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

L. GARBIN1, 2*, J. I. DIAZ2, A. MORGENTHALER3, A. MILLONES3, L. KUBA4, D. FUCHS5, G.T. NAVONE2

1Sección Ornitología, División Zoología Vertebrados (FCNyM-UNLP–CONICET), La Plata, Buenos Aires, E-mail: lgarbin@fcnym.unlp.edu.ar; 2Centro de Estudios Parasitológicos y de Vectores (CEPAVE-UNLP–CONICET), La Plata, Buenos Aires; 3Centro de Investigaciones de Puerto Deseado (UNPA-UACO), Puerto Deseado, Santa Cruz; 4Centro Nacional Patagónico (CCT CONICET), Puerto Madryn, Chubut, Argentina; 5Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (CICyTTP-UADER–CONICET), Diamante, Entre Ríos

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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. Odontesthes 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. australis, 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 host. Therefore, it is crucial to identify possible intermediate/paratenic hosts that act as first intermediate hosts for Anisakidae larvae. Cormorants and pelicans are diving birds that usually feed on small fish and crustaceans (Carr, 2009; Van Rossum et al., 2003). Pellets analysis is a useful tool to detect both parasite larval stages and prey items which could act as intermediate hosts in the environment. The main objective of this 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.
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 Bahia 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 breeding 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 (Harwich, 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.
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 (2.89 out of 26) but still significant ($\chi^2 = 7111.2, N = 2458, P = <0.0001$), 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 ($\chi^2 = 882.04, N = 296, P = <0.0001$), 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 Hysterothylacium sp. L3 (P=18.21) in Terranova spp. L3 (P=24.3), and Anisakis spp. L3 (P=43.66) in Contracaecum spp. L3 (P=18.21) in P. atriceps pellets. The highest mean intensity was detected for Pseudoterranova spp. L3 (MI=1.92), followed by Anisakis spp. L3 (MI=1.23), and Contracaecum spp. L3 (MI=1.23). 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).

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

| Taxa               | Species                        | Occurrence (%) |
|--------------------|--------------------------------|----------------|
| Ophidiidae         | Raneya brasiliensis            | 75.31          |
|                    | Genypterus blacodes            | 1.23           |
| Batrachoididae     | Trihalassothia argentina       | 65.43          |
| Clinidae           | Ribeirocinus eigenmanni        | 50.62          |
| Nototheniidae      | Patagonotothen sp.             | 19.75          |
| Engraulidae        | Engraulis anchoita             | 24.92          |
| Agonidae           | Agonopsis chioensis            | 12.35          |
| Pinguiipedidae     | Pinguipes brasilius            | 10.28          |
|                    | Pseudopercis sp.               | 12.35          |
| Cheilodactylidae   | Nemadactylus bergi             | 6.17           |
| Serranidae         | Acanthistius brasilius         | 4.94           |
| Zoaicidae          | Austrolycus laticinctus        | 3.70           |
| Paralichthyidae    | Paralichthys sp.               | 4.02           |
|                    | Xysteurys rasile               | 2.47           |
| Percophidae        | Percophis brasiliensis         | 2.43           |
| Atherinopsida      | Odontesthes sp.               | 10.48          |
| Merlucciidae       | Merluccius hubbsi              | 5.23           |
| Trigidae           | Prionotus sp.                  | 1.28           |
| Myxinidae          | Myxine sp.                     | 1.25           |
| Rajidae            | Raja sp.                      | 1.2            |
| Octopodidae        | Enteroctopus megalocyathus     | 22.22          |
|                    | Octopus tehueltchus            | 19.75          |
| Bivalvia           | Heterodonta                   | 9.88           |
| Prosobranchia      | Pegala 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      | Plectus mueller                | 1.23           |
| Polychaeta         | Eunice sp.                     | 12.35          |
|                    | Polygonidae                    | 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 Ribeirocinus eigenmanni (Jordan, 1868) (Malacalza et al., 1994; Punta et al., 2003). Fish such as S. fueguensis and
Another significant LPA found was that between Contracaecum spp. L3 and the polychaete Aphroditida 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 Asturioa (1998) also found Contracaecum L3 in Paralichthys ornibyanus (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,
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, contracaecum argentinum rastrum and Odontesthes sp. Such associations were observed in the study area (Garbin et al. 2013). Therefore, both sprats S. fueguensis and R. arcuata might play a role as intermediate/paratenic hosts of C. australis, 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 Contracaecum L3 (Smith, 1984). Not only surveys on this squid are needed but also molecular studies on Contracaecum 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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