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An optimised multi-host trematode life cycle: fishery discards enhance trophic parasite transmission to scavenging birds ☆

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ABSTRACT

Overlapping distributions of hosts and parasites are critical for successful completion of multi-host parasite life cycles and even small environmental changes can impact on the parasite's presence in a host or habitat. The generalist *Cardiocephaloides longicollis* was used as a model for multi-host trematode life cycles in marine habitats. This parasite was studied to quantify parasite dispersion and transmission dynamics, effects of biological changes and anthropogenic impacts on life cycle completion. We compiled the largest host dataset to date, by analysing 3351 molluscs (24 species), 2108 fish (25 species) and 154 birds (17 species) and analysed the resultant data based on a number of statistical models. We uncovered extremely low host specificity at the second intermediate host level and a preference of the free-swimming larvae for predominantly demersal but also benthic fish. The accumulation of encysted larvae in the brain with increasing fish size demonstrates that parasite numbers level off in fish larger than 140 mm, consistent with parasite-induced mortality at these levels. The highest infection rates were detected in host species and sizes representing the largest fraction of Mediterranean fishery discards (up to 67% of the total catch), which are frequently consumed by seabirds. Significantly higher parasite densities were found in areas with extensive fishing activity than in those with medium and low activity, and in fish from shallow lagoons than in fish from other coastal areas. For the first time, *C. longicollis* was also detected in farmed fish in netpens. Fishing generally drives declines in parasite abundance, however, our study suggests an enhanced transmission of generalist parasites such as *C. longicollis*, an effect that is further amplified by the parasite's efficient host-finding mechanisms and its alteration of fish host behaviour by larvae encysted in the brain. The anthropogenic impact on the distribution of trophically-transmitted, highly prevalent parasites likely results in a strong effect on food web structure, thus making *C. longicollis* an ideal bioindicator to compare food webs in natural communities versus those impacted by fisheries and aquaculture.

1. Introduction

Trophically transmitted parasites are central elements in most aquatic food webs (Lafferty et al., 2006, 2008) and exert strong effects on host community and food web structures when infecting a high number of host species (Thompson et al., 2005). Such parasites can manipulate the behaviour of intermediate hosts, fundamentally altering the patterns of contact between predator and prey. In gammarids, infection by the acanthocephalan *Echinorhynchus truttae* alters both attack rates for predators and consumption of prey, with a 30% increase in maximal predation rates (Dick et al., 2010). Changes in predator–prey links can strongly impact food web structure and dynamics, thereby altering energy flow through the food chain (Marcogliese, 2004; Lagrue et al., 2011). In multi-host parasites, host distribution strongly affects parasite transmission and successful life cycle completion (Esch and Fernandez, 1994; Fredensborg et al., 2006). Nowadays, marine habitats are influenced considerably by human activities such as fishing or aquaculture (e.g. Edgar et al., 2005; Grigorakis and Rigos, 2011). The impact of fisheries on the distribution and transmission of parasites that use fish as one of several hosts has

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☆ Nucleotide sequence data reported in this paper are available in GenBank™ under the accession number KT454991.

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been little studied. Wood et al. (2010) hypothesised that fishing would drive a long-term, global decline in fish parasites due to the reduction of host density, food web complexity and thus parasite transmission efficiency; however, the response of parasites to fishing depends on their life cycle strategy and the fishing status of the host (Wood and Lafferty, 2015). On the other hand, discards from fishing, which are known to attract piscivorous birds and other wild animals that serve as final hosts (Oro and Ruiz, 1997; Morton and Yuen, 2000; Arcos et al., 2001; Bozzano and Sardà, 2002), may result in increased parasite transmission. However, in the long run discards affect fish host distribution and availability (e.g. Tasker et al., 2000; Coll et al., 2008).

Here, we investigate the case of the trematode *Cardiocephaloides longicollis* (Rudolphi, 1819) Dubois, 1982 (Strigeidae), which is widespread along the Mediterranean coast. *Cardiocephaloides longicollis* has a complex life cycle. Adult parasites colonise the digestive tract of their definitive hosts, predominantly seagulls. Eggs are released via the host's faeces and drop to the seafloor where free-swimming miracidia hatch. Following its burrowing entry into the first intermediate host, nassariid snails, *C. longicollis* proliferates by massive asexual reproduction within sporocysts and forms actively swimming cercariae as the next transmission stage. Cercariae emerge from the snails and penetrate the skin of their second intermediate host, fishes. Thereafter, cercariae migrate to and encyst in the fish brain as metacercariae (Prévot and Bartoli, 1980). *Cardiocephaloides longicollis* is trophically transmitted to the definitive host when infected fish are consumed by seabirds (Bartoli and Prévot, 1986; Fredensborg and Longoria, 2012).

Parasites use a variety of transmission strategies to ensure the successful completion of their life cycle. Cercariae of *C. longicollis* overcome the distance between their benthic snail hosts and their target fish hosts, and disperse by following a strategy of alternating swimming and resting periods, using positive phototaxis for orientation (Bartoli and Prévot, 1986; Combes et al., 1994). Metacercariae of *C. longicollis* infect the optic lobes of the fish's mesencephalon (Prévot and Bartoli, 1980; Osset et al., 2005; Bartoli and Boudouresque, 2007), causing a fundamental change in host behaviour due to impaired vision and motor control (Prévot, 1974. Recherches sur les cycles biologiques et l'écologie de quelques trématodes nouveaux parasites de *Larus argentatus michaelis* Naumann dans le midi de la France. Thèse Université d'Aix-Marseille, France; Barber and Crompton, 1997). Infected fish dwell in the upper part of the water column and show body oscillations that make their bright flanks visible to flying birds (Osset et al., 2005; Bartoli and Boudouresque, 2007), and are thus more prone to predation (Poulin, 2001; Seppälä et al., 2004; Osset et al., 2005; Fredensborg and Longoria, 2012). In other trematode species, the number of metacercariae located in the eye or the brain of fish has been directly related to the degree of conspicuous behaviour induced (Barber and Crompton, 1997; Fredensborg and Longoria, 2012). In this scenario, anthropogenic influences such as aquaculture and fisheries are of special importance as they may further enhance the transmission of the parasite to their final hosts. Osset et al. (2005) first hypothesised that an increased transmission of *C. longicollis* could occur in areas of intensive commercial fishing, since discards are frequently consumed by seabirds (Witt et al., 1981; Christel et al., 2012). Subsequently this would result in increased egg release from final hosts and, long term, in a general increase in parasite biomass in the ecosystem.

We focused on *C. longicollis* as a model for studying the influence of biological and anthropogenic factors on generalist multi-host life cycles in coastal areas. Our aim was to provide a comprehensive survey of the occurrence of *C. longicollis* along the Mediterranean coastline, a better understanding of its life cycle strategies and the impact of anthropogenic activities on trematode transmission and abundance. First, the host spectrum used by *C. longicollis* was determined by assessing its occurrence and degree of specialisation at all levels, i.e. in snail, fish and bird hosts. Secondly, the marine microhabitat targeted by cercariae was determined by comparing infection levels in benthic, demersal and pelagic fish species. Thirdly, the effect of fish size on the accumulation of *C. longicollis* metacercariae was analysed, evaluating the possible implications of different parasite loads. Finally, it was tested whether increased transmission of *C. longicollis* occurs in areas of intensive commercial fishing as well as in aquaculture installations, and the impact of these human activities on trophically transmitted parasites, their hosts and the food web, was discussed.

2. Materials and methods

2.1. Sampling sites and habitat definition

An opportunistic sampling strategy was adopted for this study, focussing on the examination of a diverse range of hosts suspected to be part of the life cycle of *C. longicollis*. All sampling sites are indicated in Fig. 1 and Supplementary Tables S1–S3. Molluscs were hand-collected in shallow areas that are likely important for *C. longicollis* transmission but differ in the intensity of local anthropogenic activities. Fish species were sampled in 14 localities along the western Mediterranean coast (Spain) and in three lagoons in southern Sardinia (Italy) (see Fig. 1), including one *Sparus aurata* aquaculture site (netpens). The largest number of fish individuals and species were collected in Carboneras and Santa Pola (Spain), areas of intensive fishing and aquaculture activities. Demersal, benthic and pelagic fish (caught by trawling, netting and trapping) were obtained from commercial fishers or fish markets. Birds were obtained from bird sanctuaries, one in the Ebro Delta and one in Valencia (Spain) (approximately 200 km apart). Birds move over large distances so that their site of capture is not indicative of their distribution and feeding sites. No formal ethics approvals were required for this as both fish and bird hosts were obtained post-mortem.

2.2. Host taxa and screening methodology

A total of 3351 molluscs belonging to 24 species and 18 families (Supplementary Table S1) were collected and screened for sporocysts and mature cercarial infections using standardised methods (see Born-Torrijos et al., 2014). A total of 2108 fish belonging to 25 species and 10 families was examined for the presence and quantity of metacercariae of *C. longicollis* in the brain (Supplementary Table S2). Fish were measured and weighed before dissection. Two samples (see Supplementary Table S2) were examined as a pool, for which the total number of parasites was determined after chloropeptic tissue digestion. The bird sanctuaries provided 154 birds belonging to 17 species and four families (Supplementary Table S3), which were frozen until necropsy. The alimentary tract (from oesophagus to cloaca) was removed and examined for adult specimens of *C. longicollis*. Given the opportunistic nature of sampling, sample sizes of some species are low.

2.3. Parasite identification

All life cycle stages of *C. longicollis*, i.e. adults as well as sporocysts, cercariae and metacercariae, were identified following the descriptions by Dubois (1968) and Prévot and Bartoli (1980), and preserved in 100% ethanol for molecular analyses. Eight random samples of metacercariae from *Dentex dentex*, *Chromis chromis*, *Diplodus puntazzo* and *Lithognathus mormyrus*, cercariae from *Nassarius reticulatus* and *Cyclope neritea*, and adults from *Larus*

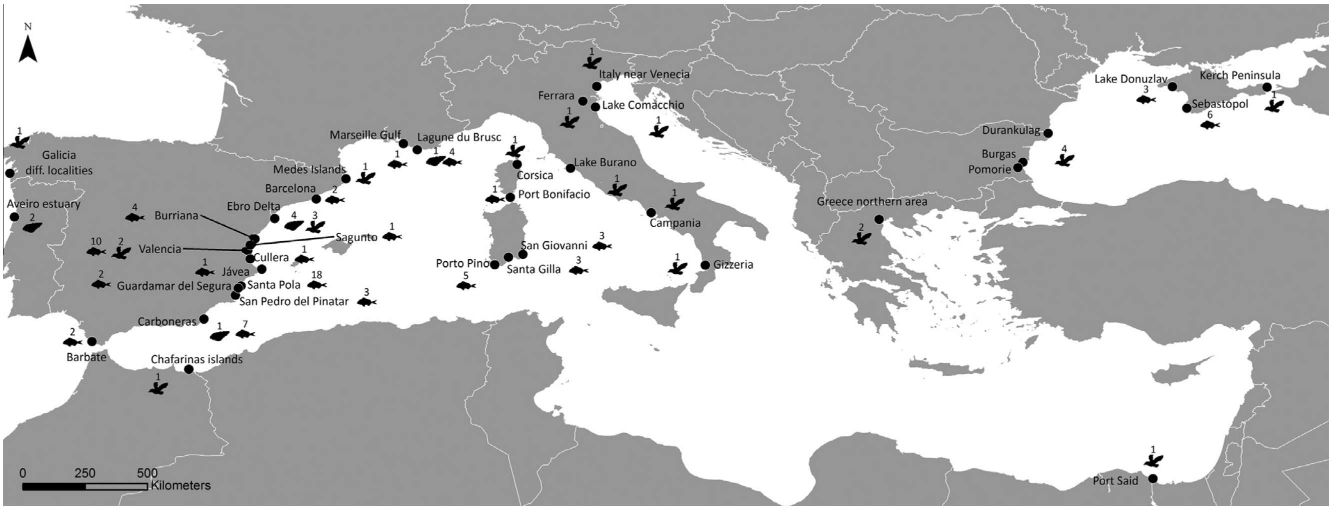

Fig. 1. Map showing the geographical distribution of *Cardiocephaloides longicollis* in Mediterranean and Black Seas and reports of it in different hosts, including published data and those of the present study. The silhouettes represent reports of infection in fish, seagulls and gastropods, and numbers indicate the numbers of infected host species that have been found in the sampling localities.

michahellis were analysed by rDNA sequencing, to confirm microscopic parasite identification. DNA extraction, 28S rDNA amplification and sequencing was performed as described in Born-Torrijos et al. (2012). For additional internal transcribed spacer region 2 (ITS2) rDNA amplification and sequencing of the adults, primers 3S (forward 5′-GGT ACC GGT GGA TCA CGT GGC TAG TG-3′) (Morgan and Blair, 1995) and ITS2.2 (reverse 5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′) (Cribb et al., 1998) were used with an annealing temperature of 54 °C for 50 s.

2.4. Statistical analyses

In all hosts, infection prevalence (percentage of infected hosts in a sample), mean intensity and range (number of parasite individuals in an infected host), as well as mean abundance (±S.D.) (average number of parasite individuals per host in a host population) were calculated (Bush et al., 1997). The data acquired in the present study were combined with published data from existing records, obtained through a search of the Web of Science (ThomsonReuters, https://webofknowledge.com/) (search string: Cardiocephal∗), expanded by a manual bibliographic search of references in publications from the primary search. For statistical analyses, only true zero prevalences were included, i.e. the local prevalence is zero but there are existing records that show this host species can actually be infected by the studied parasite. Nevertheless, host species examined in this study or in the existing literature found to be uninfected were added to Supplementary Table S2, to indicate host taxa where *C. longicollis* has never been found. In order to estimate whether fish habitat (benthic, demersal and pelagic; information based on FishBase (Froese and Pauly, 2015. FishBase. World Wide Web electronic publication. www.fishbase.org, version (02/2015)) is related to changes in prevalence of *C. longicollis*, the data were analysed using generalised linear mixed models (GLMMs). The effect of season of fish capture was also investigated, but not explored here (see Supplementary Data S1, Supplementary Fig. S1). To check for congruent patterns in prevalence or abundance depending on fish habitat, our data combined with previously published records were analysed with different linear models (LMs). To examine possible differences in *C. longicollis* prevalence in fish in relation to varying fishing pressures in the Mediterranean, GLMMs were performed. The fishing activity of the sampling areas was estimated on the basis of the hold capacity of vessels from the nearest harbour, i.e. the fishing fleet size between 2010 and 2014, as presented in the European Atlas of the Seas (Directorate General for Maritime Affairs and Fisheries of the European Commission, DG MARE, http://ec.europa.eu/maritimeaffairs/atlas/maritime_atlas/#lang=EN;bkgd=6:0.5;mode=1;pos=11.535:51.462:5;theme=63:0.9:1;time=2014; last accessed 17/03/2016), measured in gross tonnage (GT, i.e. as an indicator of the fishing fleet capacity or the fleet's overall internal volume for fish captured). This is the only measure of total fishing activity available across all ports (different countries and reporting regulations) that is reported to the European Commission (EC). Three categories of GT were used, i.e. low hold capacity, <3000 GT; medium hold capacity, 3000–12,000 GT; high hold capacity, >12,000 GT. To study the influence of fisheries on parasite transmission in more detail, we aimed to compare discard data (host species, size, etc.) from different ports with the parasite data from the present study. However, while the Secretaría General de Pesca (Spanish Ministry of Agriculture, Food and Environment) kindly provided data on landings, discards are not officially recorded by either national or international organisations such as the Food and Agriculture Organization of the United Nations (FAO) but are only reported occasionally in individual local studies (Pauly et al., 2014). The latter demonstrate that the amount of discards shows great variability in terms of weight and species composition (e.g. Sánchez et al., 2004), or even depending on the season of capture, fishing strategy and number of vessels (e.g. Arcos et al., 2001; Tzanatos et al., 2007; Coll et al., 2014), and it is often not proportional to the landed tonnage. As a concrete example for more in-depth analysis we selected local discard data from a heavily fished area that overlaps geographically with our dataset (García-Rivera et al., 2015), to infer the amount of *C. longicollis* metacercariae that may be discarded (see Discussion).

Fish infection data were also analysed for differences between shallow lagoons and deeper coastal waters, using generalised linear models (GLMs). The correlation between the prevalence and mean intensity of *C. longicollis* infection was calculated for all fish samples, using a Spearman rank correlation. To investigate the relationship between fish length and *C. longicollis* abundance, two subsets of 198 *Diplodus vulgaris* and 196 *Diplodus annularis* were used. Fish size data were stratified into size classes and the effect of size on the number of metacercariae per individual fish was analysed using a negative binomial GLM. Thereafter, to detect potential parasite-induced host mortality, the degree of aggregation was calculated for each host size class using the index of discrepancy (Poulin, 1993), using the software package Quantitative

Parasitology (QP web, powered by R, Version 1.0.9) (Reiczigel et al., 2013, http://www2.univet.hu/qpweb, last accessed 17/03/2016). Similar to fish data, bird data were analysed for the correlation between parasite prevalence and intensity. All analyses were conducted in R (R Core Team, version 3.0.1: R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, http://www.R-project.org, last accessed 17/03/2016) and detailed information on all models and conditions can be found in Supplementary Data S1.

3. Results

3.1. *Cardiocephaloides longicollis* hosts

In molluscs, the occurrence of *C. longicollis* was restricted to nassariid snails, with only one new host record, *Cyclope neritea*, added to the previously recognised host species, *Nassarius corniculum* and *N. reticulatus* (Fig. 2). This indicates a high degree of host specificity at this level. Prevalence was relatively low in *C. neritea* (0.65% in the Ebro Delta, and one of two snails in Carboneras), whereas in the more abundant *N. reticulatus*, the prevalence was higher with 8.3%, exceeding all previous records of *C. longicollis* in mollusc hosts. Our data demonstrate that *C. longicollis* has a much wider host spectrum in the second intermediate host than previously reported, by adding 12 new host records: *Diplodus sargus*, *D. dentex*, *Spicara maena*, *Spondyliosoma cantharus*, *Pagellus acarne*, *Pagellus erythrinus*, *Pagellus bogaraveo*, *Oblada melanura*, *Zosterisessor ophiocephalus*, *Coris julis*, *C. chromis*, *Serranus scriba*. This includes the Sparidae, Gobiidae and Labriidae as previously recorded host families and the Pomacentridae and Serranidae as new host families, resulting in a total of 31 fish host species from nine fish families (see Supplementary Table S2 and references therein). For the first time, *C. longicollis* was detected in *S. aurata* from aquaculture netpen facilities, located in the southwestern Mediterranean region, with 53.9% prevalence. Wild *S. aurata* from lagoons in Italy showed similar infection prevalence (40–62.5%). With regard to final hosts, the infection levels found in three larid species, i.e. within the gull family, were high with ⩾50% prevalence and thus up to 22 times higher than in previous reports (Supplementary Table S3). However, *C. longicollis* was absent from terns and smaller larid species in the present study, although these had been reported as hosts in previous studies (see Supplementary Table S3).

Sequence identity of 28S rDNA sequences of *C. longicollis* with a previously published record (GenBank accession number AY222171) was 100% (bp compared: 1307 bp of *D. dentex*, 1127 bp of *C. chromis*, 437 bp of *D. puntazzo*, 607 bp of *L. mormyrus*, 1216 bp of *N. reticulatus* and 1097 bp of *C. neritea*). An ITS rDNA sequence of adults was deposited in GenBank (accession number KT454991).

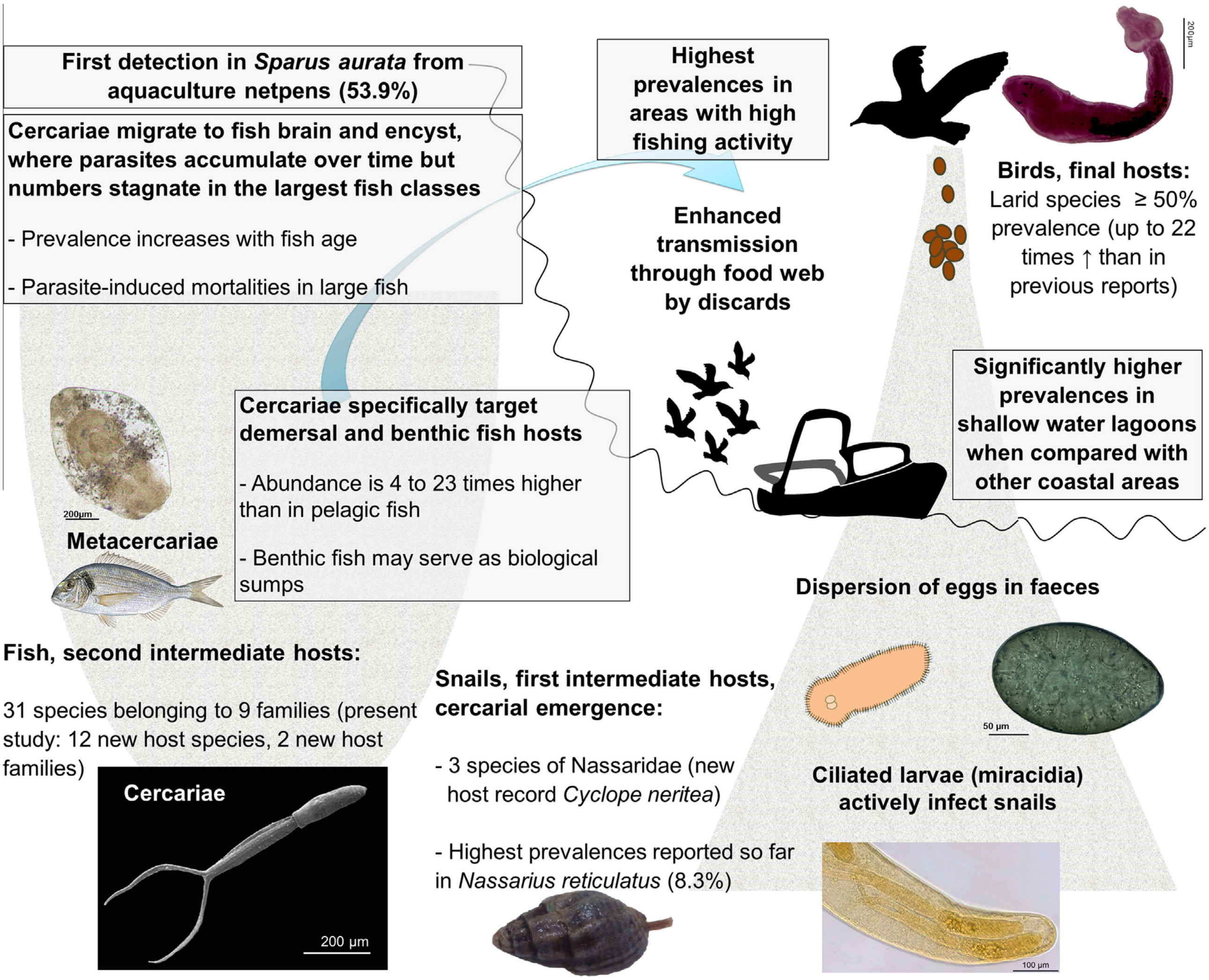

Fig. 2. Complex life cycle of *Cardiocephaloides longicollis* including the main factors that impact parasite transmission, as revealed by the present study.

3.2. Effects of habitat on infection levels in fish hosts

Using different statistical models, relationships between *C. longicollis* infection levels and biological as well as anthropogenic factors were estimated. Both prevalence and intensity of infection were used as indices for parasite quantity and we detected a strong positive correlation between these two parameters across all samples in fish (Supplementary Fig. S2), irrespective of whether data from the literature was included ($r_s = 0.69$, $P < 0.001$, $n = 51$) or not ($r_s = 0.76$, $P < 0.001$, $n = 40$), thus reflecting the consistency between indices across samples. When analysing this correlation with regard to habitat type, we found that parasite prevalence and intensity showed high variability in demersal fish, in contrast to pelagic and benthic hosts, with a medium prevalence in pelagic fishes and a high prevalence in benthic ones. Thus, overall, demersal samples showed the highest and most diverse infection levels.

No significant differences in parasite prevalences between habitats were found (GLMM, benthic $P = 0.1959$, demersal $P = 0.2245$ when compared with pelagic), possibly due to the heterogeneous infection levels in demersal and pelagic habitats, and due to a single benthic sample included in the GLMM, i.e. *Z. ophiocephalus* (GLMM, proportion of variance explained by random effect 'fish species' = 72%, Supplementary Table S4). However, demersal and benthic habitats showed higher parasite prevalence and mean abundance when ignoring the fish species effect (Table 1, Supplementary Fig. S3), with no significant differences between these two habitats. Including both new and previously published data, the mean abundance was approximately four times greater in demersal and approximately 23 times greater in benthic fish than in pelagic fish (Table 1). The difference in prevalence, although not so great, is still considerable, being 38.3% and 131.6% greater in the demersal and benthic habitats, respectively, compared with the pelagic one. Generally, pairwise comparisons were concordant with linear model results.

3.3. Factors causing increased parasite occurrence in fish

Cardiocephaloides longicollis infection levels did not differ substantially between sites, when comparing data on the same fish species captured at different sites with similar rates of fishing activity (*D. vulgaris*, *S. aurata* and *L. mormyrus*; Supplementary Table S2). *Lithognathus mormyrus* generally showed high infection rates, however the maximum of 100% prevalence was reached only in an area characterised by aquaculture netpens. Areas with intensive fishing and aquaculture activities such as Santa Pola and Carboneras showed high mean intensities of infection (up to 26 metacercariae per fish). Overall, the highest prevalences of *C. longicollis* occurred in fish captured close to harbours frequented by vessels with high fish hold capacities, being significantly higher than in harbours with low and medium fish hold capacity (74.7% in both cases; GLMM and pairwise comparisons, $P < 0.001$; Fig. 3A, Supplementary Table S5), which show no significant differences from each other ($P = 0.995$, Supplementary Table S5). Furthermore, shallow areas (lagoons) were identified as sites with high parasite numbers in fish, with mean infection intensities of 19.8–49.9 in the Italian lagoons. The highest *C. longicollis* infection rates were detected in snails from the Ebro Delta lagoon (up to 8.3%) and were found to be significantly higher in fish (40% to 100%) from lagoons than in fish from other coastal areas (GLM, $P = 0.014$, Fig. 3B, Supplementary Table S5).

3.4. Accumulation of *C. longicollis* metacercariae with host size

In *D. vulgaris*, both parasite intensity and abundance showed a tendency to increase with host size, but reached their highest values in intermediate size classes 4–6 (120–148 mm), where the highest numbers of metacercariae were recovered (average of 33–38 individuals per fish) (Fig. 4, Supplementary Table S6). Prevalence showed a steady increase with *D. vulgaris* size, however in the two largest size classes, although prevalence was the highest with 93–100% of fish infected, the abundance and the intensity of *C. longicollis* decreased relative to medium-sized classes (Fig. 4, Supplementary Table S6). The aggregation level, calculated by the discrepancy index, was similar in all size classes, except for the largest size class where it decreased markedly (Supplementary Table S6). In *D. annularis,* the mean intensity and abundance showed the same tendency to increase with host size, with the highest values obtained in the two largest size classes (140–162 mm) where, once again, the aggregation of metacercariae was the lowest (Fig. 4, Supplementary Table S7). Size class 8 contained the highest number of metacercariae with an average of 73 parasites per fish brain. Infection prevalence showed a steep increase, from 46.2 to 79.0%, in size classes 1–2. In the four largest size classes it was greater than 95%, indicating that from approximately 120 mm total length, almost every fish is infected. The highest mean abundance and mean intensity occurred in both species at standard lengths of approximately 140–150 mm. The number of metacercariae in *D. vulgaris* was significantly higher in size classes 3–8 compared with the lowest size class, i.e. 1 ($P < 0.01$), and in *D. annularis*, higher in all other size classes compared with size class 1 ($P < 0.01$) (negative binomial GLM).

Table 1

Results from statistical analyses on the fish habitat targeted by cercariae of *Cardiocephaloides longicollis*. Results evaluating the effect of the fish habitat on the response variable (i.e. prevalence or mean abundance of *C. longicollis*), obtained with different datasets and not controlling for fish species. Results of linear models (LM) (Response variable ~ fish habitat) are indicated in italics, followed by the pairwise comparison results in square brackets. The response variables were arcsin-transformed. The percentage indicates the increase of the response variable in the specified habitat, compared with the pelagic habitat. Only significant results (at $\alpha = 0.050$) are presented.

		Response variable	
		Prevalence	Mean abundance
(a) Other together with present study	(i) True zero data included (*n* = 86)	*n.s.* [n.s.]	***Demersal*** *(t = 2.495, P = 0.015) 488.7%,* ***benthic*** *(t = 2.426, P = 0.018) 731.4%* [dem > pel; bent > pel](t = 2.495, *P* = 0.039; t = 2.426, *P* = 0.046)
	(ii) True zero data not included (*n* = 77)	***Demersal*** *(t = 2.559, P = 0.013) 38.3%*[dem > pel](t = 2.559, *P* = 0.033)	***Demersal*** *(t = 2.994, P = 0.004) 697.7%,* ***benthic*** *(t = 2.699, P = 0.009) 906%* [dem > pel; bent > pel](t = 2.994, *P* = 0.011; t = 2.699,=0.024)
(b) Data present study	(i) True zero data included (*n* = 58)	***Benthic*** *(t = 2.154, P = 0.036) 74.5%* [n.s.]	***Demersal*** *(t = 2.318, P = 0.024) 407.1%,* ***benthic*** *(t = 3.176, P = 0.002) 1641.4%*[dem > pel](t = 3.176, *P* = 0.007)
	(ii) True zero data not included (*n* = 52)	***Demersal*** *(t = 3.314, P = 0.002) 55.8%,* ***benthic*** *(t = 3.485, P = 0.001) 131.6%*[dem > pel; bent > pel](t = 3.314, *P* = 0.005; t = 3.485, *P* = 0.003)	***Demersal*** *(t = 2.946, P = 0.005) 618%,* ***benthic*** *(t = 3.828, P < 0.001) 2373.3%*[dem > pel; bent > pel](t = 2.946, *P* = 0.013; t = 3.828, *P* = 0.001)

Bent, benthic; Dem, demersal; Pelag, pelagic.

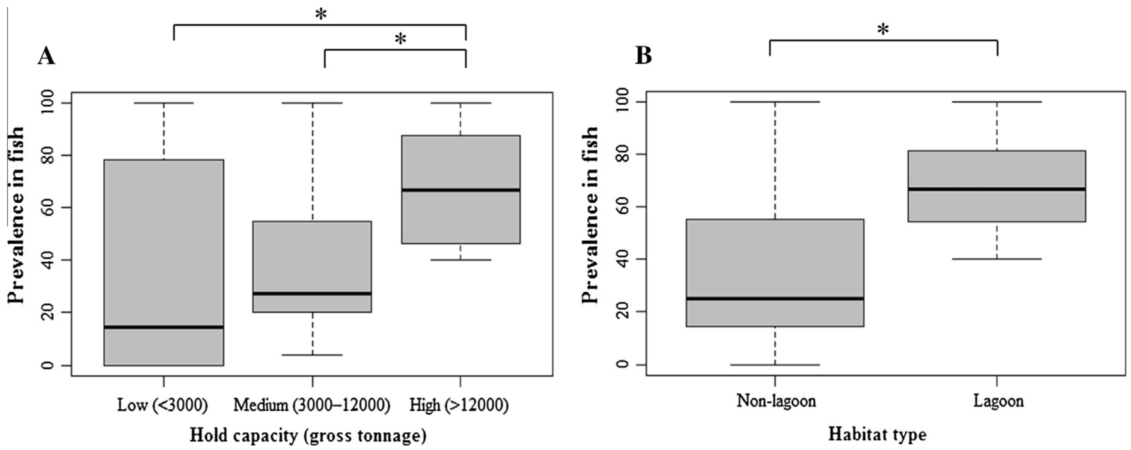

Fig. 3. Impact of fisheries and water depth on *Cardiocephaloides longicollis* numbers in fish. Parasite prevalence in relation to (A) fishing activity in the sampling area, estimated on the basis of the hold capacity of vessels from the nearest harbour and measured in gross tonnage (three categories), and to (B) habitat type, i.e. shallow water (lagoons) and other coastal areas (non-lagoons). Data obtained for the present study and true zeros are included. Statistically significant differences between levels (at $\alpha = 0.050$) are indicated with asterisks. The bottom and top of the box show the first and third quartiles and the whiskers indicate the maximum and the minimum values.

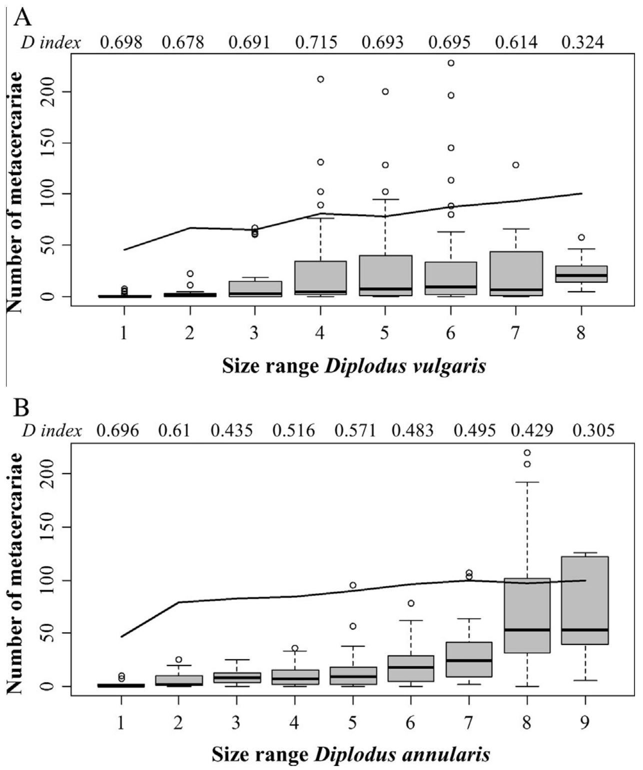

Fig. 4. Accumulation of *Cardiocephaloides longicollis* metacercariae with increasing fish size. The degree of aggregation of the metacercariae was calculated for each host size class as the index of discrepancy (D index). The number of metacercariae per size class of (A) *Diplodus vulgaris* (size class, standard length (mm)) (1, 66–98; 2,100–110; 3,111–119; 4,120–129; 5,130–139; 6, 140–148; 7,151–159; 8,160–220) and (B) *Diplodus annularis* (1,70–79; 2,80–89; 3, 90–99; 4, 100–109; 5, 100–119; 6, 120–129; 7, 130–139; 8, 140–149; 9, 150–162) are shown. Lines represent infection prevalence. The bottom and top of the box show the first and third quartiles, the circles show the outliers and the whiskers indicate the maximum and the minimum values after excluding the outliers.

3.5. Bird infection data

Among the bird species examined in this study, only larids were infected with *C. longicollis*, showing 27–69% prevalence. The species *L. michahellis*, *L. argentatus* and *L. audouinii* showed much higher prevalences than previously reported, with 69.2%, 66.7% and 50% prevalence of infection, respectively (Supplementary Table S3). *Chroicocephalus ridibundus* and terns (19 specimens) were not infected, although six previous records exist for these hosts (see Supplementary Table S3 for references). Our data, together with that in the published literature, showed no significant relationship between prevalence and mean intensity across samples ($P = 0.1302$), possibly due to the low number of samples ($n = 12$).

4. Discussion

Parasites, and more so generalists with a complex life cycle, can have strong impacts on ecosystems and food webs (Thompson et al., 2005), especially if their biomass is substantial such as that of trematodes in intertidal areas and estuaries (Kuris et al., 2008). In the present study, we investigated the distribution, life cycle strategies and successful transmission dynamics of the multi-host generalist parasite *C. longicollis* and we were able to demonstrate that shallow waters as well as fishing discards strongly impact its life cycle, leading to a considerable increase in parasite numbers in the ecosystem (results summarised in Fig. 2).

We determined that *C. longicollis* has an extremely wide host spectrum at the level of the second intermediate host, as it infects a wide range of phylogenetically distantly related fish species (see Supplementary Table S2 and references therein). Furthermore, we show that the mean abundance of *C. longicollis* in demersal and benthic fish species is four and 23 times higher, respectively, than in pelagic fish; thus cercariae appear to be more specific to the host's environment than to the host itself (Combes et al., 1994). The strong preference for the benthic habitat demonstrated in our study is possibly biased as only two benthic fish species were found to be infected in our analysis (only true zero values were included, see Section 2.4), i.e. *Z. ophiocephalus* (75.6% prevalence) and *S. scriba* (75%). Previous reports generally showed extremely low infection levels in bottom-dwelling fish (prevalences <2.9%, Supplementary Table S2), with the exception of *Neogobius melanostomus* (58%) and *Zoarces viviparus* (11%). We believe that these species, and probably some others, act as biological sinks for *C. longicollis* as they are unlikely prey items for birds, thus preventing substantial parasite numbers from completing their life cycle. The only way these fish are made available to seabirds is as discards of trawling fisheries (e.g. Oro and Ruiz, 1997; Massutí and Reñones, 2005). Overall, demersal fish clearly showed the highest infection levels, reaching 100% prevalence in *D. vulgaris* and *L. mormyrus*, and a maximum of 220 parasites in a single brain (*D. annularis*). Thus, the swimming behaviour of the cercariae increases their exposure to demersal fish, as previously hypothesised by Combes et al. (1994) and Osset et al. (2005), and thus pays off as the bulk of infected fish species come to visit surface waters and enable trophic transmission to bird hosts.

In *D. annularis* and *D. vulgaris*, an accumulative effect of metacercariae with host size was observed, similar to other trematode species (e.g. Anderson and Gordon, 1982; Thomas et al., 1995). The highest abundance and intensity occur in hosts approximately 140–150 mm standard length, a smaller size than previously determined (Osset et al., 2005). Differences in the size-related aggregation of metacercariae between different hosts can be explained by the different spatial distribution of these hosts and thus different exposure to cercariae (Poulin, 2001). However, parasite aggregation decreases in the largest size classes of both species, which is consistent with parasite-induced host mortality, i.e. heavily infected individuals are removed from the population either by death or by being preyed on more intensively (Anderson and Gordon, 1982; Poulin, 2001). The accumulation of metacercariae leads to decreasing visual capacity and increasing loss of motor control and subsequent surface ‘‘flashing” (Osset et al., 2005; Bartoli and Boudouresque, 2007), which may result in the removal of the fish with the highest infection levels by predatory seabirds, hence lowering average infection levels in the remaining fish that survive and grow into a larger size class.

In the present study, infection rates in snails and definitive bird hosts were much higher than previously reported and we were able to relate enhanced parasite transmission to two important factors: (i) close proximity of all hosts used by *C. longicollis* during its life cycle and (ii) the strong impact of fisheries on trophic transmission to definitive bird hosts.

Despite a few parasitological studies on gull species (references in Supplementary Table S2), information on *C. longicollis* distribution in final bird hosts is scarce. Following encounter and host compatibility/suitability filters (Combes, 2001), differences in bird feeding habits may explain why *L. michahellis* in the present study showed much higher parasite loads than *Larus audouinii.* The former is larger and consumes mostly fish of 150–250+ mm body length (versus <50–140 mm in *L. audouinii*, Oro and Ruiz, 1997; Arcos et al., 2001). *Larus michahellis*' prey size range encompasses the highest abundance and intensity of *C. longicollis* infection in *D. vulgaris* and *D. annularis*, with 100% infection prevalence in the latter species, which maximises parasite transmission. In contrast, smaller bird hosts such as *Chroicocephalus ridibundus* or terns include more than 50% small fish in their diet (20–100 mm, Oro and Ruiz, 1997), which transmit lower parasite numbers, thus resulting in lower infection levels or no infection, as in the present study. Moreover, *L. audouinii* and *L. michahellis* exhibit behavioural plasticity depending on food availability, both showing a clear tendency to use food resources of anthropogenic origin (Witt et al., 1981; Bartoli, 1989; Ramos et al., 2009), often fishery discards (Arcos et al., 2001; Christel et al., 2012, and references therein). Approximately 39–48% of the diet of adult breeding Audouin's gulls comes from discarded demersal fish (Navarro et al., 2010), hence supporting discards as a source of easily available food for opportunistic scavengers (e.g. Oro and Ruiz, 1997; Sánchez et al., 2004). The highest infection levels in *L. michahellis* (prevalences of 61.5 and 69.2%, Supplementary Table S3) were detected in areas of the Mediterranean region characterised by intensive fishery activities, with parasite levels 22 times higher than previously reported and in clear contrast to areas with low fishing activity (prevalence of 10.4% in the same species). Bycatch constitutes an important but highly variable fraction of the total catch (13–67% in bottom trawl fisheries in the western Mediterranean region; Tudela, 2004) (Sánchez et al., 2004; Tzanatos et al., 2007). In the Ebro Delta, which has an important commercial fishery, discards are estimated to be approximately 41% of landed fish (Oro and Ruiz, 1997; Coll et al., 2008). Discarded fish species and sizes vary somewhat (Oro and Ruiz, 1997; Machias et al., 2004; Sánchez et al., 2004; Tzanatos et al., 2007), but clearly overlap with those showing the highest *C. longicollis* infection rates (see Supplementary Table S2). A link between increased transmission and fishery activities thus becomes obvious, and was confirmed by prevalences of *C. longicollis* being approximately 75% higher in fish captured close to harbours with high fish hold capacity compared with harbours of medium or low fish hold capacity (Fig. 3A). As explained earlier, no official statistics exist on discards, with the only exception being individual local studies that give examples of the parasite biomass that is discarded into the sea. We inferred the amount of discard-associated *C. longicollis* metacercariae, combining discard data in a heavily fished area that overlaps geographically with our dataset, i.e. Santa Pola (García-Rivera et al., 2015), by analysing the four most discarded fish hosts in this area, i.e. *Boops boops*, *Pagellus acarne*, *Pagellus erythrinus* and *Diplodus annularis.* The number of *C. longicollis* metacercariae discarded at sea near Santa Pola every year is estimated to be over 100,000 individuals, i.e. eight times higher than all metacercariae collected in this study.

We can furthermore infer a strong enhancing effect of shallow water areas on the infection rates of *C. longicollis* at all host levels. Coincident with the highest prevalences of *C. longicollis* in fish (40–100%) from lagoons, the cercarial prevalence in *N. reticulatus* (8.3%) also peaked in the Ebro Delta lagoon, exceeding all previous records (Supplementary Table S1). Confined habitats are most favourable for *C. longicollis* as snail and fish hosts are concentrated in a small water volume, facilitating the parasite's transmission. Fish showed significantly higher infection levels in lagoons than in other coastal areas, however, fish are mobile and although captured in non-lagoon areas, they may have visited shallow water habitats temporarily, hence explaining the large range of infection levels observed outside lagoons (Fig. 3B). Given their sessile nature, snails are possibly the best indicator for locally variable parasite numbers. Due to insufficient infection data in non-lagoon areas, statistical comparison of snail data from different habitats was not possible; however, this should be included in future studies to confirm our preliminary results. Similar to lagoons, aquaculture installations may act as enhancers of *C. longicollis* transmission. The organic enrichment in areas close to farmed facilities and frequent mortalities in sea cages attract scavengers such as gastropods (e.g. nassariid snails, Morton and Yuen, 2000; Edgar et al., 2005) and piscivorous birds (Oro and Ruiz, 1997; Christel et al., 2012; see above). To the best of our knowledge, we report for the first time, the presence of metacercariae of *C. longicollis* in *S. aurata* cultured in netpens. High parasite prevalence in these installations (53.9%) was similar to that in wild *S. aurata* from Italian lagoons, where parasite transmission is increased. The environmental impact of increasing fishery and aquaculture activities in the Mediterranean region makes it essential to study their effects on parasites since they affect a wide range of cultured and wild host species and play an important role in food webs (Lafferty et al., 2008). We expect that, in areas of enhanced parasite transmission, trophically-transmitted generalist parasites with a complex life cycle and efficient host-finding and manipulation mechanisms will be able to substantially increase overall abundance. As a result, such parasites are expected to considerably alter the energy flow through the food web. *Cardiocephaloides longicollis* presents itself as an ideal future bioindicator to compare food web structures in natural communities with those impacted by fisheries and aquaculture.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2016.06.005.

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