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Cormorant pellets as a tool for the knowledge of parasite-intermediate host associations and nematode diversity in the environment

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Summary

Anisakids are usually acquired through the diet. Cormorant pellets are useful to detect both parasite larval stages, and prey items which could act as intermediate hosts in the environment. The current study provides information about the feeding habits of both birds and mammals, and the diversity of parasites circulating in the environment. The objective of the study was to identify Anisakidae larvae and prey items in pellets from the Imperial shag *Phalacrocorax atriceps* and the Red-legged cormorant *P. gaimardi*, suggesting possible parasite–prey associations. A total of 92 *P. atriceps*' and 82 *P. gaimardi*'s pellets were collected from both Punta León, and Isla Elena bird colonies, respectively, during the period from 2006 to 2010. Pellets were preserved in ethanol and hard prey item remnants, and nematode larvae were studied using standard techniques. Prey item occurrence, nematode prevalence, and mean intensity were calculated. A correspondence analysis was performed to evaluate the larvae-prey association. *Contracaecum* spp., *Pseudoterranova* spp,, *Anisakis* spp., *Terranova* spp., and *Hysterothylacium* spp. third-stage larvae (L3) were identified in pellets. *Pseudoterranova* spp. and *Anisakis* spp. L3 predominated in the environment of Punta León, whereas *Contracaecum* spp. and *Hysterothylacium* spp. L3 predominated in the Puerto Deseado area. The highest larvae-prey association was that of *Contracaecum* spp. L3 with *Engraulis anchoita*, followed by with *Odontestes* sp. in *P. atriceps*' pellets. *Contracaecum* spp. L3 were significantly related to both sprats, *Sprattus fueguensis* and *Ramnogaster arcuatta*, in *P. gaimardi*'s pellets. It was verified that *E. anchovy* is the main gateway of *Contracaecum* spp. L3 in *P. atriceps*. *Odonthestes* sp. might act as an intermediate/paratenic host of *Contracaecum* spp. L3 in the area. Both sprats might play a role as intermediate/paratenic hosts of *C. australe*, being the main gateway into *P. gaimardi* in the area. Thus, pellet analysis can be postulated as a good tool for indicating parasite-host associations between anisakids, and the prey items which act as intermediate hosts.
Keywords: Anisakidae; pellets; *Phalacrocorax atriceps*; *Phalacrocorax gaimardi*, parasite-host association; Argentinean sea

Introduction

Most helminth parasites that occur in the marine environment have complex (indirect) life cycles. Among them, anisakid nematodes are important components in marine ecosystems (Rhode, 2005). Usually, the first step in the life cycle of the Anisakidae is an invertebrate (e.g. copepods) as first intermediate/paratenic host. Then, they frequently parasitize a fish as second intermediate/paratenic

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host, increasing the chances to reach their definitive host (DH), particularly piscivorous birds and mammals (Anderson, 2000; Rhode, 2005; Moravec, 2009). Therefore, the food web is the key to understanding the parasite community organization (Price, 1990; Anderson, 2000; Rhode, 2005).

The identification of host-parasite relationships and the role of each trophic item as intermediate, paratenic or definitive host are severely affected by difficulties associated in detecting endoparasites in each link of their life cycle. In such concern, the analysis of the stomach contents, pellets, and regurgitates of fish-eating birds, could be useful in detecting both larval parasites, and prey items that could act as intermediate/paratenic hosts in the environment.

The Imperial cormorant or shag *Phalacrocorax atriceps* (King, 1828), and the Red-legged cormorant *Phalacrocorax gaimardi* (Lesson & Garnot, 1828), are two of the five cormorant species nesting along the Argentinean coast. *Phalacrocorax atriceps* is distributed from Punta León, Chubut, to the Beagle Channel, Tierra del Fuego on the Argentinean coast (Harrison, 1983; Frere *et al*., 2005). *Phalacrocorax gaimardi* nests from Bahía Sanguinetto to Monte León, Santa Cruz Province (Frere *et al*., 2005). They are top predators in the marine food chain of the Patagonian coast, including mainly fish, and also mollusks or crustaceans in their diet, constituting an excellent model for the analysis of parasite-host interactions in the marine environment.

With the aim of determining if pellets of Phalacrocoracidae are indicators of larvae-intermediate/paratenic host association, the objectives of this study were to identify Anisakidae larvae and prey items found in pellets of both cormorants *P. atriceps* and *P. gaimardi*; to estimate interactions among them suggesting larvae-prey associations, and to suggest gateways of those anisakid species that are commonly found parasitizing both fish-eating birds (e.g. *Contracaecum* spp.).

Materials and Methods

From 2006 to 2010, a total of 92 pellets of *P. atriceps* were collected between bird nests at random in the Punta León cormorant colony, Chubut province, Argentina (43°05'S; 64°30'W) (Fig. 1), in two consecutive breading seasons: 47 pellets from December 2006 to January 2007, and 45 from December 2007 to January 2008. Later, 86 pellets of *P. gaimardi* were collected at random from November 2009 to January 2010 at the colony of Isla Elena, Ría Deseado, Santa Cruz province (47°45'S; 65°56'W) (Fig. 1). These 86 pellets are a subsample of a larger data set, which were used to describe *P. gaimardi* diet by Morgenthaler *et al*. 2016. In all birds, sampling was performed during the chick-rearing period from hatching up to the appearance of true feathers since this is the period of maximum activity for the food search (Yorio *et al*., 1998; Svagelj & Quintana, 2007).

Collected pellets were preserved in vials with 70 % ethanol, and once in the laboratory, they were disaggregated under a stereomicroscope. All hard prey remnants (e.g. otoliths, cephalopod beaks, etc), and nematode larvae were collected, cleared with lactophenol and studied under a light microscope (Garbin *et al*., 2007, 2008; 2011). Nematode identification was carried out following the appropriate taxonomic keys and bibliography (Hartwich, 1964, 1974; Fagerholm, 1990; Anderson, 2000). Parasite ecological indexes of prevalence (P), and mean intensity (MI) were calculated following Bush *et al*. (1997) only for larval stages. Prey items were identified by using reference collections, keys, and catalogues (Cousseau & Gru, 1982; Boschi *et al*., 1992; Gosztonyi & Kuba, 1996; Pineda *et al*., 1996; Volpedo & Echeverría, 2000). Prey occurrence was calculated for all items in pellets from both cormorant species.

A correspondence analysis (CA) was performed to evaluate the larvae-prey association (LPA) occurring in pellets from both fish-eating birds (Legendre & Legendre, 1998). This ordination technique allows the association of row (pellets) and column (species) frequencies in a contingency table. Prey item species with less than 5 % occurrence were excluded from the analysis since the rare taxa might introduce error and be placed at extreme ends of the first ordination axes relegating the major community trends to later axes (Gauch, 1982). We conducted all analyses with R 3.4.0 software (R Core Team 2017) using the vegan package (Oksanen *et al*., 2018).

Ethical Approval and/or Informed Consent

All pellets were collected without causing disturbance at both cormorant colonies of Punta León, Chubut, and Isla Elena, Santa Cruz, Argentina, with required permissions of the Dirección de Flora y Fauna Silvestre, Chubut, and the Dirección de Fauna Silvestre y Areas Protegidas, Santa Cruz, Argentina, respectively.

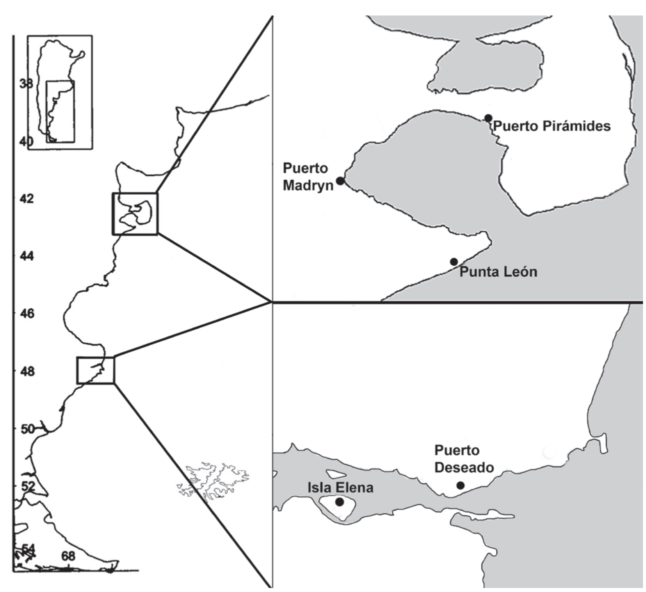

Fig. 1. Sampling sites of *Phalacrocorax atriceps* pellets at Punta León, Chubut coast, and *Phalacrocorax gaimardii* at Isla Elena, Ría Deseado, Santa Cruz Province, Argentina.

Results

From the analyzed *P. atriceps*'s pellets, 39 different prey items were identified, belonging to four different animal taxa: fish, mollusks, crustaceans, and polychaetes (Table 1). From *P. gaimardi*'s pellets, 10 different prey items were identified, belonging to the same four different animal taxa (Table 2).

Third-stage (L3), fourth-stage larvae (L4), and adults of five Anisakidae genera were identified in pellets from both cormorant species: *Contracaecum* Railliet & Henry, 1912, in both bird species; *Pseudoterranova* Mozgovoi, 1951, *Anisakis* Dujardin, 1845, *Terranova* Leiper & Atkinson, 1914, only in pellets of *P. atriceps*; and *Hysterothylacium* Ward & Magath, 1917, only in pellets of *P. gaimardi*. *Pseudoterranova* spp. L3 showed the highest prevalence (P=65.13) followed by *Anisakis* spp. L3 (P=43.66), *Contracaecum* spp. L3 (P=24.3), and *Terranova* spp. L3 (P=18.21) in *P. atriceps* pellets. The highest mean intensity was detected for *Pseudoterranova* spp. L3 (MI=4.32), followed by *Terranova* spp. L3 (MI=2.54), *Anisakis* spp. L3 (MI=1.92), and *Contracaecum* spp. L3 (MI=1.73). *Contracaecum* spp. L3 had the highest intensity and *Hysterothylacium* spp. L3 was the most prevalent anisakid in pellets of *P. gaimardi* (P=60.34, MI=3.25, and P=55.17, MI=4.56 respectively).

Only anisakid L3 were included in the CA analysis. The overall inertia was relatively low (2.893 out of 26) but still significant (χ^2 = 7111.2, N = 2458, P = <0.0001), indicating a weak association between parasites and prey items of *P. atriceps*. Moreover, the first two CA axes accounted for 37.86 % of the data. On *P. gaimardi*, the overall inertia was relatively higher (2.98 out of 7) and significant (χ^2 = 882.04, N = 296, P = <0.0001), indicating a stronger association between parasites and prey items. The first two CA axes accounted for 46.31 % of the data.

The highest significant larvae-prey association (LPA) in pellets of *P. atriceps* was revealed for *Contracaecum* spp. L3 with *Engraulis anchoita* Hubbs & Marini, 1935, followed by Afroditidae, *Odontesthes* sp., *Paralichthys* sp., Gammaridae sp., and Ostracoda (Fig. 2). *Pseudoterranova* spp. L3 showed a close relationship with Polyonidae, *Enteroctopus megalocyathus* (Gould, 1852), and *Octopus tehuelchus* d'Orbigny, 1834. *Anisakis* spp. L3 significantly associated with *Tegula* sp., followed by *Raneya brasiliensis* (Kaup, 1856), and *Percophis brasiliensis* Quoy & Gaimard, 1825. *Terranova* spp. L3 strongly associated with *Patagonotothen* sp., Eunicidae, *Triathalassothia argentina* (Berg, 1897), and *O. tehuelchus* (Fig. 2).

In *P. gaimardi*'s pellets, *Contracaecum* spp. L3 significantly associated with *Sprattus fueguensis* (Jenyns, 1842), followed by *Ramnogaster arcuatta* (Jenyns, 1842), *Loligo gahi* d'Orbigny, 1835, and *Patagonotothen* sp. *Hysterothylacium* spp. L3 associated with Nereididae, then with *S. fueguensis,* and *Odontesthes* sp. (Fig. 3).

Table 1. Occurrence of prey items in pellets of the Imperial shag *Phalacrocorax atriceps* from Punta León, Chubut province coast, Argentina.

Taxa	Species	Ocurrence (%)
Ophididae	*Raneya brasiliensis*	75.31
	Genypterus blacodes	1.23
Batrachoididae	*Triathalassothia argentina*	65.43
Clinidae	*Ribeiroclinus eigenmanni*	50.62
Nototheniidae	*Patagonotothen* sp.	19.75
Engraulidae	*Engraulis anchoita*	24.92
Agonidae	*Agonopsis chiloensis*	12.35
Pinguipedidae	*Pinguipes brasilianus*	10.28
	Pseudopercis sp.	12.35
Cheilodactylidae	*Nemadactylus bergi*	6.17
Serranidae	*Acanthistius brasilianus*	4.94
Zoarcidae	*Austrolycus laticinctus*	3.70
Paralichthyidae	*Paralichthys* sp.	4.02
	Xystreurys rasile	2.47
Percophidae	*Percophis brasiliensis*	2.43
Atherinopsidae	*Odontesthes* sp.	10.48
Merlucciidae	*Merluccius hubbsi*	5.23
Triglidae	*Prionotus* sp.	1.28
Myxinidae	*Myxine* sp.	1.25
Rajidae	*Raja* sp.	1.2
Octopodidae	*Enteroctopus megalocyathus*	22.22
	Octopus tehuelchus	19.75
Bivalvia	Heterodonta	9.88
Prosobranchia	*Tegula sp.*	8.64
Ostracoda	Ostracoda	8.64
Amphipoda	Gammaridae	2.47
Anomura	*Pachicheles chubutensis*	2.47
Caridea	*Atlantopandalus* sp.	4.94
	Nauticaris sp.	1.29
	Chorismus sp.	1.26
	Campylonotus sp.	1.23
	Betaeus sp.	1.21
	Pterygosquilla sp.	1.19
Brachiura	*Coenophthalmus tridentatus*	4.93
Solenoceridae	*Pleoticus muelleri*	1.23
Polychaeta	*Eunice* sp.	12.35
	Polyonidae	6.17
	Aphrodita sp.	3.70

Discussion

The Imperial shag in Punta León colony showed a preferably piscivorous diet with common prey items being *R. brasiliensis, T. argentina* and *Ribeiroclinus eigenmanni* (Jordan, 1888) (Malacalza *et al*., 1994; Punta *et al*., 2003). Fish such as *S. fueguensis* and

Table 2. Occurrence of prey items in pellets of the Red-legged cormorant *Phalacrocorax gaimardi* from Isla Elena, Ría Deseado, Santa Cruz province coast, Argentina.

Taxa	Species	Ocurrence (%)
Clupeidae	*Sprattus fueguensis*	32.86
	Ramnogster arcuata	14.29
Nototheniidae	*Patagonotothen sp.*	2.86
Pinguipedidae	*Pinguipes brasilianus*	1.43
Atherinopsidae	*Odontesthes sp.*	10.68
Zoarcidae	Zoarcid	1.43
Loliginidae	*Loligo gahi*	8.57
	Loligo sp.	12.86
Crustacea	*Eurypodius latreilli*	4.29
Nereididae	Nereidid	4.23

R. arcuatta were also the most frequent items in the Red-legged cormorant diet (Morgenthaler *et al*., 2016). Recorded anisakid prevalences suggested that *Pseudoterranova* spp. L3. and *Anisakis* spp. L3 predominated in the environment over the other two anisakid genera in the Punta León sea area, Chubut coast, whereas *Contracaecum* spp. L3 and *Hysterothylacium* spp. L3 predominated in the environment of Puerto Deseado, Santa Cruz coast. Thus, this study showed that pellets might serve to indicate the general diversity of the environment.

Contracaecum is the only genus of Anisakidae in this study that matures to adults in birds. The most significant LPA found in *P. atriceps* pellets was that of *Contracaecum* spp. L3 with *E. anchoita*. Previously, Diaz (2006), and Garbin *et al*. (2007) had suggested that *E. anchoita* might act as a transmitter of *Contracaecum pelagicum* L3 to the Magellanic Penguin *Spheniscus magellanicus* (Forster, 1781) and *P. atriceps*. Later, Garbin *et al*. (2013) proved this presumption analyzing phylogenetically through molecular markers (mtDNA cox2, rrnS, ITS-1, ITS-2 genes) specimens of *Contracaecum* spp. L3 from *E. anchoita*, and *C. pelagicum* of both sympatric fish-eating birds *P. atriceps* and *S. magellanicus* from Península Valdés (Garbin *et al*., 2013), the same area where the present pellets were collected. From these results, we can verify that *E. anchovy* is the main gateway of *Contracaecum* spp. L3 into *P. atriceps*. Therefore, the analysis of pellets was efficient to suggest possible parasite-host associations.

In the present analysis, *Contracaecum* spp. L3 also showed an association with *Odontesthes* sp. Carballo *et al*. (2011) reported *Contracaecum* L3 parasitizing the silversides *Odontesthes smitti* (Lahille, 1929), and *Odontesthes nigricans* (Richardson, 1848) in Península Valdés coast, Argentinean Sea. Present results reinforced the idea that *Odonthestes* sp. also should act as an intermediate/paratenic host of *Contracaecum* spp. L3 in the area. Phylogenetic molecular studies should be carried out to confirm this LPA hypothesis (Garbin *et al*. 2013).

Another significant LPA found was that between *Contracaecum* spp. L3 and the polychaete *Aphrodita* sp. Some records of *Contracaecum* parasitizing polychaetes exist but not in the Aphroditidae to date (Peoples, 2013).

Lower LPA were those between *Contracaecum* spp. L3 and *Paralichthys* sp., Gammaridae and Ostracoda. Incorvaia & Díaz de Astarloa (1998) also found *Contracaecum* L3 in *Paralichthys orbignyanus* (Valenciennes, 1839), and *Paralichthys patagonicus* Jordan, 1889, from the Argentine sea. Some authors have suggested gammarid amphipods and ostracods as intermediate/paratenic hosts of *Contracaecum* spp. larvae (Bartlett, 1996; Anderson, 2000; Moravec, 2009). Related to this, some ostracod species are prey items of *E. anchoita* and *Paralichthys* sp. (Capitanio *et al*., 2005; Ide *et al*., 2006). Therefore, it is possible to suggest that these arthropods are involved in some stage of the *Contracaecum* life cycle in the study area.

In this study, *Pseudoterranova* spp. L3 were the most abundant anisakid found showing the highest association with Polyonidae. McClelland *et al*. (1990) found *Pseudoterranova* L3 in polychaetes after they ingested copepods experimentally. Also, Martell & McClelland (1995) pointed out polychaetes as transmitters of *Pseudoterranova* L3. Associations of *Pseudoterranova* spp. L3 with *Enteroctopus* sp., and *Octopus* sp. are strange since there are no records of this anisakid parasitizing those cephalopods. *Terranova* spp. L3 strongly associated with *Patagonotothen* sp, Eunicidae,

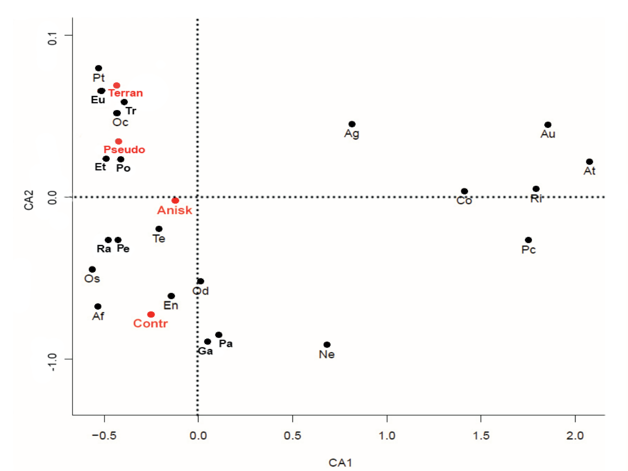

Fig. 2. Results of Correspondence Analysis (CA) revealing the larvae-prey associations in pellets of *Phalacrocorax atriceps* from Punta León, Chubut coast, Argentina. Contr: *Contracaecum* sp. L3; Anisk: *Anisakis* sp. L3; Pseud: *Pseudoterranova* sp. L3; Terran: *Terranova* sp. L3; En: *Engraulis anchoita*; Od: *Odontesthes* sp.; Ra: *Raneya brasiliensis*; Tr: *Triathalassothia argentina*; Ri: *Ribeiroclinus eigenmanni*; Pt: *Patagonotothen* sp.; Ag: *Agonopsis chiloensis*; Au: *Austrolycus laticinctus*; Pa: *Paralichthys* sp.; Pe: *Percophis brasiliensis*; Et: *Enteroctopus megalocyathus*; Oc: *Octopus tehuelchus*; Os: Ostracoda; Ga: Gammaridae; At: *Atlantopandalus* sp.; Co: *Coenophthalmus tridentatus*; Eu: *Eunice* sp.; Po: Polynoidae; Af: *Aphrodita* sp.

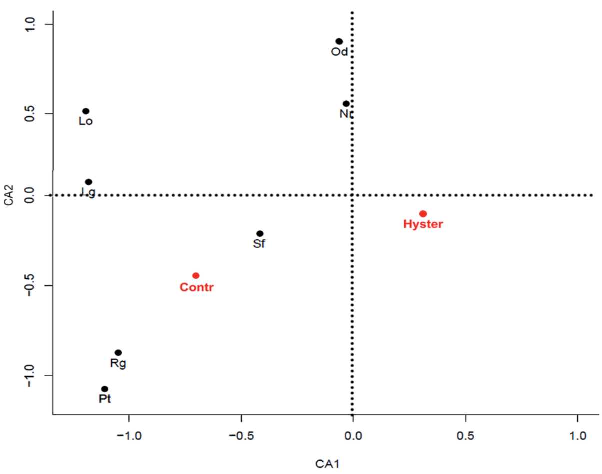

Fig. 3. Results of Correspondence Analysis (CA) revealing the parasite-pray associations in pellets of *Phalacrocorax gaimardii* from Isla Elena, Cruz Province, Argentina. Contr: *Contracaecum* sp. L3; Hyster: *Hysterothylacium* sp. L3; Sp: *Sprattus fueguensis*; Rg; *Ramnogster arcuata*; Od: *Odontesthes* sp.; Pt: *Patagonotothen* sp.; Lg: *Loligo gahi*; Lo: *Loligo* sp.; Ne: Nereididae.

and *T. argentina*, and *Octopus* sp. However, there are no records of this anisakid genus parasitizing any of the latter prey items up to date. Despite *Anisakis* spp. L3 closely associated with *Tegula* sp., it is not possible to speculate a parasite-host association because there are no records of gastropods as intermediate/paratenic host of anisakid species. None of the mentioned genera are parasites of birds.

The analyses carried out on *P. gaimardi*'s pellets from the Ría Deseado showed the two highest LPA of *Contracaecum* spp. L3 with both sprat species *S. fueguensis* and *R. arcuata*. Other studies have confirmed the parasitism of *Contracaecum* in the genus *Sprattus* (Skrzypczak & Rolbiecki, 2015; Zuo *et al*., 2016). In addition, *Contracaecum australe* Garbin *et al*., 2011, was reported previously parasitizing *P. gaimardi* in the same area (Garbin *et al*. 2014). Therefore, both sprats *S. fueguensis* and *R. arcuata* might play a role as intermediate/paratenic hosts of *C. australe*, being the main gateway to *P. gaimardi* in the study area. As mentioned before, phylogenetic molecular studies should be carried out on these nematodes (Garbin *et al*. 2013).

Also, significant LPA between *Hysterothylacium* spp. L3 with Nereididae, *S. fueguensis* and *Odontesthes* sp. were observed in *P. gaimardi*'s pellets. No records of *Hysterothylacium* parasitizing this polychaete are available. However, there are some records on *Hysterothylacium aduncum* (Rudolphi, 1802) isolated from *Sprattus sprattus* (Linnaeus, 1758) in different geographical areas in the Northeastern Atlantic and Southern Baltic Sea (Klimpel *et al*. 2007; Skrzypczak & Rolbiecki, 2015). In addition, *Odontesthes bonariensis* (Valenciennes, 1835) is parasitized by *Hysterothylacium* sp. larvae from two Argentinean lagoons (Drago, 2012). Thus, it is possible to suggest *S. fueguensis* and *Odontesthes* sp. as first intermediate hosts of *Hysterothylacium* spp. L3 since the adult nematodes parasitize other teleost fish.

In this study, *Contracaecum* also showed LPA with the Patagonian squid *L. gahi* in *P. gaimardi*. Some cephalopods have been recorded to be parasitized by *Contracaecum* L3 as paratenic -transport- hosts (Shukhgalter & Nigmatullin, 2001; Salati *et al*., 2013). However, only *Loligo forbesi* (Steenstrup, 1856) was shown to be infected with *Contacaecum* L3 (Smith, 1984). Not only surveys on this squid are needed but also molecular studies on *Contacaecum* L3 must be carried out.

According to the present results it is possible to postulate pellets as good tools to indicate parasite-host associations between anisakids and the prey items which act as intermediate/paratenic hosts. These kind of studies also provide information about the feeding habits of both birds and mammals, and about the diversity of parasites circulating in the environment.

Conflict of Interest

Authors state no conflict of interest.

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