos ambientales y la especificidad por el huésped de *O. humesi.* Así pues, sugerimos que la zona intermareal estudiada podría tratarse del límite de la distribución ecológica de este simbionte en la región de la Bahía de Cádiz. El hesiónido simbionte parece mostrar un patrón estacional de prevalencia que puede estar relacionado con su reproducción (los períodos con más hembras fértiles tendieron a coincidir con aquellos de menor prevalencia). A partir de estos datos, proponemos que los machos emprenden migraciones, probablemente para incrementar las posibilidades de fertilizar un elevado número de hembras, mientras que éstas tienden a permanecer durante todo el ciclo vital dentro del huésped. El ciclo de vida de *O. humesi* sigue siendo desconocido desde su descubrimiento en las costas de la República del Congo; no obstante, nuestros datos nos han permitido sugerir que su esperanza de vida puede ser de un año, con la muerte de los adultos después del apareamiento.

Palabras clave Hesionidae · Simbiosis · Bivalvia · Dinámica poblacional · Reproducción · Bahía de Cádiz · NE Atlántico

Resum *Oxydomus humesi* és un endobiont de bivalves, probablement un paràsit, que viu en simbiosi amb *Tellina nymphalis* en el nord de Pointe-Noire (manglars del Congo, Oest d'Àfrica) i *Scrobicularia plana* (intermareal) i *Psammotreta cumana* (submareal) als aiguamolls de la Badia de Cádiz (costes Atlàntiques del sur de la Península Ibèrica). Els simbionts mostren una distribució regular (un, excepcionalment dos o tres simbionts per hoste, sent un mascle, un femella i un de talla petita) amb competència intraespecífica i un comportament complex d'entrada a l'hoste observat en condicions experimentals. Aquest treball té com a objectiu estudiar la dinàmica poblacional de *O. humesi* allotjada per *S. plana* en la Badia de Cádiz per augmentar el coneixement sobre la biologia d'aquesta espècie i contribuir a la creixent informació existent sobre poliquets simbionts. La població del simbiont en el medi intermareal del Río San Pedro (Badia de Cádiz) sembla que es reprodueix activament durant tot l'any, amb un elevat nombre de femelles fèrtils durant primavera i, especialment, a l'estiu. Malgrat *S. plana* és molt abundant a la zona, amb una gran quantitat d'individus amb la talla preferida pels simbionts (de 26 mm a 36 mm de llargada), la prevalència tendeix a ser molt baixa (normalment <5% arribant fins a ~20%). Aquest fet, juntament amb la presència d'una població altament infestada (>85% en espècimens més llargs de 20 mm) de *P. cumana* a l'entrada del Río San Pedro, ens permet discutir sobre els requeriments ambientals i l'especificitat per l'hoste d'*O. humesi*. Així doncs, suggerim que la zona intermareal estudiada pot tractar-se del límit de la distribució ecològica d'aquest simbiont a la regió de la Badia de Cádiz. El poliquet hesiònid sembla mostrar un patró estacional de prevalència, el qual pot estar relacionat amb sa reproducció (els períodes amb major quantitat de femelles fèrtils tendiren a coincidir amb aquells de menor prevalència). Com a conseqüència, proposem que els mascles duen a terme migracions, probablement per incrementar les possibilitats de fecundar un alt nombre de femelles, mentre que aquestes tendeixen a romandre durant tot el cicle de vida dins l'hoste. El cicle vital d'*O. humesi* continua sent desconegut des de la seva trobada a les costes de la República del Congo; no obstant això, les nostres dades ens han permès suggerir que l'esperança de vida pot ser d'un any, amb la mort dels adults després de l'aparellament.

Paraules clau Hesionidae · Simbiosi · Bivalvia · Dinàmica poblacional · Reproducció · Badia de Cádiz · NE Atàntic

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1. INTRODUCTION

1.1. Symbiosis in polychaetes

The term symbiosis may be considered a synonymy of mutualism, but it may also generically refer to any kind of close association between two organisms, to the extent that at least one of the involved partners can no longer be considered a free-living organism (Martin & Britayev, 1998). Here we use the generic meaning and, thus, symbiosis includes mutualism, commensalism and parasitism, depending on the degree of affectation to the partners, +:+, +:0 or +:-, respectively. The partners involved in a "symbiotic" association are referred to as "the symbiont" (or "the guest") and "the host". The host harbours the symbiont, which usually is smaller than the host and always obtains benefits from the association. In the particular case of commensalisms and parasitisms, the symbiont may also be specifically referred to as "the commensal" and "the parasite", respectively.

Despite the above definitions, it is often difficult to identify the borderlines and nature of the symbiotic associations due to the lack of appropriate observations and the difficulty in applying experimental approaches. Quite often an association first catalogued as being of a particular type, is later redefined with the increasing knowledge about that relationship. Many different aspects may contribute to the knowledge required to define the nature of an interaction. Among them, the degree of association among the species, the degree to which the association is necessary for survival, the kind of benefits, the extent of reciprocal specialization by one species in response to the other, the temporal pattern, the stage of the life cycle at which the interaction occurs, or the location of the guest on or inside the host (Martin & Britayev, 1998). In practice, it is difficult to infer unequivocal demonstrations of all factors affecting a given interaction, so no matter the terms used, they should be better considered as stepping stones in helping to understand the real relationships involved in each particular association (Table 1).

Polychaete annelids are more often free-living organisms that lives crawling, burrowing or tube-dwelling in virtually all aquatic environments, particularly being among the most frequent and abundant marine metazoans in benthic environments (Martin & Britayev, 1998). Accordingly, symbiosis with other marine invertebrates is not a strange phenomenon in polychaetes. However, the literature on symbiotic polychaetes remained largely anecdotal for a long time, with the information often scattered among studies dealing with different subjects (e.g. systematics, descriptive ecology, biology). It was not until the review by Martin and Britayev (1998) that the current knowledge was summarised (at the time, there were 292 species of commensals from 28 families involved in 713 relationships and 81 species of parasites from 13 families involved in 253 relationships) and numerous studies with modern approaches started to appear.

The most numerous symbiotic polychaetes are, still to date, the commensals (often including potential parasites lacking clear parasitic characteristics and mutualists where the mutual benefits are not clearly proven). However, there are also some evident cases of parasitism.

Table 1. List of terms used to define the nature of symbiotic association (from Martin & Britayev 1998).

Term	Definition
Commensalism	Association where is clearly to the advantage (not necessarily from a trophic point of view) of one of the members without seriously inconveniencing or harming the other.
Mutualism	Interaction in which two (or more) species reciprocally benefit from the presence of the other species.
Parasitism	A partnership in which the advantage is wholly on one side, usually at the expense of the other partner.
Ecto-	For guests living outside the host.
Endo-	For guests living inside the host.
Obligatory	For guests that can not survive without the host.
Facultative	For guests that can also be free living.
Permanent	For guests living as symbionts during their whole life.
Temporary	For guests living as symbionts only during one phase of their life cycle.
Monoxenous	For symbionts inhabiting one or a few hosts.
Polyxenous	For symbionts inhabiting many different hosts.
Prevalence of infestation	The relationship between the number of infested hosts and the total number of hosts.
Intensity of infestation	The number of symbionts present in each infested host.
Mean intensity	The mean number of individuals of a particular symbiotic species per infested host in a sample.
Abundance or relative density	The mean number of symbiont individuals per host examined (i.e. equal to prevalence multiplied by mean intensity).

1.1.1. Commensal polychaetes

As mentioned above, commensalism is the most abundant relationship among symbiotic polychaetes. However, the low level of knowledge and scattered available information on the biology of the involved species may artificially exaggerate its relevance (Martin & Britayev, 1998). The family Polynoidae includes more than a half of the currently known commensal species, and are followed by Syllidae, Hesionidae and Spionidae, and up to 28 more families with much less numerous representatives (Martin & Britayev, 1998). Among them, the Iphitimidae, Histriobdellidae and Antonbruuniidae are entirely symbiotic, with most of their species being commensals.

Commensal polychaetes are associated with virtually all the main taxa of marine metazoans (excluding flatworms, nemerteans and nematodes), and even with protozoans (i.e. foraminifers) (Martin & Britayev, 1998). Generally, they prefer as host organisms providing shelter and protection. Among them, there are organisms having holes, grooves, chambers or channels (e.g. sponges, starfishes), as well as those showing good chemical or physical defences (e.g. sponges, cnidarians, sea urchins). Most commensal polychaetes are monoxenous (i.e., associated to a single host or to a few, closely related species). However, as repeatedly mentioned, the often vague and accidental information available may contribute to exaggerate this almost uniform pattern of distribution.

In turn, most adult symbiotic polychaetes with known distributions seem to occur alone on their hosts (Martin & Britayev, 1998). However, there are a few species showing either a random pattern (i.e., adults may occur from 1 specimen to massive densities in a single host specimen) or an aggregated distribution (i.e. adults are always located forming clusters on the host). Real regular distributions are often driven by specific mechanisms, among which the main one is intraspecific competition (including territorial behaviour and intraspecific aggressiveness). Examples of regular distributions occur among symbiotic polynoids, nereids, pilargids and amphinomids, but also in some hesionids, syllids and sphaerodorids. Quite often, however, the juveniles of a given species with solitary adults display random or even aggregated distribution patterns (Dimock, 1974, Britayev & Smurov, 1985). Also, as most polychaetes are dioecious, regular distributions must be associated with mechanisms raising the chance of commensal males and females to be in close proximity during the reproductive period, which in some cases lead to couples sharing the same host species, such as *Robertianella synophthalma*, reported as *Harmothoe hyalonemae* by Martin et al. (1992).

Most commensals have a defined location on the host surface or, less frequently, inside the host. There are many areas that may be attractive for the symbiont. For example, the host's branchial chambers, the gastropod shells inhabited by hermit crabs or the tubes or burrows (even of other free-living polychaetes), while the commensals of bivalves or limpets, such as *Oxydromus humesi* (Pettibone, 1961), often live inside the mantle cavity.

The specialised symbiotic mode of life lead to most, or even all, commensals to have more or less defined morphological features allowing to distinguish them from their free-living relatives (Martin & Britayev, 1998). In addition, their behaviour should undoubtedly be re-adapted, quite often based on the normal habits of the free-living relatives. Some examples of behavioural adaptations are the co-ordination of movements with those of their host when feeding and moving, the development of mechanisms to find a new host (i.e. during recruitment or if they are moved out from their current host, either as a result of intraspecific competition or death of the host), the finding of a reliable way of attaching themselves to their hosts, and the selection of the most protected area in the host body (Martin & Britayev, 1998). Indeed, there are a few commensals that seem to have developed a long-distance host-detection mechanism, which seems to be chemically mediated in the few cases that its presence has been experimentally assessed (i.e. the so-called host-factor) (Davenport & Hickok, 1951). The chemically mediated host factor is probably the main mechanism contributing to the maintenance of marine symbiotic partnerships. However, the own adult symbionts may produce chemical signals that could be recognised by the incoming individuals, while there are other kinds of stimuli produced by the hosts that may also trigger characteristic adaptive responses from commensal polychaetes (Martin & Britayev, 1998). For instance, the singular association between *Neanthes fucata* and hermit crabs is mediated by mechanical stimuli. Settlement of this species occurs directly on the sea bottom, as in their free-living soft-bottoms relatives. After four months living in the sediment, the worms start to find a host, which presence is recognized by perceiving the substratum vibrations caused by the host legs, a behavioural adaptation that persist during the whole life of the commensal. This fact, together with other behavioural adaptations such as the specialized feeding behaviour that mimics the movements of the host bucal appendages to steal food, is the main argument allowing to consider this particular association as a true commensalism, although, to some extent, it may also be considered as kleptoparasitic (Gilpin-Brown, 1969).

1.1.2. Parasitic polychaetes

The most important family of parasitic polychaetes are the Spionidae, which include mostly boring species, followed by the Oenonidae and Syllidae. Less numerous, but not less relevant, the Ichtyotomidae (one species) is entirely a parasitic family and the single representative of the old Calamyzidae family (actually synonymized with Chrysoptelidae; Aguado et al., 2013) is an ectoparasite.

Parasitic polychaetes are always associated with benthic invertebrates, except for three fishes and one plant (Martin & Britayev, 1998). The most infest species are gastropod and bivalve molluscs. Most known parasitic relationships involving polychaetes tend to be monoxenous and show regular patterns of distribution (i.e., each host harbours only one parasite adult). Although less frequent, aggregated distributions have also been reported, for instance, in polydorid spionids and in syllids, often in connection with particular reproductive features (such as asexual reproduction allowing the colonization of the host after being first infested by a sexual propagule).

Most parasitic polychaetes are endoparasites, either inhabiting the tissues of their hosts, the peri-intestinal blood sinus, the coelomic cavities, the aquiferous system, the hydrothecae or special galls formed by the host induced by the presence of the parasite. As a general rule, endoparasites show a reduction or specialization of locomotive appendages and mouthparts, and they are usually smaller than their free-living relatives (Clark, 1956). The ectoparasitic polychaetes, in turn, live more or less intimately attached to different parts of the host's body. They may simply live on the surface, attached to an external appendix or penetrate the tissues (e.g. the body wall, the branchiae or the fins). Surprisingly, it is within the ectoparasites where we may find the highest degree of structural and behavioural adaptations.

It is commonly accepted, and is implicit in the definition of a parasitic relationship, that the parasite must be smaller than the host. In some cases, the mere fact that the presence of the symbiont caused a significant stress to the host was the basis allowing to consider the relationship parasitic, whereas in other cases there were significant, measurable effects such as a decrease of host biomass. For instance, the feeding activity of *Haplosyllis* spp. infesting in high densities two closely related sponges, *Aplysina cauliformis* and *Verongula reiswigi*, apparently caused a decrease in the growth rate of the second (Reiswig, 1973). However, the labelling of an association as parasitic, it not so evident and the fact that symbionts are small, colonize in high density the host and feed on their tissues (i.e., parasitic characteristics) does not prevent the same worms to be apparently able to defend the host, to contribute to host cell renewal or to provide extra feeding resources to the host (i.e., clear mutualistic characteristics), as postulated for the association between *Haplosyllis* spp. and their host sponges (Lattig & Martin, 2011).

1.2. Family Hesionidae: Symbiotic species

The hesionids are well-known polychaetes currently including 11 commensal species. This represents about 3% of the known commensal polychaetes and around 7.5% of the known hesionid species. No parasitic hesionids have been reported to date and commensalism seems to be restricted to the clade Ophiodrominae, which includes *Oxydromus* (a recent synonymy of *Ophiodromus*; Villalobos-Guerrero & Harris, 2012), but has certainly multiple origins within the family. In fact, *Oxydromus* and *Gyptis*, two of the most representative Ophiodrominae genera including commensal species (Table 2), are not closely related (Ruta et al. 2007). Moreover, both are species-rich genera and, still nowadays, there are no evidences on whether commensalism arose once or more times within each of them.

Commensal hesionids are involved in about 37 different associations. Except for the polyxenous species of *Oxydromus* (i.e., *O. humesi, O. obscurus, O. flexuosus,* and *O. puguettensis*), which are associated with 3, 3, 10 and 12 hosts, respectively), most symbiotic hesionids are monoxoneus, occurring in only one (6 species) and two (1 species) hosts. Hesionid hosts include species from very different taxonomic groups, which is relatively rare among symbiotic polychaetes, except in this family and in polynoids. The most common hosts are echinoderms (particularly starfishes), followed by other polychaetes (Table 2).

In addition to *O. humesi*, there is only one more hesionid living in association with a bivalve, and this is *O. pugettensis*, which seems to be able to detect at a certain distance the presence of at least two of its host starfishes, *Patiria miniata* and *Luidia foliolata* (Davenport et al., 1960). Also there are some indications of mutualistic behaviour in their relationships with one of its echinoid hosts, the sand dollar *Clypeaster humilis* (Storch and Niggemann, 1967). The existing analyses of the behaviour of *O. humesi* in experimental conditions do not allow proving the existence of a host-factor (similar to that existing in the association between *O. puguettensis* and its host starfishes) in their relationships with *Scrobicularia plana* (Martin et al., 2015). In turn, the same experiments demonstrate the existence of an elaborated and complex host-entering behaviour, which leads the worm to enter inside the host bivalve mainly through the inhalant siphon.

Table 2. List of known symbiotic species of Hesionidae, updated from Martin et al. (2012).

Species	Authority	Host Group	Host species	Source
Anoplonereis herrmanni (nomen dubium)	(Giard 1882)	Entereopneust	*Balanoglossus robinii*	Giard (1882)
		Entereopneust	*Balanoglossus salmoneus*	Giard (1882)
Gyptis ophiocomae	(Storch and Niggemann 1967)	Ophiuroid	*Ophiocoma scolopendrina*	Storch and Niggemann (1967)
Gyptis vittata	(Webster and Benedict 1887)	Polychaete	*Notomastus lobatus*	Gardiner (1976)
Podarkeopsis brevipalpus	(Hartmann-Schröder 1959)	Polychaete	*Glycera robusta*	Gardiner (1976)
		Holothuroid	*Leptosynapta tenuis*	Gardiner (1976)
Parahesione luteola	(Webster 1879)	Decapod	*Upogebia affinis*	Pettibone (1956)
Oxydromus humesi	(Pettibone 1961)	Bivalve	*Tellina innominata*	Martin (2015)
		Bivalve	*Scrobicularia plana*	Martin (2012)
		Bivalve	*Psammotreta cumana*	Martin (2015)
Oxydromus angustifrons	(Grube 1878)	Asteroid	*Pentaceros hedemanni*	Jones (1964)
Oxydromus flexuosus	(Delle Chiaje 1827)	Asteroid	*Astropecten aranciacus*	Barel and Kramers (1977)
		Asteroid	*Astropecten bispinosus*	Barel and Kramers (1977)
		Asteroid	*Astropecten platyacanthus*	Barel and Kramers (1977)
		Asteroid	*Astropecten irregularis*	Barel and Kramers (1977)
		Asteroid	*Luidia ciliaris*	Barel and Kramers (1977)
		Hemichordata	Balanoglossus sp.	Clark (1956)
		Echiuroid	*Maxmuelleria lankesteri*	Anker et al. (2005)
		Holothuroid	Leptosynapta sp.	Barel and Kramers (1977)
		Polychaete	*Neoamphitrite edwardsi*	Barel and Kramers (1977)
		Polychaete	*Euclymene lumbricoides*	Barel and Kramers (1977)
Oxydromus obscurus	(Verrill 1874)	Polychaete	*Lysilla alba*	Pettibone (1963)
		Echinoid	*Lytechinus* sp.	Hartman (1951)
		Holothuroid	Thyone sp.	Pettibone (1963)
Oxydromus pallidus	(Claparède 1864)	Echiuroid	*Lissomyema exilii*	Anker et al. (2005)
Oxydromus pugettensis	(Johnson 1901)	Asteroid	*Patiria miniata*	Bartel and Davenport (1956; Davenport et al. (1960; Lande and Reish (1968; Ricketts et al. (1985)
		Asteroid	*Luidia foliolata*	Davenport et al. (1960; Stewart (1970; Hiblig (1994)
		Asteroid	*Luidia magnifica*	Storch and Rosito (1981)
		Asteroid	*Pteraster tesselatus*	Storch and Niggemann (1967)
		Asteroid	*Oreaster occidentalis*	Steinbeck and Ricketts (1941)
		Asteroid	*Pisaster ochraceus*	Davenport and Hickok (1957; Hickok and Davenport (1957)
		Holothuroid	*Protankyra bidentata*	Okuda (1936)
		Decapod	*Eupagurus* sp.	Hickok and Davenport (1957)
		Gastropod	*Aletes* sp.	Storch and Niggemann (1967)
		Bivalve	*Chama* sp.	Storch and Niggemann (1967)
		Echinoid	*Clypeaster humilis*	Storch and Niggemann (1967)
Unidentified hesionid		Echinoid	*Linopneustes longispinus*	Miller and Wolf (2008)

Contrary to *O. puguettensis,* however, there was a negative effect of the presence of *O. humesi* inside *S. plana*, as the infested hosts shows a significantly reduced body mass compared to the non-infested ones (Martin et al., 2012). The fact that the presence of symbionts may affect the metabolism of their host bivalves, and thus their normal growth (Bierbaum and Ferson, 1986), has been previously reported for other polychaete species living in the mantle chamber of bivalves, such as *Branchipolynoe seepensis* living inside *Bathymodiolus* spp. (Britayev et al., 2007). However, contrary to *B. seepensis*, no damages in the tissues of *S. plana* have been observed in the case of *O. humesi*.

1.3. *Oxydromus humesi* and our study

In our study, we analyse the population dynamics of a hesionid polychaete that was first described as *Parasyllidea humesi* Pettibone, 1961. Recently, both morphological and phylogenetic analyses supported that *Parasyllidea* is a junior synonym of *Oxydromus* (Martin et al., 2015) and must be referred to as *Oxydromus humesi* (Pettibone, 1961). Accordingly, the other two known species of *Parasyllidea* should be revised in order to check its taxonomic position: *P. australiensis* (Hartmann-Schröder, 1980), known from a single record (Hartmann-Schröder 1980), and *P. blacki* (Knox, 1960), an eyeless species from deep water originally described as *Nereimyra blacki* and later transferred to *Parasyllidea* by Pleijel (1998). Both species are not symbionts (Martin et al., 2012).

Oxydromus humesi is a strict bivalve symbiont, probably a parasite according to Martin et al. (2012), living in association with *Tellina nymphalis* (Bertin, 1878) in Loango (Congo mangrove swamps) and *Scrobicularia plana* (Da Costa, 1778) (intertidal) and *Psammotreta cumana* (O.G. Costa, 1829) (subtidal) in Cádiz Bay salt marshes (Martin et al., 2015). The later is common in the subtidal of Cádiz Bay (E. Pascual, personal observations), whilst it is very rare in the intertidal (Subida et al., 2013) and has never been previously reported from Río San Pedro (Arias & Drake, 1999; Carvalho et al., 2013). However, according to Martin et al. (2015), the highest prevalence of *O. humesi* occurs in this host, suggesting that both *P. cumana* and its habitat could be more favourable for the hesionid. In turn, the worms associated to the intertidal population of *S. plana* show a much lower prevalence, despite the host is very abundant in the zone (Subida et al. 2011; Drake et al. 2014) suggesting that both this host and its habitat are less preferred by *O. humesi*. However, despite the differences in habitat, both host species share a similar morphology and mode of life, and the association may be considered as an obligate symbiosis, as proposed by Martin et al. (2012), who argued that *O. humesi* did not occur inside any other bivalve coexisting in the studied area and that the worm has never been reported as free-living.

Whenever reported, the relationship between size structure of symbiotic and host populations is, if anything, unclear (Martin et al., 1991, 1992, Emson et al., 1993, Rozbaczylo & Cañete, 1993, Britayev & Zamishliak, 1996). This suggests that the life histories of symbiotic polychaetes are usually independent of their hosts, which tend to live longer than the symbionts (Martin & Britayev, 1998). In many cases, this means that same host populations may successively harbour different polychaete populations. However, there are also cases where symbiotic polychaetes and their hosts may well reveal the existence of positive correlation between the respective size structures, as occurs among other symbiotic animals (e.g. the pontoniin shrimp *Anchistus custos* associated with the host bivalve *Pinna bicolor* in Britayev & Fahrutdinov, 1994).

In accordance to the existing literature, we postulate that there is no size-correlation between the two partners under study or, in other words, that the symbiont did not show preference for a given host's size. Martin et al. (2012) proved that bivalves measuring less than 20 mm were not infested so that the host and the symbiont did not grow together from the smallest to the largest respective sizes. Limiting host-sizes have been also reported for the starfish *Asterias rathbunae*, where specimens with disc radi lower than 35 mm were not inhabited by *Arctone vittata*, whereas those with radi up to 90 mm harboured only one symbiont, and the largest starfish were infested with 1-4 symbionts (Britayev et al., 1989). Also, the scaleworm *Harmothoe commensalis* was not present in shells of the clam *Gari solida* less than 60 mm long (Rozbaczylo & Cañete, 1993). In addition, we want also to analyse the temporal pattern of the prevalence and intensity of the infestation and, whenever possible, the biological factors influencing this pattern (e.g. symbionts' reproductive dynamics, hosts' size-structure).

Oxydromus humesi does not show sexual dimorphism, but females can be distinguished during the reproductive period by the presence of intracoelomic oocytes (of about 140 µm in diameter) visible through the body wall (Martin et al., 2015). However, no other information on the life cycle of *O. humesi* has been reported since it was discovered in the coasts of the Republic of Congo (hosted by *Tellina nymphalis*). Therefore, in this study, we attempt to clarify the population dynamics of the symbiotic population associated to *S. plana* from Río San Pedro, based on a monthly sampling carried out from April 2011 to May

2012.

Accordingly, among the numerous open questions dealing with this symbiotic polychaete, we attempted to solve the following ones: Are the hosts smaller than 20 mm infested during the seasonal cycle? Do the symbiont show a preference for a particular host size larger than 20 mm? Are the prevalence and intensity of infestation constant along time or is there a temporal variability? Which are the possible biological factors that may influence the infestation pattern (e.g. symbionts' reproductive dynamics, hosts' size-structure)?. Additionally, we compare the population inhabiting *S. plana* with the few available data on the worms infesting *P. cumana* collected in January 2013.

2. OBJECTIVES

The main objective of this work is to study the population dynamics of the symbiotic polychaete *Oxydromus humesi* hosted by the bivalve *Scrobicularia plana* in Cádiz Bay. Therefore, we will analyse different biological descriptors of this association (i.e. host-symbiont size relationships, infestation prevalence and intensity and symbiont's reproduction). It should be pointed out that there are no environmental data collected during the study period, which will prevent to define the possible influences in the observed patterns.

Several sub-objectives may be distinguished within the main one:

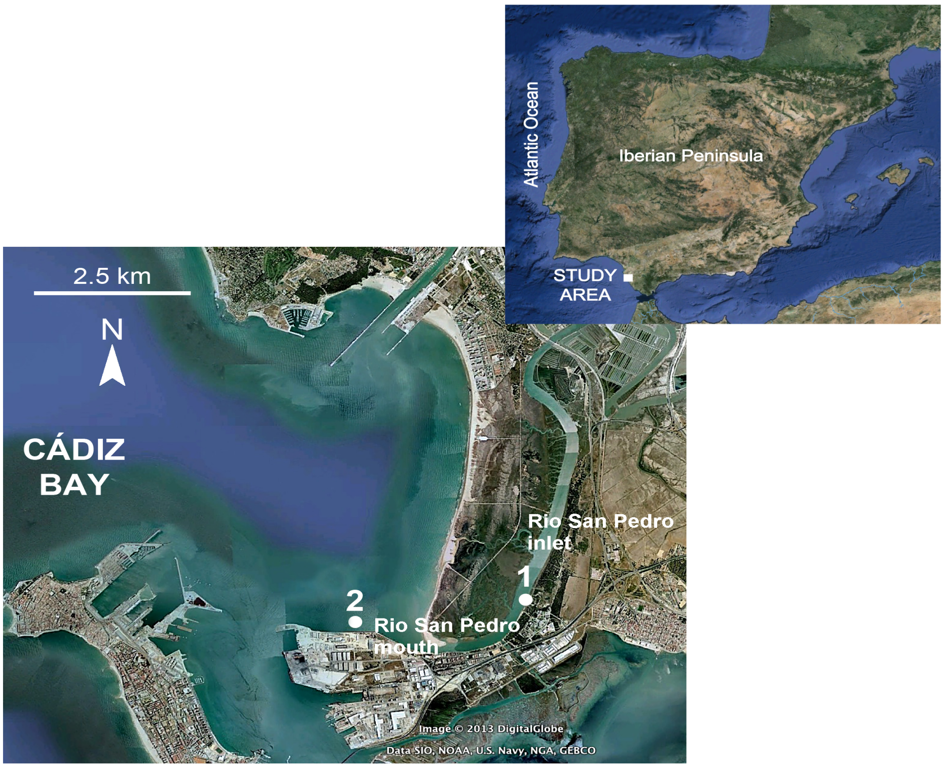
- To analyse the size structure of the studied population and its relationships with host's size.
- To study the seasonal patterns of prevalence and intensity of the infestation.
- To analyse the reproductive patterns of the symbiont along the seasonal cycle, based on the identification of the presence of ripe females and the implication in the reproductive behaviour of the species.
- To relate all above mentioned patterns together.

In addition to the approach to the population dynamics of the species, we also attempt to clarify, as far as possible, the life cycle of the population of *O. humesi* from Cádiz Bay.

3. MATERIAL AND METHODS

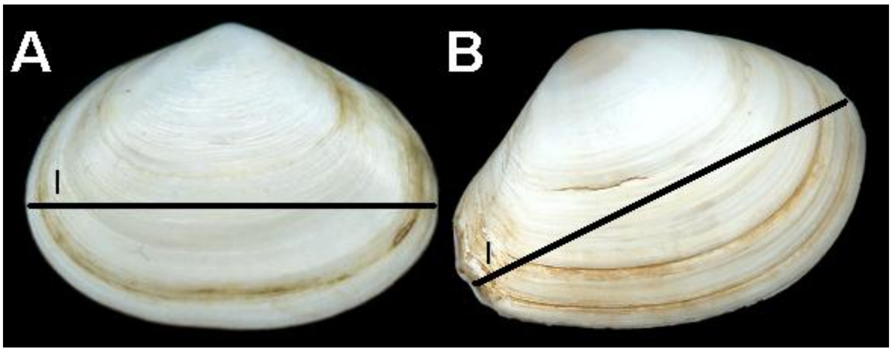
Specimens of the host bivalve *Scrobicularia plana* were collected monthly from April 2011 to May 2012 by hand digging at low tide in Río San Pedro (Cádiz Bay, southern Atlantic coast of the Iberian Peninsula) (Fig. 1). The host specimens from May 2011 were not measured as they were damaged during sample handling. In January 2013, as a result of the monitoring of the association between *Afropinnotheres monodi* Manning, 1993 and *Cerastoderma glaucum* (Bruguière, 1789) at the low subtidal of Río San Pedro mouth (about 3 km downstream the location of the studied *S. plana* population) (Fig. 1), additional specimens of a second host, *Psammotreta cumana*, were collected (also by hand digging) together with specimens of the usually much more abundant *Cerastoderma glaucum*. Some more specimens of *S. plana* were also collected this January at the intertidal of Río San Pedro.

Fig. 1. Sampling sites of the bivalve host populations harbouring the symbiotic hesionid *Oxydromus humesi* in the Bay of Cádiz: (1) Intertidal (*Scrobicularia plana*); (2) Subtidal (*Psammotreta cumana*). Images obtained from Google Earth, version 7.1.2.2041, (c) Google 2015.



Once collected, the specimens of *S. plana* and *P. cumana* were kept alive and transported to the laboratory. These were then opened to check for the presence of the symbiont and to estimate the prevalence and intensity of the infestation. All obtained worms were then counted, relaxed in isotonic magnesium chloride (7.2g $MgCl2 \cdot 6H_2O$ in 100 ml of distilled water) and preserved in 70 % ethanol. One preserved worm was selected and photographed in detail with a CMEX digital camera, linked to a Zeiss Stemi 2000-c, stereomicroscope and using the ImageFocus 4.0 software by Euromex (Fig. 3). All bivalves harbouring a worm inside were counted and measured (length, in mm; Fig. 2) with the help of a caliper. A relevant sample of *S. plana* (i.e. 100 to 300 specimens) was collected and measured (independently of the presence of the symbiont) to define the size structure of the native population each month.

Fig. 2. Length (l) of shells of host species of *Oxydromus humesi* in Cádiz Bay. **a** *Scrobicularia plana* (Da Costa, 1778). **b** *Psammotreta cumana* (O.G. Costa, 1829). Images extracted from www.idscaro.net.



According to Martin et al. (2012), there is a significant positive correlation between width and length of the worms. Thus, the size of the symbionts was expressed as the width of the tenth segment (parapodia included, in mm). To measure symbionts' size and to identify the ripe females, we used a Nikon SMZ645 binocular microscope equipped with a micrometric ocular (Fig. 4). The host's size was measured as the length of the longest shell diameter (in mm; Fig. 2).

Ripe females were identified by the presence of intracoelomic oocytes, which are visible through body wall. In preserved specimens, the worm tissues became more opaque than in living ones (Fig. 3), this slightly hindering the oocyte observation. Thus, we confirmed its presence by cutting the worm tissues and then gently pressing to force the oocytes to exit the coelomic cavity so that they could be observed floating freely in the Petry dish (Fig. 3).

The worm/host size and the ripe females/prevalence relationships were assessed by linear regression. The sizes of *O. humesi* (ripe females highlighted) and *S. plana* were used to estimate the monthly size-class frequencies of each species. The frequency of infested bivalves and that of the whole population was estimated separately and then expressed as % to compare them. All these analyses have carried out by means of the software OpenOffice.org version 2010.3.2.

Fig. 3. *Oxydromus humesi*. Digital photograph of a preserved specimen from Cádiz Bay. **a** Whole body, dorsal view. **b** Detail of broken tissue in dorsal view, showing the oocytes floating freely in the Petri dish.

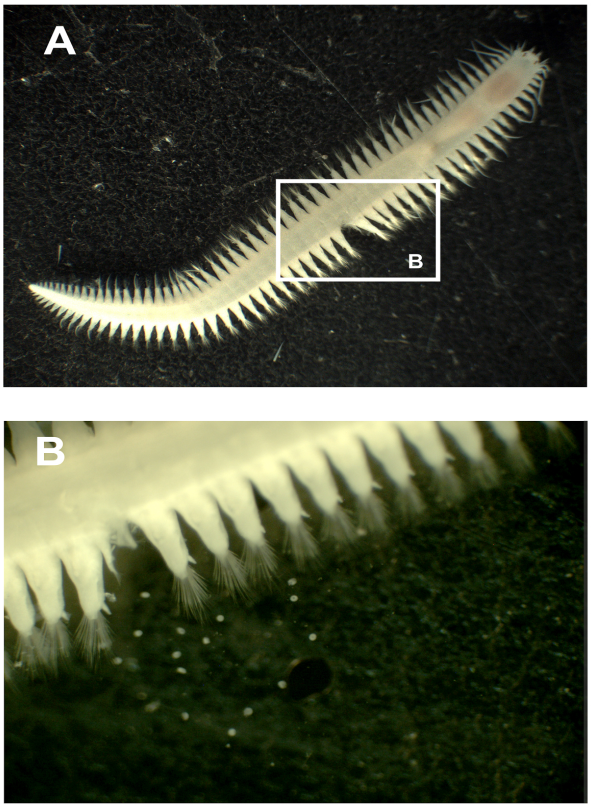


Fig. 4. Miguel A. Meca at workplace, in the Marine Ecology Department of the CEAB-CSIC, using the aforementioned binocular microscope to study the populations of the symbiotic hesionid *Oxydromus humesi*.

4. RESULTS

4.1. Size structure in symbiont and host populations: Relationships with the infestation descriptors

A total of 275 worms were collected during this study, 241 inside *Scrobicularia plana* from April 2011 to May 2012 and 26 inside *Psammotreta cumana*, 5 in *S. plana* and 3 outside the host in January 2013 (Fig. 8; Table 3). The size classes varied from 1.8 to 3.6 mm. The small-sized worms seem to be better represented mainly in late autumn and also in winter, while large worms were more or less constantly present all along the studied period, except during late winter and early spring (Fig. 8). It should be pointed out that ripe females were all placed within the largest size-classes (i.e., from 2.2 to 3.6 mm) (Fig. 8).

Concerning the hosts, 6917 *S. plana* and 39 *P. cumana* were collected along the seasonal cycle (Fig. 9; Table 3). In *S. plana,* the size classes ranged from 20 to 40 mm, with the exception of one infested host measuring 40.65 mm. However, intermediate sizes (i.e., from >26 mm to about 36 mm long) were available during the whole year, being always the most abundant and also the most infested ones (Fig. 9). The most balanced size class frequency distribution occurred in September 2011. In *P. cumana*, the size-class range was restricted between 20 and 30 mm and the most infested ones were slightly smaller (i.e., from >20 to about 28 mm long) than in *S. plana* (Fig. 9).

4.2. Host/symbiont size relationships

There were non-significant size relationships between *O. humesi* (Ox) and *S. plana* (Sc) (254 host-symbiont pairs, Ox = 0.01Sc + 2.16, $R^2 = 0.02$, $p > 0.05$; Fig. 5). The monthly pattern was the same, except for April 2012 (Ox = 0.1Sc - 0.68; $R^2 = 0.76$, $p < 0.05$ Fig. 6).

The specimens of *O. humesi* associated to *P. cumana* (26 host-symbiont pairs), also show non-significant relationships (Ox = 0.07Sc + 0.98; $R^2 = 0.33$, $p > 0.05$; Fig. 7).

4.3. Infestation characteristics

The studied population of *O. humesi* showed a seasonal variability in prevalence, with the higher percentages occurring during late autumn-mid winter (Fig. 10; Table 3). The highest peak occurred in December 2011 (i.e. 19.2 %), in coincidence with the presence of the highest number of symbionts (Table 3). Intermediate prevalences were observed in November 2011 (i.e. 7.2 %), January 2012 (i.e. 8.2 %) and February 2012 (i.e. 6.1 %) (Fig. 10; Table 3). The remaining prevalences were always lower than 5%, which agrees with the percentages reported by Martin et al. (2012). The lower prevalence was found in April 2011 (i.e. 1.6%) (Fig. 10; Table 3). In *P. cumana* the prevalence was 66.7% and, in bivalves longer than 20 mm, risen up to 89.3% (Table 3).

The intensity of the infestation was always a single worm per host.

4.4. Proportion of ripe females and its relationships with infestation characteristics

Mature females occurred during all the studied period, except in April 2012 (Fig. 8; Table 3). The highest percentages of ripe females occurred in mid spring and summer, being August 2011 the single month where the proportion of ripe females versus the rest of specimens was 1:1 (Fig. 10; Table 3). In turn, the lowest percentages occurred in autumn and winter (Fig. 10; Table 3).

Despite the relationships between ripe females (Rf) and prevalence (Pr) in *S. plana* along the studied months were non-significant ($Pr = -0.11Rf + 7.70$, $R^2 = 0.17$, $p > 0.05$), the presence of ripe females tended to be higher when the infestation was lower (Fig. 11).

Table 3. Monthly-synthesized data: total number and infested hosts, percentage of ripe females and prevalence. *Based on all collected symbionts (including those from the two hosts and the three found outside).

Year	Month	N hosts	Infested	% Ripe Females	Prevalence	Host
2011	April	1000	16	37.5	1.6	*S. plana*
	June	468	22	40.9	4.7	
	July	469	15	40.0	3.2	
	August	370	10	50.0	2.7	
	September	370	17	41.2	4.6	
	October	306	11	9.1	3.6	
	November	389	28	10.7	7.2	
	December	255	49	8.2	19.2	
2012	January	280	23	4.3	8.2	
	February	328	20	5.0	6.1	
	March	320	8	25.0	2.5	
	April	219	7	0.0	3.2	
	May	469	15	13.3	3.2	
2013	January	39	26	2.9*	66.7	*P. cumana*

Fig. 5. Size relationship between the all studied pairs of *Oxydromus humesi* and *Scrobicularia plana* (April 2011 to May 2012); n = 254.

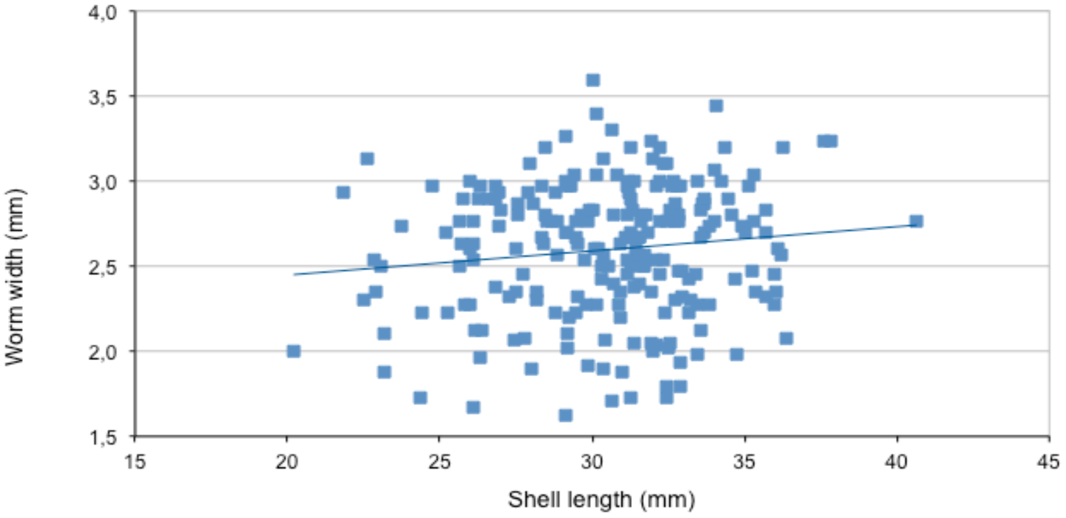


Fig. 6. Size relationship between the pairs of *Oxydromus humesi* and *Scrobicularia plana* collected in April 2012; n = 7.

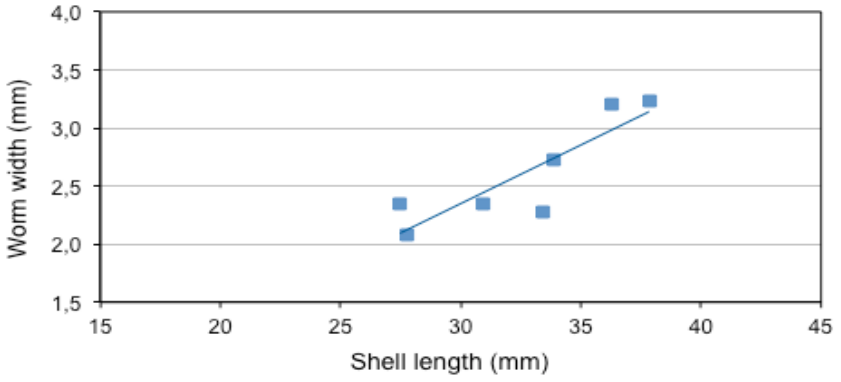


Fig. 7. Size relationship between the pairs of *Oxydromus humesi* and *Psammotreta cumana* collected in January 2013; n = 26.

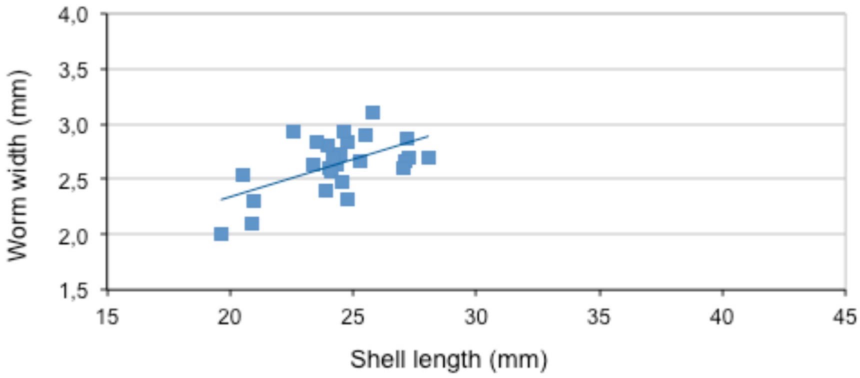


Fig. 8. *Oxydromus humesi.* Size-frequencies of the specimens hosted by *Scrobicularia plana* from April 2011 to May 2012, and *Psammotreta cumana, S. plana* and the three found outside the host in January 2013. 2011: April, n = 16; June, n = 22; July, n = 15; August, n = 10; September, n = 17; October, n = 11; November, n = 28; December, n = 49. 2012: January, n = 23; February, n = 20; March, n = 8; April, n = 7; May, n = 15.

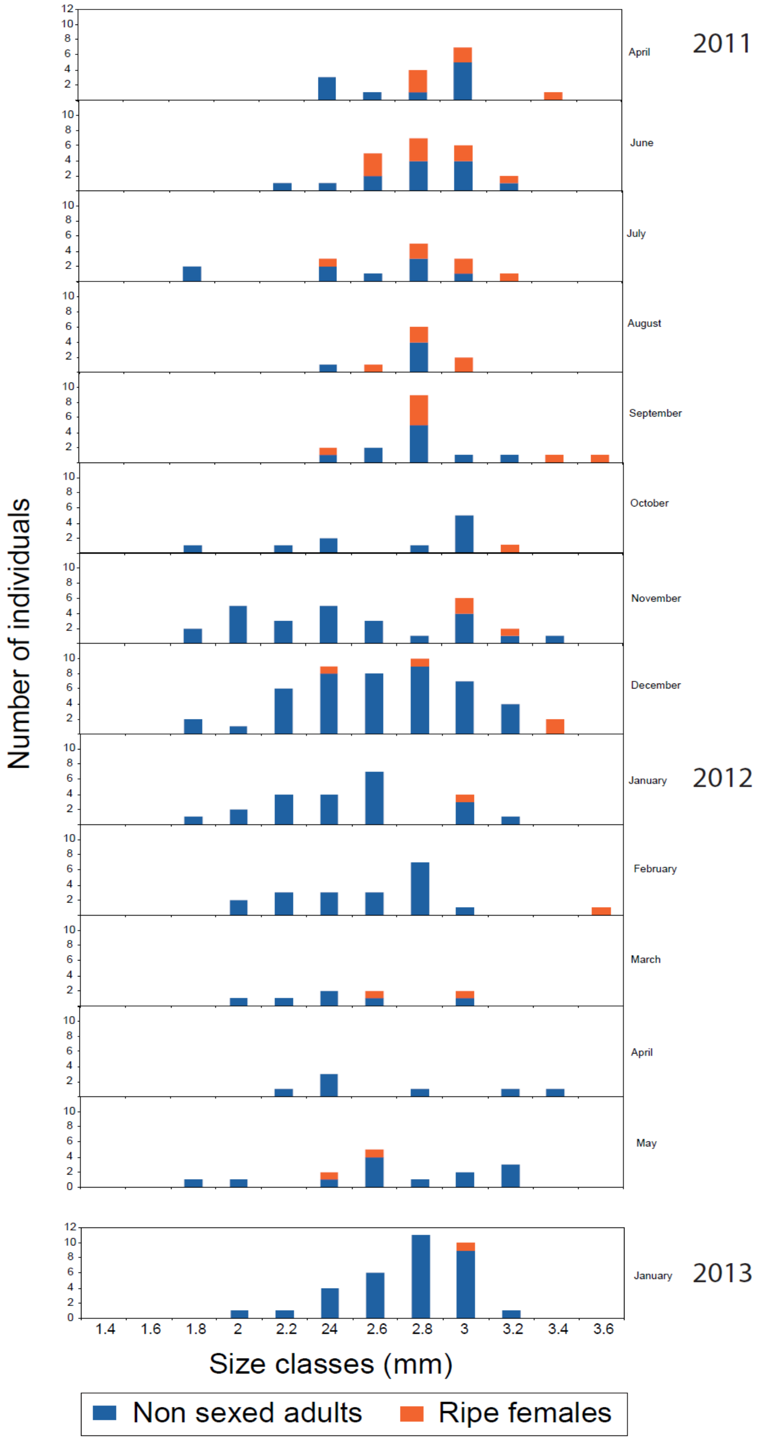


Fig. 9. Size-frequencies of the whole population of *Scrobicularia plana* from April 2011 to May 2012 and *Psammotreta cumana* in January 2013 versus the infested bivalves. The total number of individuals of *S. plana* population month by month has been: 2011: April, n = 300; June, n = 200; July, n = 94; August, n = 100; September, n = 108; October, n = 99; November, n = 99; December, n = 124. 2012: January, n = 100; February, n = 98; March, n = 150; April, n = 102; May, n = 100.

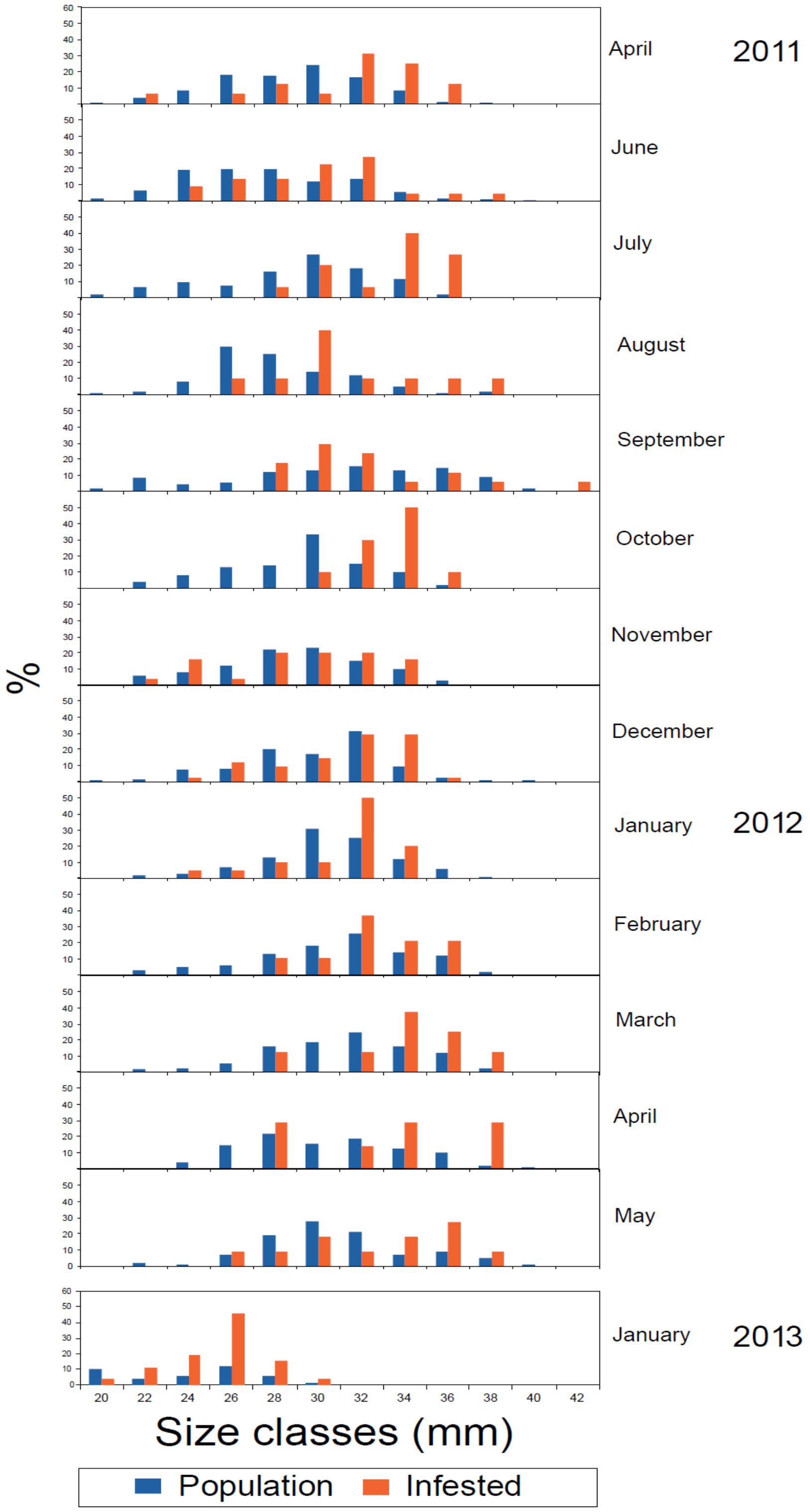


Fig. 10. Monthly percentages of ripe females of *Oxydromus humesi* inside the infested *Scrobicularia plana* during the study period.

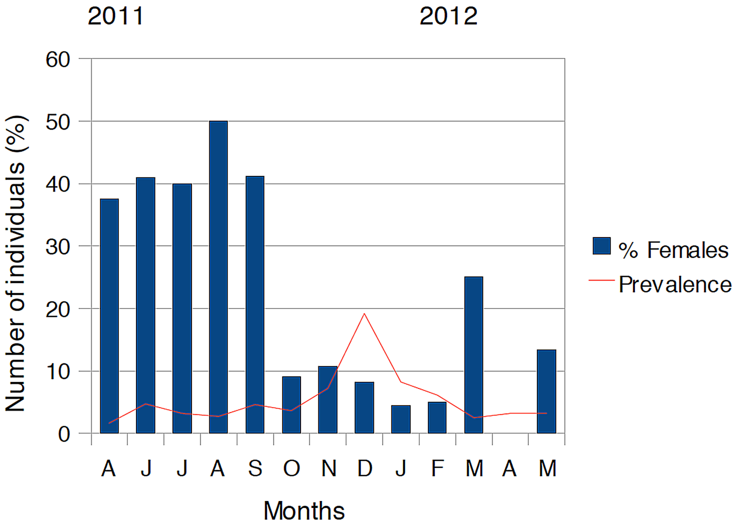
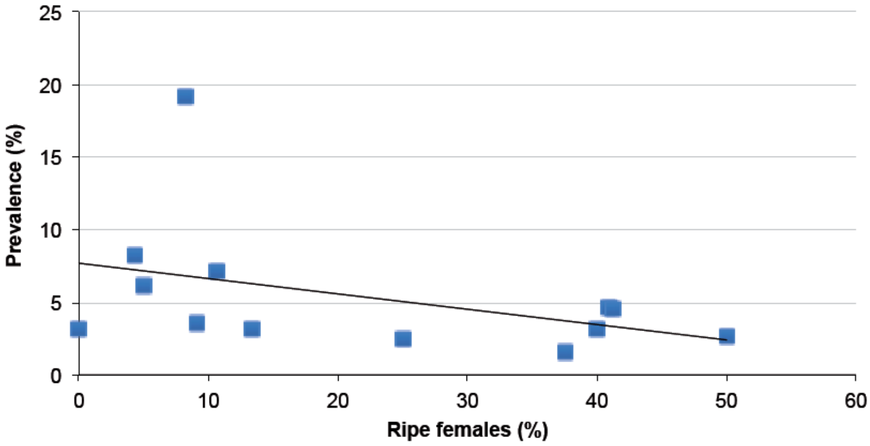


Fig. 11. Relationship between ripe females of *Oxydromus humesi* and prevalence in *Scrobicularia plana* through all the annual cycle.



5. DISCUSSION

5.1. Host/symbiont size relationships

Positive host/symbiont size correlations are not common among symbiotic polychaetes (Martin & Britayev, 1998). Two hypotheses have been suggested to explain such positive elationships. First, there may be active size segregation behaviour, as reported both for the symbiotic coral dwelling crab *Trapezia ferruginea* (Adams et al., 1985) and the fish *Gobiodon* *histrio* (Hobbs & Munday, 2004), which seem to be able to migrate from colony to colony to choose one of an appropriate size. Secondly, there may be a parallel growth of hosts and symbionts, as reported both for the nemertean *Malacobdella grossa*, hosted by *Arctica islandica* (Sundet & Jobling, 1985) and the pontoniin shrimp *Anchistus custos*, hosted by the bivalve *Pinna bicolor* (Britayev & Fahrutdinov, 1994). In turn, the absence of host/symbiont size correlation could be caused by symbionts that either colonize the hosts during a single phase of their life-history (e.g. juveniles, adults) or are highly mobile (and, thus, colonize the available host independently of their size). An additional hypothesis was postulated by Britayev et al. (2007), who suggested that a symbiont's growth faster than that of host would explain the absence of correlations in the case of symbiont whose infestation starts with the juveniles colonizing in small-sized hosts. In some cases, the host/symbiont size-correlation may be modified by the differential behaviour linked to reproductive activities. For instance, in the case of *Branchipolynoe seepensis* an obligate symbiont of mytilids of the genus *Bathymodiolus* from deep-sea hydrothermal vents, females have a longer life-span than males and remains inside the same host along their life, while males leave their original hosts for mating and then colonize the available hosts independently of their size, so that a positive host/symbiont size correlation occurs only for non-mature males (Britayev et al., 2007).

Oxydromus humesi inhabits the mantle cavity of their host bivalves. As previously reported (Martin et al., 2012), in *S. plana* they always occurred in specimens longer than 20 mm long, and were more frequent in intermediate-sized shells (i.e., from >26 mm to about 36 mm long). In turn, the infested specimens of *P. cumana* collected in January 2013, include considerably lower-sized hosts (Fig. 9). Despite the low number of bivalve individuals collected for that species, the higher infestation rates (Table 3) allowed us to suggest that *P. cumana* may be a preferred host for *O. humesi*, although a more detailed study of the populations of this host species in Cádiz Bay is required prior to confirm this statement.

As previously mentioned, we observed non-significant size-correlations between worms and bivalves in the *S. plana* population (Fig. 5), except for April 2012, which may be more likely connected to the low number of individuals (<10), lower than most remaining months (Fig. 8; Table 3). However, other months with a similar number of individuals (e.g. 8 to 10 in March, October, and August) (Fig. 8; Table 3) showed non-significant correlations. Accordingly, we agree with Martin et al. (2012) in that the worms did not grow together with their hosts, while the absence of juveniles in small-sized hosts allowed us to discard the fast growth hypothesis for this species.

5.2. Infestation characteristics

5.2.1. Prevalence

The prevalence of commensal polychaetes are highly variable, ranging from very low up to host populations being infested as a whole, and has been considered as a species-specific characteristic. However, a commensal species may also show different prevalences, which may vary according to bathymetric, spatial and temporal (i.e. seasonal and annual) patterns (Martin & Britayev, 1998).

The studied population of *O. humesi* seems to show a seasonal variability in prevalence, with the highest percentages ocurring during late autumn-mid winter (Fig. 10; Table 3). Our data set includes a single year, so we cannot infer inter-annual regularities. With the exception of the impressive fidelity of the year-to-year counts of a regular seasonal trend demonstrated for the infestation prevalence in *Branchiosyllis exilis* (Hendler & Meyer, 1982), the only known data on year-to-year variability are on *Arctonoe vittata* infesting the starfish *Asterias amurensis* (Britayev, 1991), who reported a progressive increase in prevalence from 0% in 1975-76 to 8.4% in 1978 and to 79.1% in 1980.

Theoretically, we may expect an influence of the host's populations structure on that of the symbiont (Martin & Britayev, 1998). A positive relationship between host density and infestation characteristics was reported for the starfish *Asterias rathbunae* and its commensal *Arctonoe vittata* (Britayev et al., 1989). The highest prevalence, mean intensity and abundance occurred in the area most densely populated by the host, which could be caused by the accumulation of a chemically-mediated host-cue more effectively attracting the settling symbiont than in less densely populated areas (Martin & Britayev, 1998). High host densities may also reduce the influence of external factors. For instance, commensals associated with less abundant host populations may experience a more relevant decrease in fitness than those harboured by dense host populations (Martin & Britayev, 1998).

Among the few relationships with known data, high infestation indexes tended to be positively correlated to the presence of large and more numerous hosts available to be occupied by the symbionts (Martin & Britayev, 1998). In our case, the low prevalence observed during the whole studied period would lead us to expect a low number of *S. plana* exceeding 20 mm long to be present in the study area. Nevertheless, *S. plana* was very abundant independently of size (Subida et al., 2011; Drake et al., 2014) and so numerous adequate hosts were available through the whole study period (Fig. 9; Table 3). Therefore, the prevalence seems not to be connected with the habitat availability. In turn, this low prevalence could be related to the restrictive environmental conditions at the intertidal environment (e.g. extreme temperatures changing diary and/or long desiccation periods). The high prevalence observed in the subtidal population of *P. cumana*, higher than 85% in specimens longer than 20 mm in January 2013, seems to confirm this hypothesis, with the condition of permanent immersion clearly favouring the presence of the symbiont. On the other hand, the fact that we did not found small-sized (<18 mm in shell length) specimens of *S. plana* during the annual cycle agrees with all previous information obtained in punctual periods of the year (Martin et al. 2012, 2015). Our approach prevented us to be able to explain this peculiarity of the studied bivalve population. However, the high density of specimens restricted to the narrow intertidal area at Río San Pedro led previous authors to postulate the existence of a negative interaction between the established population and the larvae trying to settle in this area, which could be actively ingested by their conspecific adults during their normal feeding activities (i.e. passive cannibalism) (Cargnin-Ferreira, 2005; P. Drake, personal communication).

Additionally, the association with various hosts in the same locality could affect the prevalence, which strongly depends on the level of affinity of the symbiont for the different hosts. Despite the symbiotic population of *O. humesi* at Río San Pedro only inhabited *S. plana*, this factor could not be eventually discarded due to the presence of the nearby subtidal population of *P. cumana* at the opening of Río San Pedro. Therefore, more data on the population structure and distribution of the different hosts are required to address both the symbiont's preferences and the possible interconnections of the life cycles of the populations inhabiting these two host species.

Despite *O. humesi* seems to live only as endobiont of bivalves sharing both mode of life and morphology all along their geographical range of distribution, further studies are required to confirm the possible existence of other hosts, as well as to clarify the pattern of preferences among the currently known ones.

5.2.2. Infestation intensity

The infestation intensity of commensal polychaetes has been more widely reported than the prevalence. It may range from 1 to 648 symbionts per host, but is clearly dominated by the association of a single symbiont per host either due to the usually low symbiont densities or to the influence of an intraspecific aggressive behaviour (Martin & Britayev, 1998). Like the prevalence, intensity may also oscillate within the same population due to seasonal changes in relative abundances, linked or not to reproduction and recruitment events. A common situation for symbionts with 1:1 regular distributions like *O. humesi* is that adults may occasionally share the host with one to several juveniles, as previously reported for species of the genera *Acholoe, Adyte* or *Branchiosyllis* (Martin & Britayev, 1998).

In the case of *O. humesi*, previous studies reported a single finding of one male and one female (Martin et al., 2012), as well as one male, one female and one small worm, likely a juvenile (Martin et al., 2015), sharing the same host. These authors attributed the constant regular distribution pattern to the intraspecific aggressive behaviour of the species. There are several cases of symbiotic polychaetes with male and female couples living together inside the same host, such as the Mediterranean species *Ichthyotomus sanguinarius* (symbiont of fishes) or *Robertianella synophthalma* (symbiont of deep-sea hexactinellid sponges) (Martin & Britayev, 1998). However, in both cases, most hosts harboured couples, while in *O. humesi* couples seem to be the exception.

5.2.3. Relationships between infestation characteristics and reproduction

The marked seasonal pattern found in the population of *O. pugettensis* infesting the starfish *P. miniata* at Dana Point (California) seemed to be connected with the commensal reproductive dynamics (Lande & Reish, 1968). This species reached the highest (≥80%) and lowest (≤30%) prevalence in November-December and summer (prior to the decrease of water temperature), respectively, and his abundance was maximum in winter (2-3 worms per host) and minimum in the middle of summer (<0.5 worms per host). In the case of *O. humesi* the prevalence seems also to be connected with the species reproduction, as the months with lower percentages (i.e., mid spring and summer) tended to show a markedly higher presence of ripe females (>30%) (Fig. 8, 10, 11; Table 3). Moreover, as previously mentioned, August 2011 was the single month where the proportion of ripe females versus the rest of population was 1:1. However, there were some relevant deviations: December 2011 showed the highest prevalence and there was an 8% of ripe, while November 2011 and January and February 2012 showed very low prevalence and the percentage of ripe females ranged from 5 % to about 10% (Fig. 8 and 10; Table 3). However, sampling on different years would be required in order to infer regularities in the relationships between prevalence and the reproductive cycle of *O. humesi*.

The fact that the lower prevalence tended to coincide with the higher percentages of ripe females suggests that ripe females may have a reduced mobility and so they tend to remain inside the host during the whole life, whereas males could be more mobile and thus leave the host, likely to increase fertilization success. This behaviour was previously reported for *B. seepensis,* and, as mentioned above, also contributed to explain the lack of host-bivalve size relationships for adult males (Britayev et al., 2007). A similar situation was reported for *Haplosyllides floridana*, whose male stolons were never found inside the host sponge *Neofibularia nolitangere* and, conversely, were found free-swimming in the water column (Martin et al., 2009). Logically, the highest prevalence in the studied population of *O. humesi* occurred when both males and females occupied their respective hosts.

Despite this general pattern of reproduction vs. prevalence, the species seems to reproduce actively during the whole year, but with an increasing effort during spring-summer and a higher intensity in summer. The absence of ripe females in April 2012 contrasted with the 37.5% found in 2011. However, the ripe females occurred both before and after April 2012, allowing us to suggest that the lack of ripe females in April 2012 could have been biased by the low number of symbionts found this month (Fig. 8; Table 3). Persistent high temperatures can stimulate oocyte growth, subsequently causing the advancement of reproductive period in polychaetes, as reported for the Mediterranean populations of *Eupolymnia nebulosa* (Cha et al., 1997), which show ripe adults during spring and early summer and spawn in egg-masses three to four times during this period, in contrast with Atlantic English Channel population that concentrate the reproductive efforts in summer to spawn during early July (Lenaers & Bhaud, 1992; Martin et al., 1996). However, our data does not allow assessing whether environmental constraints such as differences in temperature could affect the reproductive cycle of *O. humesi* during the studied period. Further studies during several consecutive years must be carried out to confirm the regularity of the observed pattern, as well as to unravel the possible influences of the environment.

5.3. Life-cycle in *Oxydromus humesi*

Little is known about the life cycle of most commensal polychaetes. The available data, however, suggest that, in fact, the life cycle structure of most species does not significantly differ from those of their free-living relatives and so we may expect to find planktonic larvae (responsible of dispersal and colonization) and benthic adults (with a somewhat reduced mobility). There is, however, a single main difference: the symbiotic mode of life replaces the free-living one during the benthic phase (Martin & Britayev, 1998). Commensals' larval settlement may occur on the bottom, being then followed by a juvenile migration towards the respective hosts (Davenport, 1966). However, as previously mentioned, it seems more likely that chemically mediated cues (either generated by the host or by the own symbiotic adults) driving larval settlement could be the most widespread behaviour among symbiotic polychaetes (parasites included) (Martin & Britayev, 1998).

As it occurs for the free-living species, this basic life-cycle scheme may vary in many cases as an adaptation to the symbiotic mode of life. The life cycles may be simplified by reducing, or even eliminating, the free-living pelagic stage, the responsible of dispersion being the adults themselves. Conversely, other symbionts may increase the complexity of their life-cycles by having one or more intermediate hosts, which are occupied when the preferred ones are not available or because they have more room to host several juveniles during the growth period (Martin & Britayev, 1998). For instance, *Arctonoe vittata* which, in the northwest part of the Sea of Japan (Vostok Bay), lives in association with the limpet *Acmaea pallida* (which may harbour a single host), while juveniles often occur on the starfish *Asterias amurensis* and, more occasionally, on the sea-cucumber *Stichopus japonicus* and the starfish *Aphelasterias japonica* (Britayev, 1991). Herewith, intraspecific competition and aggressiveness probably plays a major role in the associated relocation processes.

As previously mentioned, the only existing information on the juvenile phase of *O. humesi* species is that juveniles have not been found inside the studied population of *S. plana*, neither free-living. Accordingly, the size (ranging from 1.6 to 3.6 mm) and number of segments of the worms found during this study allowed us to consider them as adults.

The starting date for the symbiont’s recruitment into the host bivalves occurred in late autumn (after being more actively reproducing during summer), while the large adults tended to disappear from the population around mid winter. This suggests that the life span of *O. humesi* may be of one year, with the adults dying after reproducing. However, sampling during successive years would be required to confirm this hypothesis, as well as the regularity of the recruitment events.

The life cycle of the studied population of *O. humesi* may be related to the tidal regime characteristic of the intertidal habitat. The presence of a highly infested population of *P. cumana* in the subtidal area of Cádiz Bay facing the opening of Río San Pedro suggests that recruitment may occur mainly in the more favourable subtidal conditions. Thus, we suggest a possible scenario in which the symbionts mainly inhabit certain areas of the Bay not submitted to periodical desiccation by tides, living inside *P. cumana* (and we may not discard other possible unknown hosts), then reaching intertidal areas such as those at Río San Pedro either as larvae through to tidal currents or by adult migration. Therefore, the intertidal area here studied could be at the limit of the ecological distribution of this endobiotic species in the Cádiz Bay region.

The absence of juveniles inside the studied hosts suggests that this phase may be free living and that the colonization of *S. plana* occurs during the benthic phase of the life cycle of *O. humesi*, whose adults are able to move up along Río San Pedro with tides. However, neither juveniles, nor adults have been found in the sediments surrounding the studied areas (P. Drake, personal communication), likely because they may be quite rare. Therefore, further studies on the infauna of these subtidal and intertidal sediments must be carried out in order to confirm our postulates.

As a summary, the possible life cycle of *O. humesi* may consist of a planktonic larval phase, settling on soft bottoms, free-living juveniles, and adults that are able to enter the hosts at a given size (i.e. >1.6 mm wide according to our data) using thigmotaxis and the highly specific host entering behaviour described by Martin et al. (2015).

A similar life cycle was described for *Neanthes fucata*. The planktotrophic larvae of this nereidid settle directly on the soft bottoms. The juveniles live in tubes for several months feeding on detritus and small benthic animals exactly as many of their free-living relatives. Then, the 4-month old worms start to develop the ability to recognize the presence of potential hosts by the substratum vibrations produced by the hermit crab legs bouncing on the sediment surface, which triggers a characteristics host-entering behaviour that allow the worms to crawl on the hermit crab shell following the shell spirals by thigmotaxis (Gilpin-Brown, 1969). This complex life cycle uses different mechanisms that characterize the free-living nereidids (such as thigmotaxis or mucus production) as specific adaptations to the commensal mode of life, while the worm itself has no relevant morphological adaptations. Therefore, in addition to the similarity of the hypothesized life cycle of *O. humesi*, both species also share the lack of evident morphological adaptations (except for the reduction of the central antennae in the hesionid, whose significance in terms of adaptation to the symbiotic mode of life remains unclear) and a similar specific host entering behaviour. In turn, the host-recognition behaviour in *O. humesi* is currently unknown, and the presence of a host-factor has not been demonstrated (Martin et al., 2012, 2015). However, it is well known that the bivalves hosting *O. humesi* may alternate between direct water filtration and deposit feeding by tapping on the sediment surface with their inhalant siphons. Thus, we may hypothesize that *O. humesi* may recognize the presence of a potential host by the movements of the inhalant siphon.

Despite aforementioned similarities between known hosts of *O. humesi*, there are no direct evidences of that the symbiont enters into *T. nymphalis* or into *P. cumana* (or any other potential host) in a similar way as into *S. plana*, this also being an interesting topic for future studies.

6. CONCLUSIONS

1.- The population of *Oxydromus humesi* hosted by *Scrobicularia plana* in the intertidal area of Río San Pedro (Cádiz Bay) seems to reproduce through all the year, with ripe females being particularly numerous in summer.

2.- The observed seasonal pattern of prevalence seems to be related with the reproductive cycle of *O. humesi*, as the lowest summer values tended to coincide with the highest percentages of ripe females. This led us to suggest that males are temporally and more actively leaving their hosts, likely to increase the possibilities of fertilizing more females.

3.- The prevalence in *S. plana* tends to be very low (usually <5% and reaching up to ~20%) during the whole year. Accordingly, the density of *O. humesi* is also very low so that we consider the intertidal as an unfavourable habitat and *S. plana* as a non-preferred host.

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