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Larval Helminth Parasites of Fishes and Shellfishes from Santa Gilla Lagoon (Sardinia, Western Mediterranean), and Their Use as Bioecological Indicators

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Abstract Qualitative and quantitative data of the larval stages of 30 helminth species infecting fish and shellfish in the Santa Gilla lagoon (southern Sardinia, western Mediterranean Sea) were collected and analysed to determine their usefulness as biological and ecological indicators. Parasitological data were collected from 2001 to 2011 from 12 fish species, from the families Anguillidae, Atherinidae, Cyprinodontidae, Gobiidae, Moronidae, Mugilidae, Soleidae and Sparidae and from bivalve molluscs of the families Cardiidae, Mytilidae and Veneridae. Digenean metacercariae dominated the larval parasitofauna; nevertheless, the most abundant parasite species was a nematode, i.e. the third-stage larvae of the anisakid *Contracaecum rudolphii*. Based on their life cycle, the species detected were divided into autogenic or allogenic parasites, which have fishes and birds/mammals as their definitive hosts, respectively. Data on the prevalence, intensity and abundance of infections and the parasitic diversity in each host species were combined with previous knowledge on the life history of the parasites detected, to build a model food web based on the different trophic relations, especially predator–prey interactions between hosts marked by the transmission of parasites. The results will contribute to the knowledge of species diversity and to the evaluation of general environmental changes occurring in brackish Mediterranean ecosystems. In combination with other analytic tools in a multidisciplinary approach, this will enable us to better understand the functioning of other Mediterranean brackish waters that are comparable to the Santa Gilla lagoon.

Keywords Brackish waters · Digenea · Nematoda · Indicators · Food web · Mediterranean lagoon

Introduction

The life cycle of heteroxenous helminth parasites is mostly based on the transmission of their developmental stages through prey–predator relations in the food web. Together with the sequence of morphological and functional changes, the interactions that occur between the host–parasite system and its environment must be also considered in the study of the parasites as natural indicators of hosts' biology and ecology (Polyanski 1961; MacKenzie 1987). Consequently, the disappearance of a parasite from its known host(s) might indicate unfavourable environmental factors not only for the parasite itself but for one or more of its other host species. In fact, the detection of a parasite species in a host indicates that all the host species required for the life cycle of that parasite must be present in the community (in case of stationary species), or that the hosts are in the same place and at least for the time necessary for the transmission of the parasite. Larval stages in particular can be highly sensitive to many types of impact, e.g. pollution, low oxygen stress, variations of salinity and temperature, affecting directly their presence (resulting in an increase or decrease of richness and/or abundance) or acting on the population of their hosts (MacKenzie 1995, 1999; Huspeni et al. 2005). In addition, parasites can have a variety of effects on their hosts, influencing their

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behaviour, population dynamics and food-web processes (Marcogliese and Cone 1997). Thus, studies on larval parasites are a useful addition to standard techniques for monitoring biodiversity and ecosystem function (Lafferty et al. 2006).

Parasites as biological indicators of aquatic ecosystems have been studied in marine, freshwater and brackish waters, including the Mediterranean. This has mainly been in relation to the sensitivity of these organisms to pollutants (Williams and Mackenzie 2003; Sures 2008; Shea et al. 2012), or investigating their use as biological tags for host populations and/or stock levels (Williams et al. 1992; Balbuena et al. 1995; Ferrer-Castello et al. 2007).

To date, reports derived from large surveys on helminth parasitofauna in brackish areas of the Mediterranean either are in the form of checklists and synopsis of life cycles (Bartoli and Gibson 2007), investigate the epidemiology of infections in their respective aquatic hosts or consider the zoonotic risk associated with some of the parasite species detected (Merella et al. 2004; Culurgioni et al. 2010a, 2013). In some cases, such data are related to changing environmental conditions (Dzikowsky et al. 2003) or to the effects of parasites on their hosts; thus, they can also be used to study the consequences of these effects on relevant food webs (Bartoli and Boudouresque 2007).

The main studies of parasites as indicators of free-living communities, diversity and ecosystem health in wetlands deal principally with adult stages in fish (Rueckert et al. 2009), or, as in the case of digenean trematodes, with early larval stages in Gastropoda, their first intermediate hosts (Huspeni et al. 2005; Hechinger et al. 2006; Lafferty et al. 2008). The same authors underline the positive correlations between the diversity of trematode sporocysts and cercariae infecting snail populations and those of birds, because the abundance of these parasites indicates the efficiency of trophic linkages between these definitive hosts and benthic invertebrates.

Unlike the cercariae, which in most cases (depending on the digenean species) actively penetrate the skin of the second intermediate hosts, the final larval stages that precede the adult stage need to be transmitted through predation or ingestion (Williams and Jones 1994). Therefore, data concerning these stages could directly reveal the trophic relations between the second intermediate hosts and their predators (i.e. fish, birds or mammals), which have a role as the definitive host for the parasite. Using metacercariae, as well as cestode plerocercoids, nematode third-stage larvae and acanthocephalan cystacanths as indicators is therefore advantageous over the use of the earlier larval stages such as sporocysts and cercariae.

In this study, we investigate the intermediate stages of helminth parasites in fishes and bivalves, their second intermediate hosts, in the brackish-water Santa Gilla lagoon, in the Mediterranean. We make preliminary conclusions based on these data of the biological and ecological nature of these parasites and propose a parasitological data-based model of the food web of a typical Mediterranean brackish-water system.

Materials and Methods

Study Site

The sampling of larval helminth parasites was carried out as part of various research projects, conducted by our research group during a period of 11 years (2001–2011), which mostly concerned the epidemiology and morphology of parasites of fish and shellfish from the Santa Gilla lagoon (southern Sardinia, western Mediterranean). This brackish-water lagoon ‘Stagno di Cagliari’ was included in the Ramsar List of Wetlands of International Importance in December 1976, and between 1990 and 2008, it was also listed in the Montreux Record relating to wetlands at risk of adverse changes in ecological character, ‘because the ecology of the site has been seriously damaged by thermal, industrial and urban effluent from the city of Cagliari, resulting in nutrient enrichment and major port development projects’ (Anonymous 2008).

The current morphology of the lagoon is the result of a series of alterations that started approximately 100 years ago and that strongly impacted on the ecosystem. The lagoon is approximately 4500 ha and serves a saltworks, an airport, a channel port and the fishing and aquaculture industry with an exploitable surface area of 1200 ha (Fig. 1). The intake of fresh water is restricted because the flow of the two main tributaries has been consistently diverted directly into the sea, via the main river mouth, which is 300 m wide. As a result, the water salinity ranges from 19 to 45, determined mainly by rainfall; during the summer and autumn months, the salinity is often higher than the mean salinity of the Gulf of Cagliari, which is 38.

Host Sampling

All host samples were obtained fresh from local fishermen of ‘Consorzio Ittico di Santa Gilla’, according to the current needs of each individual research carried out in the period between September 2001 and July 2011, and also according to the relative availability of each species in the landings of fisheries. Parasitological analyses were carried out on a total of 515 fishes belonging to 13 species (see Table 1): *Anguilla anguilla* (103 specimens), *Atherina boyeri* (90), *Aphanius fasciatus* (89), *Chelon labrosus* (six), *Dicentrarchus labrax* (56), *Gobius niger* (86), *Gobius paganellus* (three), *Liza aurata* (15), *Liza ramada* (12), *Liza saliens* (nine), *Mugil cephalus* (10), *Solea solea* (10) and *Sparus aurata* (26); and from 2255 specimens of four bivalve species: *Cerastoderma glaucum* (391), *Mytilus galloprovincialis* (123), *Ruditapes decussatus* (1484), and *Polititapes aureus* (257).

Fig. 1 Map of the Santa Gilla lagoon. The sampling area which is subjected to fishing activities is shaded in *grey*

Extraction and Identification of Parasites

The fish and shellfish specimens were transported to the laboratory and classified. Total fish length and weight, and shell length, were measured before dissection. The surface and lumen of organs and the visceral cavity were microscopically examined for the presence of metazoan parasites. In order to extract the digenean metacercariae encysted in muscles, each eviscerated fish was grinded and artificially digested for 2 h at 37 °C in a stirred water solution containing NaCl (0.85 %), pepsine (0.7 %) and HCl (0.5 %). The resulting material was first sieved at 500 μm to separate large particles, then at 70 μm in order to retain cysts of different sizes. These were selected and cleaned in a drop of saline and worms were extracted using two needles, or by slight pressure on the cover glass. Cystacanths of acanthocephalans were transferred to freshwater until the spined proboscis was everted, enabling classification of the worms.

After collection, helminths were counted and preserved in 70 % ethanol, or in AFA (alcohol, formalin, acetic acid). Their taxonomic position and life cycles were determined using morphological identification keys and descriptions available in the published literature (Palombi 1933, 1934; Paggi and Orecchia 1965; Pearson 1973; Schmidt 1973; Stunkard 1977; Maillard and Saad-Fares 1981; Youssef et al. 1987; Moravec 1994, 2009; Jousson and Bartoli 2001; Scholz et al. 2001; Spakulova et al. 2002; Laffargue et al. 2004; Bartoli and Gibson 2007; Pina et al. 2009; Sohn 2009). In addition, nematodes of the specie *Contracaecum rudolphii* were genetically identified by sequential analyses of the mitochondrial gene cytochrome oxidase II (mtDNA cox2), according to Mattiucci et al. (2008).

Data Analysis

Based on their life cycle, the parasites detected were divided into two main categories according to Esch et al. (1988): autogenic, which use aquatic invertebrates as first intermediate hosts, fish (or invertebrates) as second intermediate hosts and mature in aquatic hosts (i.e. predatory fish), or allogenic, which complete their life cycle in nonaquatic definitive hosts (i.e. piscivorous and benthivorous birds, or mammals).

Since the origin of the informations collected is heterogeneous in time as it depended on separated projects conducted in multiple years, the data were aggregated in a single set.

Prevalence of infection (P), intensity range (IR), mean intensity (MI) and mean abundance (MA) were calculated according to Bush et al. (1997), for each parasite/host complex. Richness (R) of larval parasite species was calculated overall and for each host, also considering the distinction of parasites in the two groups above described.

Except for R, the two species of *Ascocotyle* (*Phagicola*) observed in mugilids were numerically considered as one, because in many cases, the specimens lost easily some of their oral spines, making it impossible to determine their morphology accurately enough to separate the two species.

Table 1 Temporal distribution of sampling of fish and shellfish from Santa Gilla Lagoon, Sardinia, Italy, divided by years and season

Host species (*n*)	No. of hosts (season)										
	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Teleostea											
Anguilla anguilla (103)	58 (S) 25 (A)									20 (W)	
Aphanius fasciatus (89)							71 (A)	18 (W)			
Atherina boyeri (90)	24 (S) 31 (A)	35 (W)									
Chelon labrosus (6)									6 (s)		
Dicentrarchus labrax (56)										18 (W) 24 (s) 8 (S) 6 (A)	
Gobius niger (86)		7 (S)		11 (s) 16 (A)	11 (s) 8 (A)	14 (W)					19 (W)
Gobius paganellus (3)										3 (W)	
Liza aurata (15)							2 (W) 2 (A)	11 (W)			
Liza ramada (12)							12 (W)				
Liza saliens (9)								2 (A)	5 (W) 1 (s)	1 (W)	
Mugil cephalus (10)							2 (W)	5 (W)	3 (W)		
Solea solea (10)										1 (s) 3 (S) 6 (A)	
Sparus aurata (26)										11 (s) 15 (A)	
Bivalvia											
Cerastoderma glaucum (391)		21 (S) 39 (A)	15 (W) 19 (A)	12 (W) 10 (s) 10 (S) 29 (A)	17 (W) 19 (s)				80 (A)	80 (W) 40 (s)	
Mytilus galloprovincialis (123)			41 (s)	30 (W) 30 (s) 22 (S)							
Ruditapes decussatus (1484)	158 (S) 203 (A)	23 (W) 30 (s) 25 (S) 35 (A)	25 (W) 24 (s) 48 (A)	11 (W) 36 (s) 20 (S) 54 (A)	32 (W) 46 (s) 47 (S) 75 (A)	25 (W) 53 (s) 35 (S) 39 (A)	41 (W) 27 (s) 31 (S) 44 (A)	27 (W) 22 (A)	25 (W) 55 (A)	85 (W) 40 (s)	25 (W) 18 (S)
Polititapes aureus (257)	32 (A)	22 (W) 50 (s)	1 (W) 14 (s)	11 (W) 23 (A)	21 (W) 38 (s) 20 (S)	12 (W) 13 (s)					

W winter, *s* spring, *S* summer, *A* autumn

Results

Total Data

Of the 68 helminth species totally identified, 30 were found as larval stages in the fishes and/or in the shellfishes. The ratio between autogenic (Table 2) and allogenic (Table 3) parasite species was 14:16.

Digeneans dominated the larval parasite fauna with 25 species. However, the most abundant and widespread parasite was the nematode *C. rudolphii* (Anisakidae): 5515 third-stage larvae (L3) were collected from five fish species (Table 3), primarily *D. labrax* (MA=79.84) in which parasite intensity reached values of up to 584 worms in a host. Among the Digenea, metacercariae of *Timoniella imbutiforme* and *Bucephalus minimus* infested the widest host range (five fish species), whereas those of the heterophyid *Ascocotyle* (*Phagicola*) sp. 1 and sp. 2 showed the highest prevalence (90.0 % in *M. cephalus*) and the highest MI (116.7 in *L. ramada*). Except in *C. labrosus*, this infection was heaviest in the musculature of all the mugilid species, with a total abundance of 2685 (Table 3).

Table 2 Larval stages of autogenic parasite species found in fish and shellfish from Santa Gilla Lagoon, Sardinia, Italy

Parasite	Intermediate host	Stage	%	No. of parasites collected	Mean intensity±SE	Intensity range	Definitive host
Digenea							
Bucephalidae							
Bucephalus anguillae Spakulova et al. 2002	*A. fasciatus*	MC	20.2	55	3.1±0.5	1–9	*A. anguilla*
B. baeri Maillard and Saad-Fares 1981	*P. aureus*	S+C	0.8	–	–	–	*D. labrax*
	S. aurata	MC	3.8	1	1.0	1	
B. labracis Paggi and Orecchia 1965	*L. aurata*	MC	6.7	3	3.0	3	*D. labrax*
	L. saliens	MC	22.0	220	110.0±100.0	10–210	
	S. aurata	MC	19.2	39	7.8±6.0	1–23	
B. minimus (Stossich 1887)	*C. glaucum*	S+C	0.5	–	–	–	*D. labrax*
	A. boyeri	MC	87.8	525	6.6±0.1	1–70	
	A. fasciatus	MC	78.7	960	13.7±2.0	1–73	
	G. niger	MC	4.7	10	2.5±1.0	1–5	
	G. paganellus	MC	33.3	21	21.0	21	
	S. aurata	MC	19.2	39	7.8±2.6	3–17	
Prosorhynchus aculeatus Odhner, 1905	*G. niger*	MC	1.2	1	1.0	1	*Conger conger*
	S. solea	MC	10.0	1	1.0	1	
Cryptogonimidae							
Timoniella imbutiforme (Molin, 1859)	*A. fasciatus*	MC	4.5	6	1.5±0.5	1–3	*D. labrax*
	A. boyeri	MC	1.1	1	1.0	1	
	D. labrax	MC	1.8	2	2.0	2	
	G. niger	MC	4.7	13	3.3±0.9	1–5	
	S. solea	MC	60.0	402	67.0±22.0	1–123	
T. praeterita (Looss, 1901)	*A. fasciatus*	MC	11.2	18	1.8±0.3	1–3	*D. labrax*
	L. aurata	MC	6.7	4	4.0	4	
	M. cephalus	MC	20.0	7	3.5±2.5	1–6	
Faustulidae							
Bacciger bacciger (Rudolphi, 1819)	*P. aureus*	S+C	18.5	–	–	–	*A. boyeri*
	R. decussatus	S+C	20.3	–	–	–	
Fellodistomidae							
Proctoeces maculatus (Looss, 1901)	*M. galloprovincialis*	S+C	4.1	–	–	–	Labridae, Sparidae, Gastropoda, Bivalvia, Polychaeta
Monorchiidae							
Lasiotocus longicystis Bartoli, 1965	*R. decussatus*	MC	52.3	776	4.7	1–18	*A. anguilla*
	P. aureus	MC	30.7	79	17.1	1–39	
Opecoelidae							
Cainocreadium labracis (Dujardin, 1845)	*A. fasciatus*	MC	7.9	12	1.7±0.4	1–3	*D. labrax*
Cestoda							
Tetraphyllidea							
Scolex pleuronectis Müller, 1788	*A. anguilla*	P	1.9	3	1.5±0.5	1–2	Chondrichthyans
	G. niger	P	3.5	4	1.3±0.3	1–2	
	D. labrax	P	3.6	2	1.0	1	
Nematoda							
Cystidicolidae sp.	*D. labrax*	L3	3.6	3	1.5±0.5	1–2	Teleosts
Acanthocephala							
Iliosentidae							
Telosentis exiguus (Von Linstow,1901)	*A. fasciatus*	C	2.2	4	2.0±1.0	1–3	*A. anguilla*

The intensity was not evaluable in first intermediate hosts, due to the high number of sporocysts and cercariae detected

% prevalence, *MC* metacercaria, *S+C* sporocysts and cercariae (in first intermediate host), *P* plerocercoid, *L3* third-stage larva, *C* cystacanth, – intensity not evaluable in first intermediate hosts, due to the high number of sporocysts and cercariae detected

Table 3 Larval stages of allogenic parasite species found in fish and shellfish from Santa Gilla Lagoon, Sardinia, Italy

Parasite	Intermediate host	Stage	%	No. of parasites collected	Mean intensity±SE	Intensity range	Definitive host
Digenea							
Echinostomatidae							
Himasthla elongata (Mehlis, 1831)	*C. glaucum*	MC	0.7	12	4.0	1–7	*Larus* spp.
H. quissetensis Miller & Northup, 1926	*R. decussatus*	MC	0.1	3	3.0	3	*Larus michaellis*
Gymnophallidae							
Gymnophallus choledochus Odhner, 1900	*C. glaucum*	S+C	2.3	–	–	–	*L. michaellis*
		MC	2.3	38	4.2	1.9	
Gymnophallus fossarum† Bartoli, 1965	*R. decussatus*	MC	1.8	54	2.0	1–4	*L. michaellis*
Strigeidae							
Cardiocephalus longicollis Rudoplhi, 1819	*S. aurata*	MC	46.2	37	3.1±0.8	1–10	*L. michaellis*
Heterophyidae							
Ascocotyle (*Phagicola*) sp. 1 and sp. 2 Faust, 1920	*L. aurata*	MC	86.7	1116	85.9±28.7	2–268	Mammals (dog, cat, fox, human)
	L. ramada	MC	58.3	817	116.7±26.7	12–207	
	L. saliens	MC	55.6	104	20.1±10.1	2–40	
	M. cephalus	MC	90.0	648	72.0±23.3	3–208	
Ascocotyle (*Phagicola*) sp. 3 Faust, 1920	*A. fasciatus*	MC	5.6	9	1.8±0.6	1–4	Unknown, likely as above
Ascocotyle (*Ascocotyle*) sp. Looss, 1899	*D. labrax*	MC	1.8	14	14.0	14	*Pelecanus onocrotalus*, *Nycticorax nycticorax*
Heterophyes heterophyes Travassos, 1928	*G. niger*	MC	1.2	1	1.0	1	Human, dog, cat, fox, *Sterna hirundo*
Pygidiopsis sp. Looss, 1907	*A. fasciatus*	MC	1.1	1	1.0	1	*P. onocrotalus*, *Ardea cinerea*
Galactosomum lacteum (Jägerskiöld, 1896)	*D. labrax*	MC	1.8	1	1.0	1	*A. cinerea*, *Phalacrocorax* spp.
	S. aurata	MC	3.8	1	1.0	1	
Galactosomum timondavidi Pearson & Prevot, 1971	*S. solea*	MC	50.0	40	8.0±4.3	1–25	*Larus* spp.
Stictodora sawakinensis Looss, 1899	*S. aurata*	MC	11.5	256	85.3±66.0	4–216	*Larus* spp.
	G. paganellus	MC	66.7	5	2.5±0.5	2–3	
Nematoda							
Anisakidae							
Contracaecum rudolphii Hartwich, 1964	*A. anguilla*	L3	5.8	16	2.7±1.28	1–9	*Phalacrocorax carbo*, *P. aristotelis*
	D. labrax	L3	83.9	4471	95.1±22.2	1–583	
	G. niger	L3	8.1	8	1.1±0.1	1–2	
	G. paganellus	L3	33.3	5	5.0	5	
	S. solea	L3	40.0	5	1.3±0.3	1–2	
Acanthocephala							
Polymorphydae							
Southwellina hispida Meyer, 1931	*A. anguilla*	C	1.0	1	1.0	1	*P. carbo*, other piscivorous birds
	D. labrax	C	8.9	13	2.6±0.9	1–6	
	S. aurata	C	3.8	2	2.0	2	

† species extinct together with its 1st intermediate host (*S. plana*), *%* prevalence, *MC* metacercaria, *S+C* sporocysts and cercariae (in first intermediate host), *L3* third-stage larva, *C* cystacanth, – intensity not evaluable in first intermediate hosts, due to the high number of sporocysts and cercariae detected

The highest parasite richness (Table 4) was observed in *A. fasciatus*, which harboured eight helminth species, followed by *S. aurata* and *D. labrax* (seven species each). Despite these values, each of these fish species showed a particularly high MA for only one parasite species each: *B. minimus*, *Stictodora sawakinensis* and *C. rudolphii*, respectively.

Autogenic Parasites

Four of the 14 parasite species with entirely aquatic life cycles, which were all digeneans, were found in four bivalves: *Bacciger bacciger* (in *R. decussatus* and *P. aureus*), *B. minimus* (in *C. glaucum*) and *Proctoeces maculatus* (in *M. galloprovincialis*) occurred as sporocysts and cercariae, whereas *Lasiotocus* *longicystis* occurred only as metacercariae in *R. decussatus* and *P. aureus*. Adult *B. bacciger* were also found in its definitive host *A. boyeri*.

Figure 2 illustrates the steps of the autogenic parasitic biological cycles known in the Santa Gilla lagoon, highlighting the trophic links between hosts used for their development.

Eight digenean species were found as generalist metacercariae in small-sized prey fish (*A. boyeri*, *A. fasciatus*, *D. labrax*, *S. aurata*, *M. cephalus*, *Liza* spp., *Gobius* spp. and *S. solea*). Seven were also found as species-specific adults in their definitive fish hosts inhabiting the lagoon: *Bucephalus anguillae* occurred in *A. anguilla*; *Bucephalus baeri*, *Bucephalus labracis*, *B. minimus*, *Cainocreadium labracis*, *Timoniella praeterita* and *T. imbutiforme* infected *D. labrax*.

Three non-digenean species were also detected: *Telosentis exiguus* (Acanthocephala) was found in the visceral cavity of *A. fasciatus*; plerocercoids of the complex *Scolex pleuronectis* were found in the intestine of eel, sea bass and black goby; third-stage larval Cystidicolidae (Nematoda) were only found in the visceral cavity of *D. labrax*. The latter two parasites, together with the digeneans *P. maculatus* and *Prosorhynchus crucibulum*, have typical marine cycles, occurring as adults in chondrichthyans and teleost predators (such as *C. conger*), respectively (Fig. 2).

The highest autogenic larval richness (6) was observed in *A. fasciatus*. Another common second intermediate host was the black goby (R=4), followed by *S. aurata* and *D. labrax* (R=3).

Among the autogenic parasites, the family Bucephalidae was represented the most with five species, and the highest infection values were observed among these. In fact, *B. minimus* (the most widespread, found in six host species) and *B. labracis* had a prevalence of 87.8 % in *A. boyeri* and an intensity of 10–210 in *L. saliens*, respectively. The highest abundance (40.20) was recorded for *T. imbutiforme* in *S. solea* (Table 4).

Allogenic Parasites

Four digenean species maturing in birds occurred in the two bivalve species *R. decussatus* and *C. glaucum*: *Gymnophallus choledochus* occurred as sporocysts with cercariae and metacercariae; *Gymnophallus fossarum*, *Himasthla elongata* and *H. quissetensis* occurred as metacercariae. All these infections showed low values in their hosts (Table 3).

Figure 3 illustrates the steps of the biological cycles of allogenic parasites known in the Santa Gilla lagoon and highlights the trophic links between hosts used for their development.

Metacercariae of ten fluke species were found in 11 species of prey fish. In the order of specimens collected, these were *Ascocotyle* (*Phagicola*) sp. 1 and sp. 2, *S. sawakinensis*, *Galactosomum timondavidi*, *Cardiocephalus longicollis* (the only species belonging to the Strigeidae), *Ascocotyle* (*Ascocotyle*) sp., *Ascocotyle* (*Phagicola*) sp. 3, *Pygidiopsis* sp., *Galactosomum lacteum* and *H. heterophyes*.

Other two larval parasites species were identified: the most widespread generalist *C. rudolphii* (Nematoda, Anisakidae), found in five fish species with particularly high levels in *D. labrax*; and *Southwellina hispida* (Acanthocephala, Polymorphidae), which cystacanths were harboured by three species (Table 3). The adults of these worms usually infect *Phalacrocorax carbo*.

S. aurata and *D. labrax* showed the highest richness (4) of larval allogenic parasites, whereas all the other fish species harboured only two parasites (Fig. 3). Among these, some were infected only by Heterophyidae, the most represented family of allogenic parasites: four mugilids were infected by *Ascocotyle* (*Phagicola*) sp. 1 and sp. 2 with high levels, and *A. fasciatus* harboured *Ascocotyle* (*Phagicola*) sp. 3 and *Pygidiopsis* sp, but at low levels. The same was true for *Ascocotyle* sp. and *H. heterophyes* in *D. labrax* and *G. niger*, respectively.

Discussion

The parasitological data obtained allowed to make qualitative considerations about the trophic linkages between their second intermediate and definitive hosts, providing important information about the structure of the food web. Moreover, the changes in abundances of the parasites more sensitive to salinity variations can help us to confirm the evidences of the ongoing environmental changes in the Santa Gilla lagoon.

In Fig. 2, both autogenic and allogenic 'systems' are included in the same model of the Santa Gilla lagoon, which schematises all the relations between hosts and parasites, preys and predators and between predators, as competitors.

In terms of the autogenic parasites, the six fluke species of *D. labrax* highlight its role as the main fish predator in the lagoon. The sea bass feeds on several small teleosts, which are important as second intermediate hosts for digeneans such as *B. minimus* and *T. imbutiforme* (Figs. 2 and 4), the generalist metacercariae of which were both found in five fish species. Regarding the sporadic occurrence of metacercariae of *T. imbutiforme* in the musculature of small-sized sea bass may mean that they are also dead end hosts for these parasites, in case of incidental infection. But, in some cases, the life cycle of the parasite can be completed normally, given that a cannibalistic behaviour of this species may occur, as already observed under farmed and wild conditions (Smith and Reay 1991; Henderson and Corps 1997).

The high values of larval abundance observed in some fish suggest that even generalist parasites have a host preference,

Table 4 Abundance and richness of larval parasite infections in fish and shellfish from Santa Gilla lagoon, and their definitive hosts

Autogenic parasite species	Intermediate host	*V.aurea*	*R.decussatus*	*C.glaucum*	*M.galloprovincialis*	*A.fasciatus*	*L.aurata*	*L.ramada*	*L.saliens*
T. imbutiforme D						0.07			
T. praeterita D						0.20	0.27		
C. labracis D						0.14			
B. minimus D				1st [d]		10.79			
B. labracis D							0.20		**24.44**
B. baeri D		1st							
B. anguillae D						0.62			
L. longicystis D		0.31	0.52						
*T. exiguus*A						0.04			
B. bacciger D		1st	1st						
Cystidicolidae sp. N									
S. pleuronectis C									
P. aculeatus D									
P. maculatus D					1st				
R [a]		3	2	1	1	6	2	0	1
Allogenic parasite species									
H. elongata D				0.03					
H. quissetensis D			0.01						
G. choledochus D				0.05					
G. fossarum D [e]			0.04	0.10					
C. longicollis D									
G. timondavidi D									
S. sawakinensis D									
G. lacteum D									
C. rudolphii N									
S. hispida A									
Phagicola sp. 1 D							74.40	68.08	11.56
Phagicola sp. 2 D									
Ascocotyle sp. D									
H. heterophyes D									
Phagicola sp. 3 D						0.10			
Pygidiopsis sp. D						0.01			
R [a]		0	2	3	0	2	2	2	2

M cephalus	*S.aurata*	*A.boyeri*	*G.paganellus*	*S.solea*	*G.niger*	*A.anguilla*	*D.labrax*	Definitive host (R)[b]
		0.01		**40.20**	0.15		0.02	*D. labrax* (6)
0.70								
	1.50	5.83	7.00		0.12			

Table 4 (continued)

M cephalus	*S.aurata*	*A.boyeri*	*G.paganellus*	*S.solea*	*G.niger*	*A.anguilla*	*D.labrax*	Definitive host (R)[b]
	1.34							
	0.04							
								A. anguilla (3)
								A. boyeri
							0.05	Marine Teleosts
					0.05	0.03	0.04	Sharks/rays
				0.10	0.01			*C. conger* (2)
1	3	2	1	2	4	1	3	14 tR [c]
Allogenic parasite species								
								Larus spp. (7)
	1.42							
				4.00				
	9.84		1.67					
	0.04						0.02	
			1.67	0.50	0.09	0.16	**79.84**	*Phalacrocorax carbo* (5)
	0.08					0.01	0.23	
64.80								
							0.25	Piscivorous birds and mammals (6)
					0.01			
2	4	0	2	2	2	2	4	16 tR

A. fasciatus showed the highest richness (8), followed by *S. aurata* and *D. labrax* (7). Some high values of abundance (in bold) suggest that even generalist parasites may have a host preference

[a] R=parasite richness in each intermediate host species

[b] (R)=parasite richness in definitive hosts

[c] tR=total richness

[d] 1st=in first intermediate hosts, abundance was not evaluated due to the high number of sporocysts and cercariae

[e] =currently extinct in the lagoon

A Acanthocephala, *C* Cestoda, *D* Digenea, *N* Nematoda

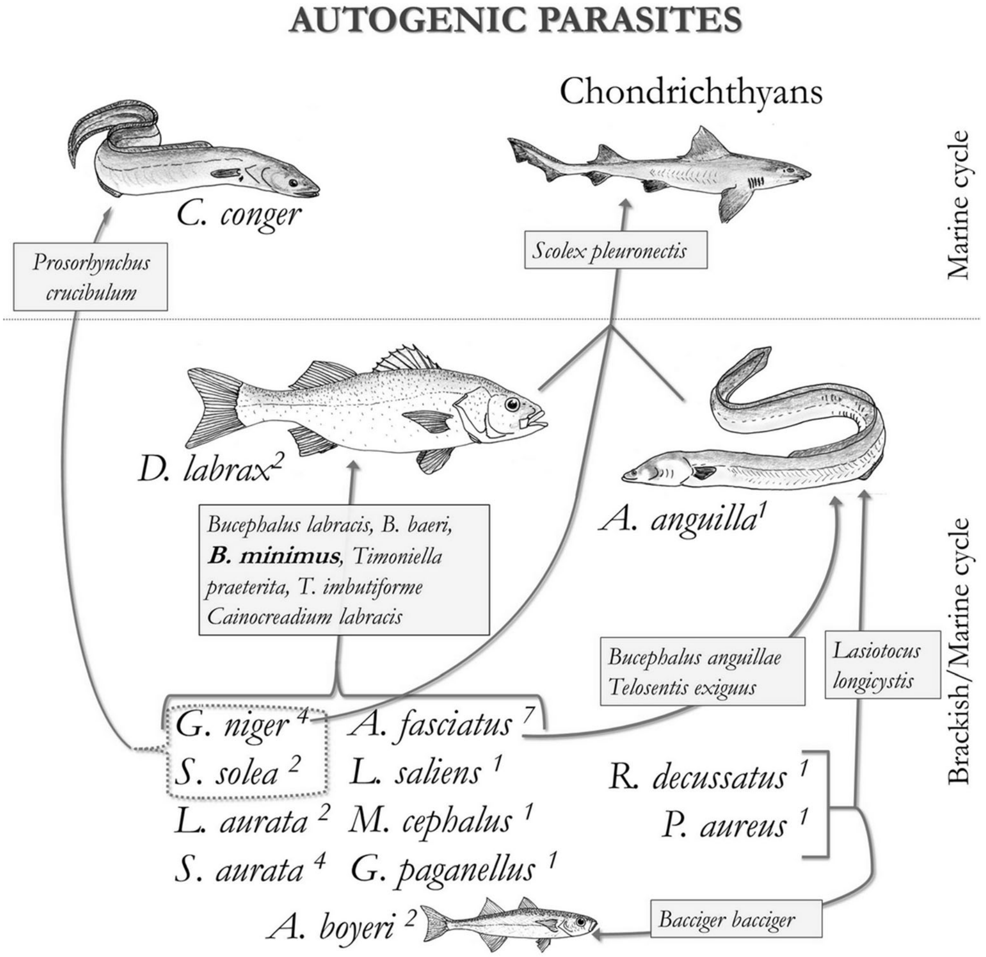

Fig. 2 Trophic relations (*arrows*) between second intermediate hosts (*in letters*; *exponents* indicate the number of parasite species harboured) and definitive hosts (*in pictures*) of autogenic parasites (*grey background*; dominant species in *bold*). In this study, autogenic parasites fullfill their life cycles entirely in aquatic habitats (Esch et al. 1988), reaching the adult stage mainly in brackish predators, but also in strictly marine fishes

as in the case of *B. labracis* in *L. saliens*, *T. imbutiforme* in *S. solea* and *B. minimus* in *A. fasciatus* (Table 4). Moreover,

parasitological data from killifish suggest that in Santa Gilla, it is the only second intermediate host shared by parasites of

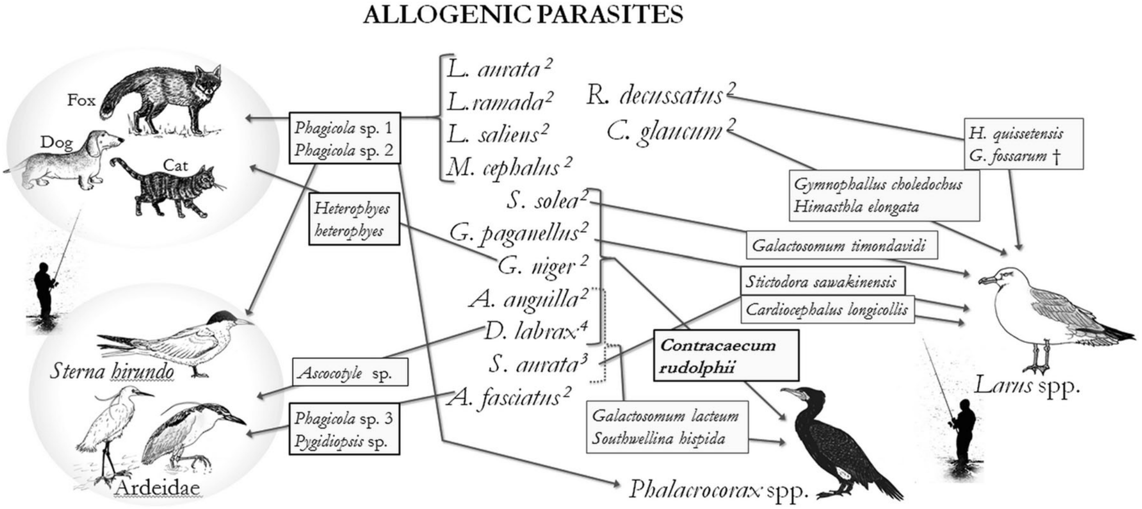

Fig. 3 Trophic relations (*arrows*) between second intermediate hosts (*in letters*; *exponents* indicate the number of parasite species harboured) and definitive hosts (*in pictures*) of allogenic parasites (*grey background*; zoonotic species in *thick-lined squares*; dominant species in *bold*). In this study, allogenic parasites complete their life cycle in nonaquatic habitats, reaching the adult stage in piscivorous and benthivorous birds or mammals, potentially including humans. *Dagger* indicates recently extinct in Santa Gilla lagoon

A. anguilla and *D. labrax* explaining that competition occurs between sea bass (*C. labracis*) and eel on this prey. In fact, killifish is the only known second intermediate host for *B. anguillae* (Gargouri-Ben Abdallah and Maamouri 2002), and also harbours cystacanths of *Telosentis exiguus*, an acanthocephalan of eels (Figus et al. 2004).

It is interesting to note that the occurrence of the larvae of typical marine parasites, even if with low infection indices, suggests the existence of trophic links between lagunar fish and marine predators. For example, the occurrence of tetraphyllid (Cestoda) plerocercoids (which are conventionally considered to be a unique species, *Scolex pleuronectis*) and of cystidicolid larvae suggests that *D. labrax* and *A. anguilla*, together with other teleosts of the lagoon, serve as prey for several species of chondrichthyans and other marine predators. The same is true for the bucephalid metacercariae of *P. crucibulum* (whose definitive host is *Conger conger*) found in *G. niger* and *S. solea*. This is owing, in part, to the migrating behaviour of some intermediate hosts, such as sea bass and sole (Gonzalez and Gerlotto 1998; Dierking et al. 2012), which might become infected with parasites in coastal seawater, but also it may depend on the increase of the dispersion of earlier larval stages (i.e. cercariae) more inside the lagoon, towards stationary hosts. This maybe one of the signs of the ongoing process of salinisation of the lagoon (Cau and Murenu, unpublished results 2010), which is resulting in whole marine host–parasite complexes to become established further into the lagoon.

Alternatively, other parasitic cycles might have disappeared as a consequence of salinisation: for example, the lack of typical freshwater parasites of *A. anguilla* as *Proteocephalus macrocephalus*, *Bothriocephalus claviceps*, *Anguillicola crassus* and *Acantocephalus clavula* in comparison to data recorded in the same area during 2000–2001 has been recently observed (Figus et al. 2002; Culurgioni et al. 2010b). These data are interesting since the parasitofauna of eels is strongly characterised and differently influenced by environmental conditions, particularly by salinity, as observed in other Mediterranean lagoons (Di Cave et al. 2001; Maíllo et al. 2005). However, according to Kennedy (1997), the use of intestinal helminths of *A. anguilla* as indicators of more specific environmental changes is difficult because they cannot provide indication on the nature of the changes such as long- or short-term pollution, or eutrophication.

Also notable was the current absence of the allogenic digenean *Gymnophallus fossarum*, the metacercariae of which were previously observed in *R. decussatus* (Figus et al. 2004). This is likely to be a consequence of the salinity-dependant process of extinction in Santa Gilla of its first intermediate host *Scrobicularia plana* (Cau and Murenu, unpublished results 2010).

In terms of the allogenic parasitofauna currently present in the lagoon, the data collected indicate that *P. carbo* is the most important ichthyophagous bird in the lagoon, since five helminth parasites of the cormorant were detected in at least ten fish species that act as intermediate hosts. Interestingly, parasites that have *P. carbo* as a definitive host, such as *S. hispida*, *G. lacteum* and *C. rudolphii*, occur as larvae in fish species usually considered as secondary prey for cormorants (Tables 3 and 4). In particular, the high occurrence of the anisakid *C. rudolphii* in *D. labrax* (with a prevalence of 83.9 % and MI of 95.1) indicates that cormorants and sea bass living in the Santa Gilla lagoon have a strong trophic link. Based on the life cycle of *C. rudolphii*, *D. labrax* seems to be an important prey organism for cormorants.

Grey mullets are also important, as they are the most abundant fish species in the lagoon. In fact, as shown in Fig. 3, the mugilid fish examined harboured larval parasites of cormorants, as *Ascocotyle* (*Phagicola*) sp. 1 and sp. 2, according to data that indicate that grey mullet is the most important prey for *Phalacrocorax carbo* in the brackish waters of the Mediterranean (Liordos and Goutner 2007; Cosolo et al. 2010), including Sardinia (Addis and Cau 1997; Cau et al. 2011).

These considerations are consistent with the results of a study carried out on the alimentary pellets of cormorants in the Cabras lagoon (western Sardinia), in which the remains of *D. labrax* had the third-highest abundance (7.09 %) among fish, immediately after those of *L. ramada* and *L. saliens* (Buttu et al. 2013). The comparison of the results of these two investigative methods, even if applied in two different sites, suggests that there may be a positive correlation between the level of infection of a parasite in its intermediate fish host and the predation activity of the piscivorous bird on that fish species. Moreover, in this correlation may play a role the behavioural changes that, as observed in some species (Dobson 1988; Combes 1991; Bartoli and Boudouresque 2007), larval parasites may induce in their host, e.g. changes in swimming depth or speed, or in body oscillation. In fact, such influences may enable predators to catch more easily the infected fishes.

Seagulls are also important definitive hosts, harbouring seven of the allogenic digenean species detected (Bartoli and Gibson 2007), but from a parasitological point of view, there is no close competition between *Larus* spp. and *P. carbo*; in fact, basing on the parasitological observations in this study, only three species of fish prey were shared by the birds (*S. solea*, *G. paganellus* and *S. aurata*). The remaining food items of gulls marked by parasites are bivalve molluscs, as indicated by the occurrence of metacercariae of Cryptogonymidae and Gymnophallidae in Veneridae and Cardiidae.

The high values of mean abundance relating to *S. sawakinensis cfr figure* in *S. aurata* (9.84) and *C. rudolphii* in *D. labrax* (79.84) indicate that even if these larvae are generalists, they do appear to have a host preference (Table 4). Thus, there is a strong trophic link between these intermediate and definitive hosts, which are seagulls and cormorants, respectively.

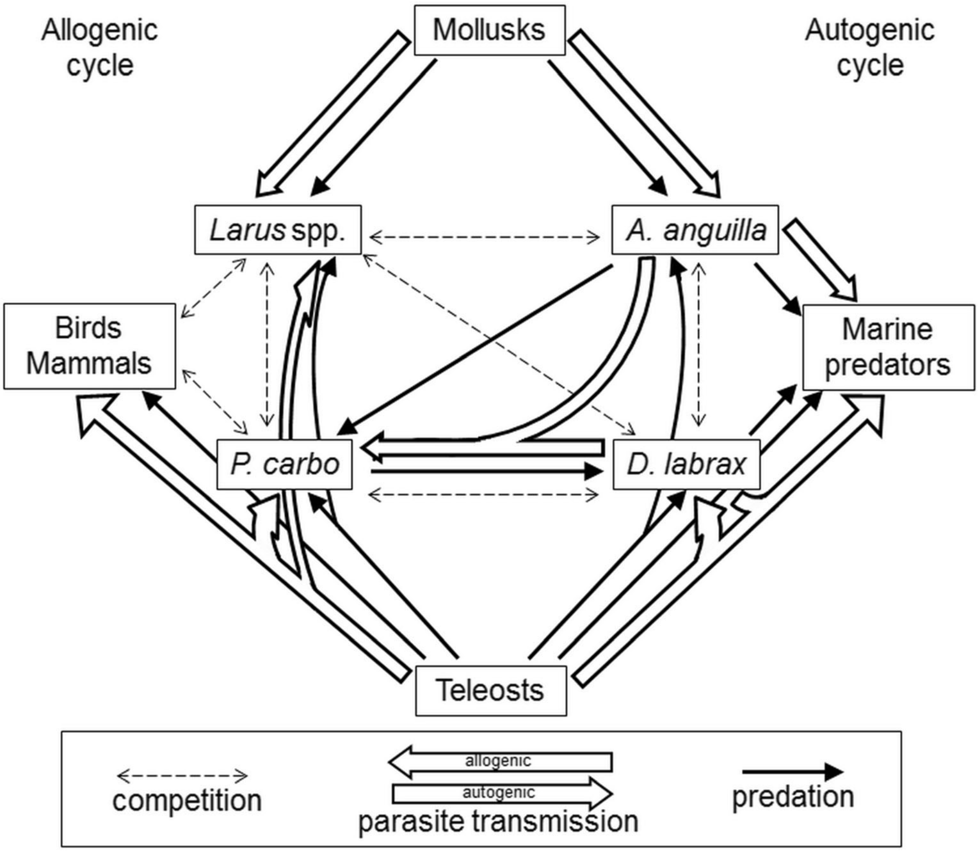

Fig. 4 Model of food web for a Mediterranean brackish area based on the parasitological analyses of second intermediate hosts (fishes and shellfishes) in the Santa Gilla lagoon. The symmetry of this scheme gives the idea of a balanced ecosystem in which the different levels are linked together by a variety of ecological relations. The hosts examined represent the base resource (lower and upper corner of the rhombus, respectively). The most important predators show competition in predation which may be related to the same group of parasites, i.e. autogenic (*A. anguilla* vs *D. labrax*) and allogenic (*P. carbo* vs *Larus* spp.) and to the two different groups (*Larus* spp. vs sea bass and eel; *P. carbo* vs *D. labrax*). In particular, *P. carbo* and sea bass show a double relation: in fact, the cormorant is the most active predator on sea bass, and both species compete on almost all fish species in the Santa Gilla lagoon

As discussed above, parasites with allogenic cycles mature not only in diving birds that are active predators in the lagoon, such as seagulls and cormorants, but also in other bird species with limicolous habits and mammals that feed close to the shallow shores of Santa Gilla. Thus, the allogenic parasitofauna of *A. fasciatus* might indicate that it is not a preferential prey for the main piscivorous birds of the lagoon. In fact, the parasites found as larvae in killifish (i.e. *Pygidiopsis* sp. and (*Ascocotyle*) *Phagicola* sp. 3) usually become adults in birds as Ardeidae, *Sterna hirundo* and/or mammals (Køie 1990); the same is valid for *Ascocotyle* sp., found in sea bass, and *H. heterophyes*, found in black goby (Elsheikha and Elshazly 2008). Moreover, their low occurrence in their respective hosts suggests that other fish species (or ones smaller than those examined) that swim near the shore of the lagoon act as second intermediate hosts of these heterophyids. However, the existence of a trophic link between the lagoon and the terrestrial environment is suggested.

It is also important to point out that, among allogenic parasites, well-known and potential zoonotic parasites were identified, such as *H. heterophyes*, *Pygidiopsis* sp., *S. sawakinensis*, *Ascocotyle* (*Phagicola*) sp. 1 and sp. 2 and *C. rudolphii*, the latter two reaching high indices of infection in largely marketed fish, such as grey mullet and sea bass, respectively This could represent a risk factor for human consumption (Chai and Lee 2002; Fried et al. 2004; Chai et al. 2005), suggesting the need for measures aimed to minimize the zoonotic threat, e.g. education programmes on the importance of cooking fish properly, protocols of veterinary checks on fish and control of the impact of warm-blooded definitive hosts (which may act as reservoir hosts) on the lagunar waters subject to fishing activities.

Comparing the links between predators and prey highlighted by parasitological data of the autogenic and allogenic groups (Fig. 2), the most noticeable emerging observation is the double ecological relation between *D. labrax* and *P. carbo*. First, the relatively high abundance of *C. rudolphii* L3 for sea bass when compared to other hosts examined adds evidence to the idea that if cormorants frequently prey on bass, they are more likely to be exposed to this nematode, suggesting that its life cycle is adapted to this trophic connection, as it is valid for many parasite species. According to the considerations of Marcogliese and Cone (1997), even if it is rarely practical to assess quantitatively the strength of links in food webs, the identification of this parasitic linkage within the food web may also help to indicate a relative importance of the coevolved predator–prey relationship between cormorant and sea bass in Santa Gilla lagoon.

Secondly, according to the parasitological data collected, *P. carbo* and *D. labrax* are linked as competitors for the food resource represented by small teleosts. In fact, at least nine of these fish species are utilized as second intermediate hosts by parasites of both cormorant and seabass. The diet of *D. labrax* overlaps with that of top predator species not only in lagunar but also in marine waters, as shown in a study conducted in the Bay of Biscay, where sea bass and common dolphin *Delphinus delphis* compete mainly for small pelagic fish of Clupeidae, Carangidae, Engraulidae and Scombridae families (Spitz et al. 2013). In addition, competition, even if less characterised by parasitological data, also occurs between seagulls and both *D. labrax* and *A. anguilla*: three small teleosts are shared as prey with sea bass and one mollusc species with eel (Table 4).

Previously, the Santa Gilla lagoon was considered at risk of eutrophication, and this was one of the reasons for its inclusion in the Montreaux record. Recently, in 2008, it was removed from the list after restoration and ameliorative measures conducted together with environmental investigations on potential targets of contamination, including fish and molluscs (source: http://www.ramsar.org). According to Esch (1971), eutrophic waters are usually rich in allogenic parasite species, because they are frequently visited by birds that shed the eggs of parasites and also feed on intermediate hosts in these waters. Conversely, environments with many autogenic parasites are considered rather oligotrophic because they are not attractive for birds. The results regarding the ratio between autogenic and allogenic larval parasites (14:16) detected in this 10-year survey indicate that the situation is currently balanced in the lagoon, which guarantees the existence of parasites of both categories in this ecosystem according to the analogue observations made by Zander (1988) in coastal Baltic waters.

In this study, the parasitological data collected mainly on the analyses of second intermediate hosts provided first a variety of information on the qualitative diet of the most important fish and bird predators of the Santa Gilla lagoon. The results also provide evidence relating to finer behavioural details, such as the occurrence of cannibalism in a fish species, or of the diets overlapping between predators. Such a data collection, especially if compared with the results of other studies in a multidisciplinary view, is able to offer a complementary point of view on the food web of a brackish ecosystem. This might be useful in the management of fishing resources, such as the sea bass, which at different age classes suffer the double negative effect of the cormorant, which is both a predatory on smaller sea bass and a competitor with larger bass.

The analyses of the parasitological data have also highlighted the general environmental changes that occurred throughout the study period. In the Santa Gilla lagoon, the recent data on parasitofauna highlight the undergoing salinisation process, determined by the massive inflow of marine water which is not regularly balanced by continental waters, because of the building, in the 1980s, of a system of dams in the mouth of the main rivers. Even if the available data on water salinity are very frammentary, the trend of this value is clear. In fact, if before the building of the dams, it did not reach the maximum of 21 ‰ (Cottiglia et al. 1973), in 1994 it ranged from 25 to 39 ‰, in 2000 from 19 to 46 ‰, in 2002 from 35 to 40 ‰ and in 2008/2009 from 17 to 33.5 ‰. During the period of this study, the minimum mean annual value was 26.5 ‰, recorded in 2010/2011 (Cannas A., personal communication). The consequences of this process may reflect in the parasitofauna of Santa Gilla lagoon: on the one hand, it results in the extinction of (or the reduction in) low-salinity species that act as first intermediate hosts, such as the bivalve *S. plana* for *G. fossarum*, and the above cited parasites of eels; on the other hand, it encourages the development of marine parasite cycles within the lagoon area, together with the occurrence of hosts in its waters. Furthermore, even if the trend to eutrophication persists (Cau and Murenu, unpublished results 2010), a balanced condition currently exists between the fully ‘aquatic’ food web, represented by 14 larval autogenic parasites, and the ‘terrestrial’ food web, represented by 16 allogenic species.

The richness and abundance of larval parasite species represents an element of the diversity and richness of the whole ecosystem, because a positive correlation exists between the diversity and abundance of parasites and their host species in wetlands (Huspeni et al. 2005). The parasites could serve as indicators for the evaluation of the diversity, structure and trophic links in a lagunar system, by providing information about the bird, fish and benthic communities.

Larvae of helminths in second intermediate hosts could also be used in combination with other techniques to understand the environmental status and its changes in variable systems such as brackish environments. The knowledge of food web components and their regulating interactions is a starting point for the valuation of the quality and the management of an ecosystem.

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