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# DIVERSITY, HABITAT AFFINITIES AND DIET OF OPHRYOTROCHA SPECIES (POLYCHAETA, DORVILLEIDAE) LIVING IN MEDITERRANEAN HARBOUR HABITATS

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OPHRYOTROCHA NICHE HABITAT AFFINITY

ABSTRACT. - Information from 40 harbours was used to investigate the diversity and habitat affinities of Ophryotrocha species living in Central Mediterranean coastal habitats. Three environmental descriptors were considered: climate, boat traffic in harbours and fouling assemblages of port wharfs. Moreover, the diet was studied through the analysis of faecal pellets. The number of Ophryotrocha species did not vary among the climatic sub-areas, while the main, high traffic harbours exhibited a higher number of species with respect to the minor harbours. Ophryotrocha species can colonise all the fouling types considered, but the highest number of species was observed in mixed, heterogeneous assemblages. In most of the places investigated, two or more species co-existed on both harbour and wharf scales. The three most common species, O. labronica, O. puerilis (Mediterranean indigenous) and O. japonica (a non-indigenous species), differed in their affinities for climatic areas and harbour typology, but no significant preferences were observed with respect to the fouling types. The examination of faecal pellets from animals collected in the field suggested that all the species are omnivorous. Ophryotrocha species can live in very different types of harbour habitats. However, the ability to successfully colonise these environments varied strongly among species, perhaps because of their "history" (especially in the case of non-indigenous species), tolerance and habitat preferences/requirements.

# **INTRODUCTION**

In the fouling of Central Mediterranean harbour and brackish habitats, eight species of Ophryotrocha (Dorvilleidae: Eunicida: Polychaeta) can be found: O. adherens Paavo et al. 2000; O. diadema Åkesson 1976; O. hartmanni (Huth 1933), O. japonica nomen nudum (Pleijel & Eide 1996); O. macrovifera nomen nudum (Åkesson 1975); O. robusta nomen nudum (Åkesson 1975); O. labronica (La Greca & Bacci 1962); O. puerilis (Claparède & Mecznikow 1889). The two latter species are represented by the subspecies O. l. labronica Paxton & Åkesson 2007 and O. p. puerilis Paxton & Åkesson 2007 (Åkesson 1973, 1975, Simonini 2002, Åkesson & Paxton 2005, Simonini et al. 2009). O. japonica and O. diadema were considered to be non-indigenous species (NIS) native of the Pacific coasts (Åkesson & Paxton 2005, Simonini et al. 2009). The other Ophryotrocha species, which were originally described or occurred historically in the Mediterranean, are regarded as indigenous (native) species (Åkesson & Paxton 2005, Simonini et al. 2009). O. robusta and O. p. puerilis may be endemic to the Mediterranean. O. hartmanni is known from the Mediterranean and North Europe. O. l. labronica is very common in the Mediterranean and in the Lusitanian province: isolated populations have been found at Aqaba (Israel) and in Sydney harbour. *O. macrovifera* has also been sampled in some places along the United States coasts. *O. adherens* has been reported in Cyprus, the Canary Islands and Hawaii (Simonini *et al.* 2009).

*Ophryotrocha* is relatively well-studied in comparison to most annelid and marine invertebrate genera. In fact, these organisms have been the object of a relatively large number of biological investigations and are among the most-studied polychaetes (Paxton & Åkesson 2007, Simonini *et al.* 2009, Thornhill *et al.* 2009 and references therein). Nonetheless, because most of the research was performed using cultured strains under laboratory conditions, we have a limited understanding of various aspects of *Ophryotrocha* biology in the field, such as habitat requirements and diet (Thornhill *et al.* 2009).

A decade of surveys has enabled us to assess the distribution of all these species along the Italian coasts and to discuss some aspects of *Ophryotrocha* biogeography (Simonini *et al.* 2009). The sites investigated belong to different biogeographical sectors and climatic areas, and ranged from intercontinental ports to marinas (Fig. 1, Table I).

Intercontinental high-trafficked harbours are particularly subject to the introduction of non-indigenous species (NIS) (Occhipinti-Ambrogi 2007). In fact, in recent years, two NIS of *Ophryotrocha*, *O. japonica* and *O. dia*-

Table I. – Geographic localization and classification of the investigated harbours/wharfs. Main climatic areas (Area): $LS = Ligurian$ Sea, SS = southern Sicily, NA = North Adriatic Sea. Harbour traffic (Traffic): main: > 1 Mt y <sup>-1</sup> traded goods; minor: <1 Mt y <sup>-1</sup> traded goods and marinas. The dominant fouling categories found at site investigated for each harbour (indicated from 1 <sup>st</sup> to 4 <sup>th</sup> ) were classified as: A = ascidians; AL = algae, B = bivalves, B&A = bivalves and ascidians in association, S = serpulids, M = mixed). The harbours
ned as: A = ascidians; AL = algae, B = bivalves, B&A = bivalves and ascidians in association, S = serptings, M = mixed). The hardburs
where <i>Ophryotrocha</i> species were not found are highlighted in italics.

	Harbour	Coordinates		1 = 2 = 2	Harbour and wharf classification				
Abbr.	Name	Latitude N Longitude E		Area	Traffic	$1^{st}$	$2^{nd}$	$3^{rd}$	$4^{\text{th}}$
Ge	Genoa	44°24'51"	8°55'05"	LS	main	В	В		-
La	Lavagna	44°18'33"	9°20'06"	LS	minor	B&A	B&A		
LS	La Spezia	44°06'19"	9°49'48''	LS	main	B&A	В	В	Μ
PV	Porto Venere	44°03'01"	9°50'07"	LS	minor	B&A	B&A		
MC	Marina di Carrara	44°02'09"	10°2'26"	LS	main	В	В		
Vi	Viareggio	43°51'49"	10°14'28"	LS	minor	S	В		
Le	Leghorn	43°32'47"	10°18'03"	LS	main	В	B&A	А	
Со	Castiglioncello	43°23'46"	10°25'31"	LS	minor	В	B&A		
Cc	Cecina	43°18'12"	10°29'21"	LS	minor	S	S		
AC	Alghero/Calich	40°35'29"	8°17'14''	-	minor	B&A	B&A		
РТ	Porto Torres	40°50'26"	8°24'10"	-	main	В	B&A		
OSG	Oristano/Santa Giusta	39°52'20"	8°36'32"	-	main	В	B&A		
Ol	Olbia	40°55'12"	9°30'06"	-	main	В	B&A		
Pi	Piombino	42°55'48"	10°32'38"	-	main	М	В	В	
Cs	Castiglione	42°45'43"	10°52'50"	-	minor	В	B&A		
Or	Orbetello	42°26'19"	11°13'7"	-	minor	В	B&A		
Ci	Civitavecchia	42°5'44"	11°47'15"	-	main	B&A	B&A		
Тр	Trapani	40°49'44"	14°14'56"	SS	main	М	М		
PE	Porto Empedocle	38°00'49"	12°30'33"	SS	main	М	М		
Gl	Gela	37°17'7"	13°31'39"	SS	minor	А	B&A		
Li	Licata	37°03'57"	14°13'58"	SS	minor	М	B&A		
Me	Messina	37°05'32"	13°55'58"	SS	main	AL	AL		
PP	Porto Palo (Syracuse)	38°11'25"	15°33'29"	SS	minor	AL	AL		
Og	Ognina	36°40'10"	15°7'28"	SS	minor	S	B&A		
Sy	Syracuse	36°58'48"	15°15'25"	SS	minor	B&A	М	AL	B&
Ca	Catania	37°3'48"	15°17'24"	SS	main	B&A	B&A	B&A	B&
Au	Augusta	37°30'5"	15°5'51"	SS	main	B&A	S		
Cr	Crotone	37°13'39"	15°13'03"	-	minor	B&A	B&A		
TMP	Taranto/Mar Piccolo	39°05'01"	17°08'08"	-	main	B&A	А		
Br	Brindisi	40°28'50"	17°13'37"	-	main	S	B&A		
Ot	Ortona	40°38'02"	17°56'55"	-	main	В	B&A		
Ra	Ravenna	42°21'26"	14°24'32"	NA	main	B&A	B&A		
VC	Venice/Chioggia	44°27'27"	12°14'59"	NA	main	B&A	М		
Ce	Cervia	45°13'25"	12°16'50"	NA	minor	B&A	B&A		
Cn	Cesenatico	44°16'02"	12°21'20"	NA	minor	В	B&A		
Ri	Rimini	44°12'6"	12°23'49"	NA	minor	В	В	А	S
Ps	Pesaro	44°4'18"	12°34'20"	NA	minor	B&A	B&A		
Gr	Grado	43°55'23"	12°54'22"	NA	minor	А	B&A	B&A	B&
An	Ancona	45°40'56"	13°23'18"	NA	main	B&A	B&A		
Tr	Trieste	43°37'23"	13°30'17"	NA	main	B&A	B&A	B&A	B&

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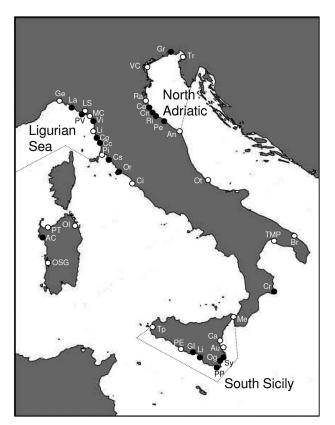


Fig. 1. – Study area and harbours considered for the analysis of the habitat requirements of *Ophryotrocha* species. White points = Main ports (> 1 Mt handled goods/year); black points = minor ports (< 1 Mt handled goods/year) and marinas. Harbours abbreviations, coordinates, and attribution to the different levels of considered environmental factors are reported in Table I. The dotted lines represent the borders of the three main climatic areas (Ligurian Sea, South Sicily, and North Adriatic Sea). Modified from Simonini *et al.* (2009).

*dema*, colonised Mediterranean harbour environments (Simonini 2002, Åkesson and Paxton 2005, Simonini *et al.* 2009). The introductions of NIS could be the result of anthropogenic dispersion by means of sea traffic. If this hypothesis was true, a greater incidence of NIS would be expected in the major harbours, which are characterized by intense international traffic, with respect to minor harbours, where most of the traffic is due to small fishing and pleasure boats. Moreover, if the NIS does not compete with the indigenous ones, more species in the major harbours would be expected.

The fouling communities in which *Ophryotrocha* species live can vary not only among harbours but also within the same port, because of the environmental heterogeneity and specificity of local situations (Cognetti 1992, Damianidis & Chintiroglou 2000, Karalis *et al.* 2003). *Ophryotrocha* species were found in fouling encrustations made by *Mytilus galloprovincialis*, bryozoans and ascidians (Parenti 1962, Prevedelli *et al.* 2005, Paxton & Åkesson 2007), but information on the capacity of *Ophryotrocha* to colonise different types of fouling microhabitats is not available.

The information on the diet of *Ophryotrocha* in the field is also quite scant. In their classic work on polychaete feeding guilds, Fauchald & Jumars (1979) reported that "*O. puerilis* may capture prey or feed on macerated meat while crawling on the surface of the mud in aquaria". This species also scrapes diatoms and filamentous algae growing on aquarium walls, using its jaws. Laboratory cultures of most species could be fed with different diets, such as diatoms, unicellular and multi-cellular green algae, cereals, spinach, freshly killed *Artemia* nauplii and dry fish food (Åkesson 1970, Fauchald & Jumars 1979, Prevedelli & Zunarelli 1998, Thornhill *et al.* 2009).

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In the present paper, the data obtained from surveys over the last decade (Simonini 2002, Simonini *et al.* 2009) were analyzed to asses whether the number of *Ophryotrocha* species (indigenous or NIS), incidence of each species and affinities of the most common species vary depending on three environmental descriptors: climate, boat traffic in harbours and fouling assemblages of wharfs. Moreover, the diet of the eight Mediterranean *Ophryotrocha* species was studied through the examination of faecal pellets. The objective is to improve the current knowledge concerning preferences and requirements, in terms of habitat type/conditions and food, of *Ophryotrocha* living in harbour environments.

### MATERIALS AND METHODS

Sampling: from 1999 to 2008, a total of 40 harbours (10-500 km away from each other) were investigated (Fig. 1, Table I). For each harbour, sampling was performed on at least two wharfs (100 m-2 km away from each other) which may differ (or not) in terms of macroscopic fouling assemblage composition, for a total of 95 wharfs (Table I). The density of most *Ophryotrocha* species varies with the season, but they can be found throughout the year: for example, in the harbour of La Spezia (Ligurian Sea), *O. labronica, O. japonica* and *O. puerilis* reached maximum densities in different seasons (autumn, winter and spring respectively but specimens of all these species were collected in all monthly surveys (with the exception of August for *O. puerilis*) (Prevedelli *et al.* 2005). The samplings were repeated at least twice for each site (with a maximum of 18 times at La Spezia 1 and 12 times at Ravenna 2), for a total of more than 100 surveys.

Samples of fouling were collected from stretches of wharfs (10-20 m length), by scraping vertical hard surfaces made of cement or cemented rocks. The samples were collected between the normal low tidal level and 1-1.5 m water depth, using a steel net with a cutting edge and extendible handle. The fouling samples were placed into small 2.5 dm<sup>3</sup> capacity aquaria filled with seawater. The aquaria were taken to the laboratory and kept at room temperature under light exposure for a few days. Anoxic conditions were established at the bottom of the tanks, forcing the animals to climb up the walls towards the light, where the organisms were collected using a Pasteur pipette. Then, the *Ophryotrocha* specimens were sorted individually, and identi-

fied to species level. More details on sampling, extraction and identification techniques, together with a description of the species found along the Italian coast, are reported in Prevedelli *et al.* (2005) and Simonini *et al.* (2009).

Analysis of faecal content: Preliminary investigations performed in September 2006 and July 2007 highlighted that at Porto Empedocle (PE, South Sicily, Fig. 1, Table I) six of the eight Mediterranean Ophryotrocha species co-occurred: O. adherens, O. diadema, O. hartmanni, O. labronica, O. puerilis and O. robusta (Simonini et al. 2009). Porto Empedocle provides a unique opportunity to investigate the diet of a relatively large number of Ophryotrocha co-existing at the same place and time. The sampling for the faecal pellet analyses at Porto Empedocle was carried out in July 2008. In addition, specimens of O. labronica and O. japonica from La Spezia (LS, Ligurian Sea, sampled in September 2007), and O. labronica, O. japonica and O. macrovifera from the Venice-Chioggia lagoon (VC, North Adriatic, sampled in October 2007) were also considered for diet examination. The faecal pellet analysis was performed by adopting the procedure used by Giangrande et al. (2000), to investigate the trophic requirements of syllid polychaetes in vivo, which was slightly modified and adapted for Ophryotrocha spp.. After extraction and identification, the Ophryotrocha specimens were transferred and isolated in clear jars containing filtered seawater (salinity 32-35) without a food source. In about 30 % of cases, the worms released the entire content of the intestine immediately after transfer. In this way, we had the opportunity to obtain faecal pellets which reflected the gut content. In order to prevent hungry worms from eating their faeces, the jars were checked every 2-3 hours and the pellets that had been excreted were collected. The faeces eliminated from 2-6 hours after isolation and those ejected immediately after the transfer were examined under a Zeiss Axioscope light microscope and a Nikon Eclipse 90i fluorescence microscope, which enabled the identification of the algae. The identification of feeding habits was performed in accordance with Giangrande et al. (2000) and Fauchald and Jumars (1979). In order to confirm that Ophryotrocha species feed on the matter occurring in the fouling of wharfs, within the sampling activity at Porto Empedocle, Chioggia and La Spezia, a fraction of fouling was washed in a bucket. Then, the material that did not pass through a 0.5 mm sieve was discarded. After decantation, the remaining fine sediment was subdivided, preserved at 4°C or frozen and given as food to the Ophryotrocha cultures.

*Classification and categorization of data:* The data on the occurrence of *Ophryotrocha* species were classified into different categories/levels of the investigated environmental descriptors (harbour climatic area and boat traffic, wharf fouling) according to published information and/or our own experience (Table I).

On most wharfs, the sessile macrofauna represented the most important part of the fouling, in terms of both biomass and cover. It consisted of bivalves (mainly *Mytilus galloprovincialis*, sometimes in association with *Mytilaster minimus* and *Ostrea edulis*), ascidians (*Styela plicata*, *Ciona intestinalis*, and *Botryllus* sp.),

serpulid polychaetes (e.g. Hydroides sp., Pomatoceros sp., Ficopomatus sp.) and cirripeds (Balanus sp.). Among the photophilic macroalgae, green algae (Ulva sp., Enteromorpha sp., Cladophora sp.), red algae (Corallina sp., Liagora sp., Pterocladia sp., Ceramium sp.), and brown algae (Cystoseira sp.) were also found. A large number of amphipods, isopods, ophiurans and polychaetes (nereidids, syllids, sabellids, dorvilleids, etc.) were living free in the fouling interstices. These fouling communities closely resemble the ones that are typically found in polluted and moderately polluted Mediterranean harbours (Relini 1993, Relini & Merello, 1999, Damianidis & Chintiroglou 2000, Karalis et al. 2003, Relini & Faimali 2003). The fouling assemblages on each sampling wharf were categorised depending on the dominance of one or more main sessile taxa: ascidians (A), algae (AL), bivalves (B), bivalves and ascidians in association (A&B), serpulids (S) and mixed (M), with the co-occurrence of serpulid, bivalves, ascidians and algae (Table I).

The harbours were classified on the basis of the port traffic, starting with the reports of the Italian Statistic Institute (ISTAT, http://www.istat.it/dati/dataset/20080429\_01/). The total traffic (national and international) generally increases with harbour size and percentage of international traffic. In accordance with ISTAT, we consider "main ports" to be the ones that trade more than 1 million tonnes (Mt) of goods every year. The other harbours and marinas were considered to be "minor ports" (Fig. 1, Table I).

The harbours considered in the present study belong to nine Mediterranean biogeographical sectors (BS, Bianchi 2007, Simonini et al. 2009). Yet, the extent of BS is very heterogeneous and the number of harbours varied among them. Moreover, several harbours lie closely to the borders between two or more adjacent BS, and climatic conditions vary gradually between adjacent BS, while geographically separated BS often exhibit very different climates. Consequently, the requirements of Ophryotrocha species in terms of climatic conditions were assessed by considering only data collected from three distinct areas: the Ligurian Sea (LS), southern Sicily (SS) and the Northern Adriatic Sea (NA). These three areas are separated by more than 1000 km and exhibit different climatic situations, as confirmed by the water temperatures observed during the sampling surveys. The NA coasts display a sub-continental temperate climate (mean annual temperature of 17°C), with cold winters (mean minimum temperatures of 6°C) and warm summers (mean maximum temperatures of 27°C). The LS coasts display a warm-temperate climate (mean annual temperature of 19°C), which is characterised by mild winters (12°C) and warm summers (28°C). The SS coasts have a sub-tropical temperate climate (mean annual temperature of 22°C), with milder winters (15°C) and hot summers (30°C) (Bianchi and Morri 2000, Peel et al. 2007; Simonini and Prevedelli 2003b).

*Data analysis:* The total number of *Ophryotrocha* species and the presence/absence of each species were estimated for each wharf/harbour depending on three environmental factors: climatic area (three levels: LS, SS, NA); harbour traffic (two levels: main, minor); fouling typology on the wharf (six levels:

Harbour	Indigenous species						Ν	IS
Harbour	O. adherens	O. hartmanni	O. labronica	O. macrovifera	O. puerilis	O. robusta	O. japonica	O. diadem
Ge	-	-	XXX	(-)	XXX	(-)	XXX	-
La	-	-	Х	-	Х	-	-	-
LS	-	Х	XXX	-	XXX	-	XXX	-
PV	-	-	Х	-	Х	-	Х	-
MC	-	-	XXX	-	Х	-	Х	-
Vi	-	-	XXX	-	-	-	-	-
Le	-	Х	XXX	-	XX	-	XX	-
Co	-	-	XX	-	-	-	-	-
Cc	-	-	-	-	-	-	-	-
AC	-	-	-	-	Х	-	-	-
PT	-	-	XX	-	Х	-	-	-
OSG	-	-	XX	-	Х	-	-	-
Ol	-	-	XX	-	Х	-	-	-
Pi	-	Х	XXX	-	XX	-	Х	-
Cs	-	-	Х	-	-	-	-	-
Or	-	-	Х	-	-	-	Х	-
Ci	-	-	XX	-	-	-	XXX	-
Тр	-	-	XXX	-	-	-	-	-
PE	XXX	Х	XXX	-	XXX	Х	-	XXX
Gl	-	-	XXX	-	-	-	-	-
Li	-	-	XXX	-	-	-	-	-
Me	-	-	-	-	-	-	-	-
PP	Х	-	XX	-	-	-	-	-
Og	-	-	-	-	-	-	-	-
Sy	-	-	XX	-	-	Х	-	-
Ca	-	-	XX	-	Х	Х	-	-
Au	-	-	XX	-	Х	-	-	-
Cr	-	-	XX	-	Х	-	Х	-
TMP	-	-	XX	-	-	-	XXX	-
Br	-	-	-	-	-	-	XX	-
Ot	-	-	-	-	-	-	XX	-
Ra	-	-	XXX	-	-	-	XXX	-
VC	-	-	XXX	Х	-	-	XXX	-
Ce	-	-	XXX	-	-	-	-	-
Cn	-	-	-	-	-	-	-	-
Ri	-	-	XXX	-	-	-	-	-
Ps	-	-	-	-	-	-	XX	-
Gr	-	-	XX	-	-	-	XX	-
An	-	Х	XX	-	Х	-	XX	-
Tr	-	-	Х	-	-	-	XXX	-
incidence								
Harbour	5 %	13 %	80 %	3 %	40 %	8 %	45 %	3 %
Wharf	3 %	5 %	78 %	1 %	26 %	5 %	41 %	2 %

Table II. –. Occurrence of *Ophryotrocha* species at investigated harbours (- = absent; (-) known only from historical data; X = less than 10 individuals; XX = 10-30 individuals; XXX = more than 30 individuals, data averaged among wharves and dates). The incidence of *Ophryotrocha* at both harbour and wharf scales is also reported in the bottom of the table. NIS = non-indigenous species.

A, AL, B, B&A, M, S). The information for each harbour were obtained through the aggregation of the data from the wharves belonging to the same harbour. Quantitative information was not available for most of the 2002-2006 surveys. Indication on the

average species densities found at each sampling site was given in Table II.

A preliminary analysis highlighted that the distribution of the number of species was extremely right-skewed, with a high fre-

quency of repeated values (most harbours/wharfs contained 1-2 species). Moreover, for some categories, the number of counts were low (< 5 counts). In these cases, re-sampling methods were often used as a robust alternative to inference based on parametric assumptions when their assumptions were in doubt (Efron & Tibshirani 1993, Simon & Oswald 1997). The percentile method was used to generate 95% confidence intervals for the number of species (total, only indigenous) for each level of environmental factors based on 2000 estimates, obtained by bootstrapping the original data. Permutation/randomisation procedures were used to test the null hypothesis that the Ophryotrocha species distribution did not vary according to climatic area, harbour traffic or wharf fouling type. In order to evaluate whether the native species were affected by the occurrence of NIS, the null hypothesis that the number of indigenous species is the same in harbours and wharfs in which NIS occurred or not, was tested. If the null hypothesis was true, the difference in the investigated statistics D (e.g. the absolute value of the differences in the mean number of species between major and minor ports) would have been be 0. The distribution of D was calculated by permuting the data between the level of the factor (e.g. permuting the harbours with their respective Ophryotrocha species number values between the two levels of the "traffic" factor), maintaining the sample sizes for each level. The whole calculation series was repeated 2000 times, to calculate the permutation distribution of D. Given the probability of type 1 error  $\alpha = 0.05$ , if the observed value of D was greater than (1-0.05) % of the value in the permutation distribution, the null hypothesis was rejected.

The incidence I and the affinity A observed for the species j with respect to a generic level k of the environmental descriptor f were calculated as:

 $I_{j,k,f} = P_{j,k,f} \ / \ N_{k,f}$ 

 $A_{j,k,f} = P_{j,k,f} / P_{j,f}$ 

where P was the number of wharfs/harbours where the species was present and N is the number of wharfs/harbours. Then, an affinity index AI was calculated as:

 $AI_{j,k,f} = A_{j,k,f} - EA_{j,k,f}$ 

where EA is the expected affinity, which was estimated as:

 $EA_{j,k,f} = N_{k,f} / N_f$ 

AI is based on the "electivity index" for selection of prey, which was initially proposed by Ivlev (1961) and modified by Strauss (1979). It defines habitat affinity based on the relative concentration of a species in a particular habitat (affinity), compared with the availability of that habitat in the study area (expected affinity). AI varies from -1 to +1 for a given species within a given habitat, where: -1 = complete absence from the habitat; 0 = neutral with respect to the habitat; +1 = exclusive presence in the habitat.

The permutation method was also adopted to test the significance of differences in the affinity index estimated for the three most common species. The other species were too rare to attempt the statistical analysis. Under the null hypothesis that a species had no particular preference for a climatic area, harbour and/or fouling typology, the observed and expected affinities are equal and the affinity index would be 0. The permutation tests were achieved by using the "Resampling Stats for Excel" software (http://www.resample.com/).

# RESULTS

#### Frequencies on harbour and wharf scales

One or more *Ophryotrocha* species were found in 90 % of the harbours and on 89 % of the investigated wharfs. Only in the main harbour of Messina (Me) and in the small marinas of Cecina (Cc), Ognina (Og) and Cesenatico (Cn) no *Ophryotrocha* were found (Table I).

At least one indigenous species occurred in more than 80 % of the investigated harbours and wharfs. The nonindigenous species (NIS) were also quite common (48 % and 43 % of investigated harbours and wharfs, respectively). The most common species was by far O. labronica, which was found on about 80 % of the investigated wharfs and harbours. The NIS O. japonica was present in 45 % of the harbours and 41 % of the wharfs. O. puerilis and O. hartmanni occurred in 40 % and 13 % of the harbours, respectively; however, they were rarer on the smaller scale because, in most of the harbours, they were not found on all the investigated wharfs. The other species were uncommon: O. robusta and O. adherens were only reported from southern Sicily, while O. macrovifera and the NIS O. diadema were only found in the Venice/ Chioggia lagoon and Porto Empedocle harbour, respectively (Table I).

## Harbour and wharf classification (expected affinities)

70 % of the harbours fit into the climatic areas of the Ligurian Sea, Northern Adriatic and southern Sicily: nine harbours (four main and five minor harbours) were investigated for each area.

Among the 40 investigated harbours, 21 (53 %) and 19 (47 %) were classified as "major" and "minor" harbours, respectively, and both types were distributed homogenously among the areas (Fig. 1, Table I).

On more than 52 % of the wharfs the fouling was composed rather exclusively of an association of bivalves and ascidians (B&A). Bivalve-dominated fouling communities (B) were found on 20 % of the wharfs. Serpulids (S), ascidians (A), and algae (AL)-dominated communities occurred on 7 %, 6 % and 5 % of the wharfs, respectively. On some wharfs (9 % of the total), mixed assemblages (M), with the co-occurrence of serpulids, bivalves, ascidians and algae, were found (Table I).

## Ophryotrocha diversity in terms of number of species

The mean number of *Ophryotrocha* species observed on harbour and wharf scales were 1.9 (lower and upper 95 % c.i.: 1.5-1.9) and 1.6 (lower and upper 95 % c.i.: 1.4-

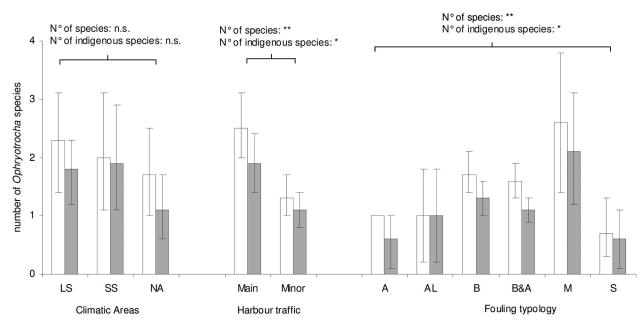


Fig. 2. – Number of species (mean and 95 % confidence intervals) of *Ophryotrocha* (total and indigenous only [in white and grey, respectively] observed in different climatic areas, harbour traffic and fouling typology (analyzed at harbour scale). Climatic areas: LS = Ligurian Sea; SS = south Sicily; NA = North Adriatic. Fouling typology: A = ascidians; AL = algae, B = bivalves, B&A = bivalves and ascidians in association, S = serpulids, M = mixed. Permutation test: n.s. = non significant; \* = p < 0.05; \*\* =p < 0.01; \*\*\* =p < 0.001).

1.9), respectively. The highest *Ophryotrocha* diversity in terms of number of species was observed at Porto Empedocle (PE), where 6 species co-existed on both investigated wharfs. Four species were found at the La Spezia (LS), Leghorn (Le), Piombino (Pi) and Ancona (An) harbours (Table I). In about 25 % of the harbours and on 40 % of the wharfs a single *Ophryotrocha* species occurred.

The number of total and indigenous *Ophryotrocha* species did not vary among the climatic sub-areas (Fig. 2). On the other hand, the main harbours exhibited a higher number of species with respect to the minor harbours, in terms of both total and indigenous taxa. *Ophryotrocha* species can colonise all the fouling types considered, but the number of species reported from mixed, heterogeneous assemblages was twice that found in assemblages that were strongly dominated by serpulid polychaetes, ascidians or algae (Fig. 2). The indigenous species did not seem to be affected by the occurrence of NIS: the number of native species was similar in harbours and wharfs whether NIS occurred or not (permutation test: harbours, p > 0.65; wharfs, p > 0.34).

## Species incidences and affinities

The incidence of *O. labronica* was high in all the climatic areas (78-89 %) and in main and minor harbours (86-74 %). It did not show preferences for climatic areas and harbour types: the affinity index ranged from -2 % to 3 % for each category (permutation test, p > 0.8, n.s.). This species colonised all the fouling typologies con-

sidered. In particular, *O. labronica* was found on all the wharfs characterised by mixed encrustation and ascidiandominated assemblages. It was very common in bivalve (incidence: 90%) and bivalve-ascidian (incidence: 73%) fouling types, while its incidence in algae and serpuliddominated communities was lower (60% and 43%, respectively) (Fig. 3).

The NIS *O. japonica* was common in the Ligurian and North Adriatic harbours, where its incidence was 56 % and 67 %, respectively, but it was never reported in southern Sicily. The incidence of *O. japonica* was about three times higher in the main harbours (72 %) with respect to the minor harbours (28 %). The affinity analyses confirmed that *O. japonica* had significant preferences for Ligurian and North Adriatic areas (AI=13 % and 22 %, respectively) and main harbours (AI = 23 %) respect to southern Sicily (AI = -35 %, p < 0,001) and minor ports (AI = -23 %, p < 0.05). This species colonised all the substrate types, with the exception of algal encrustations: the highest incidences were observed for ascidian, bivalve and bivalve-ascidian dominated communities (Fig. 4).

*O. puerilis* also showed significant affinities for specific sub-areas and harbours (p < 0.01 for both permutation tests): its incidence and affinity indices were high in the Ligurian area, intermediate in southern Sicily and very low in the North Adriatic. *O. puerilis* preferentially colonised the main harbours and was never found in ascidian (A) and algae (AL)-dominated fouling assemblages (Fig. 3).

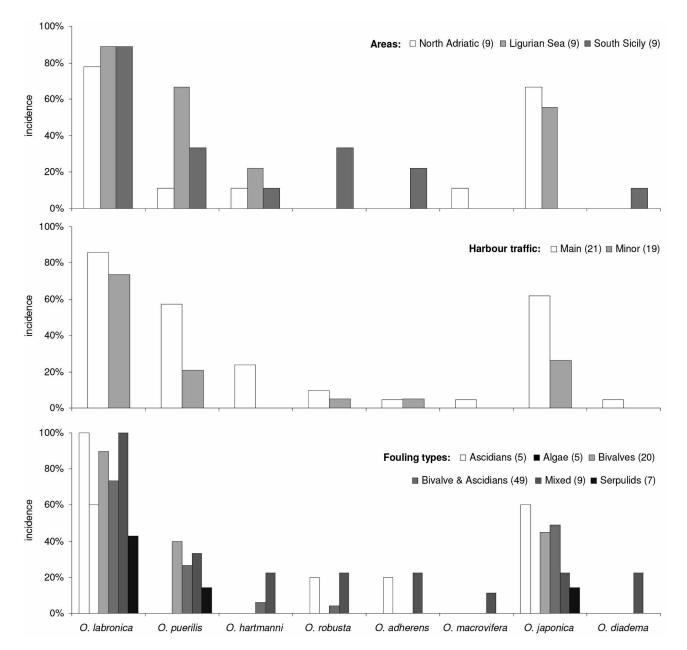


Fig. 3. – Incidence of *Ophryotrocha* species in different climatic areas, harbour traffic and fouling types. The number between parentheses indicates the replicates (number of harbours or wharfs for each group).

The other species, O. adherens, O. hartmanni, O. macrovifera, O. robusta and O. diadema, were rarer and were always found together with one or two of the most common species (Table I). Only O. hartmanni was present in all the sub-areas. This species was only reported in the main harbours, where it colonised mixed and bivalveascidian dominated substrates. O. robusta inhabited three types of substrate: algae and mixed, mixed (M), and bivalve-ascidian (B&A). Both O. diadema and O. macrovifera were only found in the heterogeneous fouling of a single main harbour, Porto Empedocle (the former) and Venice-Chioggia (the latter species). O. adherens colonised algae-dominated fouling at Porto Palo (minor harbour) and mixed assemblages at Porto Empedocle (Fig. 3).

# Diet

The faecal pellet composition was similar in the eight species of *Ophryotrocha* considered. The faecal pellets had a tubular shape and a length of 0.5-3 mm (Fig. 4). Partially or totally digested unicellular algae (diatoms and dinophyceans) could be recognised within the sediment and particulate detritus, which constituted most of the volume of the faecal pellets. Sometimes, fragments of multi-cellular algae, sponge spicules, compound chaetae

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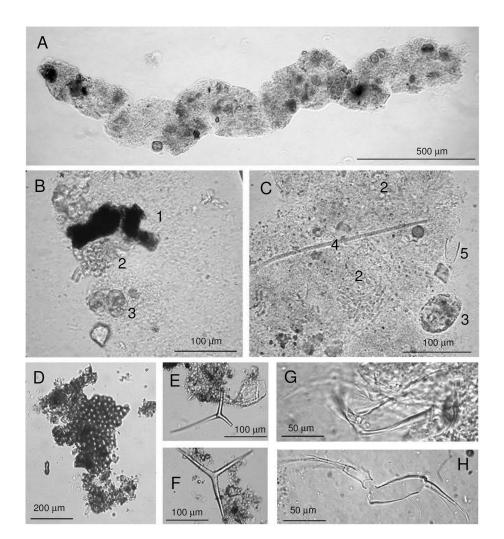


Fig. 4. – Faecal pellets from Ophryotrocha species. A. Entire faecal pellet ejected immediately after the transfer of a specimen of O. japonica from aquaria to the clean-seawater bowls, showing a large amount of sediment particles, grains and algae. B-H enlargement of different faecal pellets showing different food types. B-C: sediment grains (1), unicellular algae (diatoms [2] and dinophyceans [3]), Ophryotrocha chaetae (4); D: fragment of pluricellular alga; E-F: sponge spicules, G-H: fragments of crustacean exoskeletons.

of *Ophryotrocha* and remains of crustacean exoskeletons were observed. A large amount of live ciliates was present inside and at the edges of faecal pellets of all the species. Under laboratory conditions, individuals of the different species that were fed with the fine sediment collected from the field survived and reproduced successfully (Fig. 4).

# DISCUSSION

The results of the present study highlighted that the number of indigenous species was not affected by the occurrence of NIS, and in most of the investigated places two or more species co-existed. Under laboratory conditions, competitive exclusion among species often occurs: cultures initially composed of *O. labronica* and *O. japonica* or *O. japonica* and *O. puerilis*, over time ended up being dominated only by the non-indigenous species *O. japonica* (Prevedelli *et al.* 2005); moreover, *O. labronica* often overweighted *O. puerilis* in mixed laboratory cultures (Simonini personal observation). The co-existence of several species of *Ophryotrocha* in the field

could be due to the fact that harbours are r-selective environments, where resources are plentiful and abiotic conditions are more important than biotic interactions (Cognetti 1992, Çinar *et al.* 2008), and to the variability of environmental conditions such as temperature (Prevedelli *et al.* 2005). Moreover, the maximum density of *Ophryotrocha* observed in the field (less than 200 ind. kg<sup>-1</sup> of fouling) is two-three orders of magnitude less than in laboratory cultures (Prevedelli *et al.* 2005).

Abiotic factors, such as harbour traffic and fouling typology, seemed to affect the number of *Ophryotrocha* species on a smaller scale (harbour/wharf scales). In actual fact, differences between the levels of the environmental descriptors considered (climatic areas, harbour traffic and wharf fouling) were low in absolute terms (1-2 species), but this disparity appeared to be important in biological terms because of the relatively low number of species found throughout the investigated area.

On the small scale of wharfs, it was found that fouling assemblages dominated by one of few major macrobenthic taxa hosted fewer *Ophryotrocha* species with respect to the mixed ones. In fact, all the species exhibited relatively high affinities for heterogeneous fouling assemblages, and some of the rarer species, such as *O. diadema* and *O. macrovifera*, were only collected from this type of substrate. Heterogeneous, complex communities often exhibit high-polychaete species richness and diversity and host a large number of occasional and rare species (Somaschini *et al.* 1997, Serrano & Preciado 2007). On the other hand, communities dominated by serpulids, which often occur in the early colonization phase of hard substrata and/or are indicative of high environmental instability (Chalmer 1982, Marzialetti *et al.* 2009), exhibit the lowest number of *Ophryotrocha* species.

High-traffic harbours showed a relatively high number of Ophryotrocha. The NIS Ophryotrocha (especially *O. japonica*) has a particularly high occurrence in these environments. As pointed out above, the main harbours are more exposed to the introduction of NIS with respect to minor ports because of the relatively high level of international traffic, both in relative and absolute terms, which increases the probability of NIS being successfully transported, released and settled into the new environment. NIS have been implicated in the decline of density and distribution of several native species, leading to biodiversity loss and potential ecosystem unbalancing (Occhipinti Ambrogi & Savini 2003, Occhipinti Ambrogi 2007). Yet, at least in some areas, NIS may have been additions rather than the cause of the native species displacement (Reise et al. 1999, Occhipinti Ambrogi & Savini 2003). Stressed environments such as harbours and lagoons are relatively poor in species and are easily colonised by NIS (Wolff 1999, Cognetti & Maltagliati 2000). If the new species did not interfere with the native ones (e.g. occupying free niches or creating biogenic structures), the diversity of receiving habitats may increase (Occhipinti Ambrogi 2007). Accordingly, in some harbour environments, positive correlations between the abundance and species richness of NIS and native species have been observed (Ranasinghe et al. 2005, Cinar et al. 2006, 2008).

The NIS O. japonica preferentially colonised the main, high-traffic intercontinental harbours. Despite O. japonica being widespread along the Italian peninsula, it was not present in Sicily and Sardinia, possibly because on these islands most of the harbour traffic is headed for Italy (ISTAT port traffic statistics). O. japonica also occurred in several minor ports, which could have been colonized secondarily by means of local transport/dispersal processes (Colautti et al. 2006, Occhipinti Ambrogi 2007). O. japonica was very common along the North Adriatic and Ligurian coasts but it was never found in the relatively warm climatic area of Southern Sicily. Yet, strains of O. japonica were also collected from Ionian Sea harbours (Fig. 1, Table I; Simonini & Prevedelli 2003a), where the climatic regime is similar to the one found in Sicily. Thus, the distribution of O. japonica did not appear to be affected by the climatic conditions of the three examined areas. O. puerilis is very common in harbours on the temperate Ligurian Sea while it is very rare in the cold North Adriatic Sea. Temperature can affect survival and growth rates of *O. puerilis* (Sella 1978, Levinton & Monahan 1983), but detailed studies on the temperature limits of *O. puerilis* have not been performed. Yet, Åkesson (1973) reported that the development of larvae did not take place at temperatures below 10°C: it is possible that the North Adriatic is an unsuitable habitat for *O. puerilis* because of the low surface water temperature during winter (sometimes less than 4°C, Simonini & Prevedelli 2003b). On the other hand, *O. labronica*, which is considered a euritherm and eurihalin species (Åkesson 1975, 1976, Prevedelli & Simonini 2001), was able to colonise main harbours as well as small ports and marinas. Moreover, it exhibits high incidence in all three of the climatic areas and can live in the interstices of all types of fouling considered.

Demographic analyses performed under laboratory conditions suggested that, in the genus *Ophryotrocha*, gonochorism is more advantageous than hermaphroditism (Prevedelli *et al.* 2006). Accordingly, the present results highlighted that the two most common species, *O. labronica* and *O. japonica*, are gonochoric, while the simultaneous hermaphrodite *O. hartmanni*, *O. adherens* and *O. diadema* occurred in few harbours.

Some studies on the diet of polychaetes combine the examination of stomach content and the analysis of stable isotopes and/or fatty acids (e.g. Hentschel 1998, Bischoff *et al.* 2009). The former technique was also used to investigate the diet of *Ophryotrocha* from seep- and ventsites (Levin *et al.* 2009). Yet, when the different types of food are easily recognizable, the analyses of faecal pellets excreted immediately or within a few hours from live worms, which had just been collected from the field, can give an adequate overview of the diet (Giangrande *et al.* 2000, Caron *et al.* 2004).

The examination of faecal pellets from Ophryotrocha collected in the field suggested that the feeding requirements of the eight species are very similar. They may be considered to be a detritivorous species because of the presence of a large amount of sediment and particulate inside faecal pellets (Giangrande et al. 2000). Yet, the occurrence of unicellular algae, spicules and fragments of macroalgae and crustaceans suggest that Ophryotrocha species are omnivorous. Accordingly, in the laboratory, all species can be maintained for long periods by using various plant or animal-derived diets (Åkesson 1970, Prevedelli & Zunarelli 1999). The occurrence of relatively large fragments of food may be explained by the scraping-grazing action of the maxillary plates and jaw. Cannibalism or predation among Ophryotrocha has never been observed, even in dense, starved laboratory cultures (Simonini personal observation). Yet, a number of Ophryotrocha chaetae were observed in faecal pellets produced by both field-collected and cultured worms. These chaetae often remain attached to the surface of mucous masses which contain Ophryotrocha eggs, and can be ingested when the worms graze on the masses to care for

the embryos during their development (Simonini personal observation).

The present results suggest that *Ophryotrocha* can live in very different types of harbour habitats. Yet, the ability to successfully colonise these environments varies strongly among species, in accordance with their "history" (especially in the case of NIS), tolerance and habitat preferences/requirements. The findings of the present study could be used to extend the potential application of this model organism to new ecologically relevant questions, such as those concerning the mechanism underlying the introduction of allochthonous species into aquatic environments, the evolutionary and ecological implications of sexuality and the effects of climate change on marine organisms (Prevedelli *et al.* 2006, Massamba N'Siala *et al.* 2007, Lorenzi *et al.* 2008, Thornhill *et al.* 2009).

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