



RESEARCH ARTICLE

# Community ecology of the metazoan parasites of the Atlantic anchoveta, *Cetengraulis edentulus* (Actinopterygii: Engraulidae) from the Sepetiba Bay, Rio de Janeiro, Brazil

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**ABSTRACT.** The Atlantic anchoveta, *Cetengraulis edentulus* (Cuvier, 1829) is one of the most commercially important fish species in the littoral of Brazil. The present study evaluates the parasitic fauna of this engraulid fish from the southeastern Brazilian coast. Between October 2019 and March 2020, a total of 100 specimens of *C. edentulus* from the Sepetiba Bay, Rio de Janeiro, Brazil (22°57'44"S; 43°52'28"W), were examined. Eighty-five specimens of *C. edentulus* were parasitized by at least one species of metazoan, with a mean of  $4.32 \pm 6.12$  parasites/fish. Eleven species of parasites were collected: five digeneans, two monogeneans, two copepods, one isopod and one nematode. The nematode *Hysterothylacium* sp. was the most abundant and dominant species, representing 51.85% of the metazoan parasites collected, showing positive correlation with the host's total length and parasite abundance and prevalence. The mean abundance and prevalence of *Hysterothylacium* sp. was significantly higher in female hosts. One pair of larval endoparasites showed positive covariation. *Cetengraulis edentulus* represents a new host record for nine species of parasites. A dominance of endoparasitic larval stages is documented in the parasite community of *C. edentulus*. This may be a function of the feeding habits of engraulid fish, which feed mainly on zooplanktonic organisms. It may also have to do with the fact that *C. edentulus* is the intermediate and/or paratenic host of fish parasites, birds, and marine mammals.

**KEY WORDS.** Clupeiformes, engraulids, helminths, infracommunity, marine fish, parasitic crustaceans, structure of community.

## INTRODUCTION

The Sepetiba Bay is located west of the state of Rio de Janeiro, with an area of approximately 519 km<sup>2</sup> (Araujo et al. 2017). This bay is a microtidal estuarine lagoon, and one of the most important aquatic ecosystems in the state, with approximately 97 species of fish reported (Araujo et al. 1998). However, since the 1950s, with the expansion of urbanization and industrial development, this bay became a part of the geoeconomic center of Brazil, housing an important industrial park that includes the largest steel complex in Latin America (Araujo et al. 2017). The intensification of anthropogenic factors in recent decades has caused the emergence of new potential sources of diffused pollution (Pfeiffer et al. 1985, Araujo et al. 2017).

Species of Engraulidae are one of the most important fishing resources in many parts of the world. They represent

about 25% of the total production of fish caught commercially in the world (Vasconcellos and Csirke 2011, Vicente et al. 2020). Currently, engraulids are represented by 150 species distributed in 17 genera around the world. Most species are found in shallow coastal waters and estuaries in tropical and temperate regions (Nelson 2016, Froese and Pauly 2021). The Atlantic anchoveta, *Cetengraulis edentulus* (Cuvier, 1829) is a brackish fish, commonly found in lagoons and estuaries with low salinity or even in freshwater environments. It is distributed from Cuba to southern Brazil (Silva et al. 2003, Froese and Pauly 2021). Usually occurring in large schools, these engraulids are presumably filter-feeders. Their diet is composed of phytoplankton and zooplankton (Froese and Pauly 2021). *Cetengraulis edentulus* is one of the most commercially important fish species in the littoral of Brazil, with annual catches over 1200 t. In the Sepetiba Bay *C. edentulus* represents the bulk of engraulid fish catches, followed

by *Anchoa tricolor* (Spix & Agassiz, 1829) (Silva and Araujo 2000, Silva et al. 2003, Santos et al. 2020).

In the South Atlantic Ocean, studies regarding the metazoan parasite community of engraulids are scarce. Timi (2003) and Timi and Poulin (2003) studied the parasite population and ecological features of *Engraulis anchoita* Hubbs & Marini, 1935 from the coastal zone of Argentina, and Tavares et al. (2005) investigated the parasitic fauna of *A. tricolor* from the coastal zone of the State of Rio de Janeiro, Brazil. Studies focusing on the ecology of the parasitic fauna of *C. edentulus* are lacking. However, three species of parasites have been recorded from this fish in Brazilian waters: *Pseudanthocotyloides heterocotyle* (van Beneden, 1871) (Monogenea) off Rio de Janeiro, pranzia larvae of *Gnathia* sp. (Isopod) off Pará and *Livoneca desterroensis* Thatcher, Souza-Conceição & Jost, 2003 (Isopod) off Pará and Santa Catarina (see Kohn et al. 1992, Thatcher et al. 2003, Monfort et al. 2009, Luque et al. 2013).

In the present contribution, we analyzed the composition and structure of the metazoan parasite community of *C. edentulus* from the Sepetiba Bay, Rio de Janeiro, Brazil.

## MATERIAL AND METHODS

### Fish sampling and analysis of parasites

Between October 2019 and March 2020, 100 specimens of *C. edentulus* were obtained from the Sepetiba Bay (22°57'44"S; 43°52'28"W), Rio de Janeiro, Brazil. Specimens were necropsied for the study of their community of metazoan parasites. Fish that had been collected by local fishermen were purchased at the fish market. The specimens were identified according to Figueiredo and Menezes (1978); the nomenclature and classification were updated according to FishBase (Froese and Pauly 2021). Hosts were mostly fresh, but some specimens were kept frozen at -20 °C, until examination.

All organs (i.e., body surface, nostrils, gills, branchial, and body cavities, oesophagus, stomach, intestine, mesenteries, heart, liver, gonads, swim bladder and musculature) were individually examined for the presence of parasites, using a stereomicroscope. The metazoan parasites were picked, fixed, preserved and processed for identification, according to standard protocols (Eiras et al. 2006). Taxonomic identification of the metazoans followed specific literature, pertinent to each taxon.

### Statistical analyses of parasites and deposited specimens

The analysis included only parasite species with a prevalence higher than 10% (Bush et al. 1997). The variance-to-mean ratio of parasite abundance (index of dispersion) and the discrepancy index, computed using the program Quantitative Parasitology 3.0 (Rózsa et al. 2000), were used to detect the distribution patterns of the infrapopulations (Poulin 1993). The dominance frequency (percentage of infracommunities in which either parasite species was dominant) and the relative dominance (number of specimens of one species/total number of specimens

of all species in the infracommunity) of each parasite species were calculated according to Rohde et al. (1995). The diversity of parasite species was calculated using the Brillouin index (H), because each fish analyzed corresponded to a fully censused community (Zar 1996). The Spearman's rank correlation coefficient (rs) was calculated to determine a possible correlation between the host's total length and parasite abundance. Pearson's correlation coefficient (r) was used to indicate the relationship between the host's total length and parasite prevalence, with previous arcsine transformation of the prevalence data (Zar 1996). The effect of host sex on abundance and prevalence of parasites was tested using the Zc (normal) approximation to the Mann-Whitney test and the Fisher exact test, respectively. The probable variation of diversity in relation to host sex (Mann-Whitney test) and to host's total length (Spearman's rank correlation coefficient) was tested. Possible interspecific association between concurrent species was determined using the chi-square test. Possible covariation among the abundance of concurrent species was analyzed using the Spearman's rank correlation coefficient. The ecological terminology used follows Bush et al. (1997). Statistical significance level was evaluated at  $p \leq 0.05$ .

Voucher specimens of helminths were deposited in the Helminthological Collection of Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil; copepods were deposited in the scientific collection of the Museum of Zoology (MZUSP), University of São Paulo, Brazil.

## RESULTS

The average total length of the fish was 12.3–16.5 (14.8 ± 0.82) cm, and the weight was 18–57 (31.2 ± 6.42) g. The average total length of male (14.52 ± 0.84 n = 58) and female (15.21 ± 0.63 n = 42) fish in the studied sample were not significantly different ( $t = 4.660$ ,  $p = 0.998$ ).

### Component community

Eleven species of metazoan parasites were collected (Table 1). *Cetengraulis edentulus* is a new host record for nine of the eleven species collected, with exceptions made for the monogenean *P. heterocotyle* (Kohn et al. 1992) and for the isopod *L. desterroensis* (Luque et al. 2013). The nematode *Hysterothylacium* sp. was the most abundant and dominant species, representing 51.85% of the metazoan parasites collected, with greatest values of mean relative dominance and frequency of dominance (Table 2). The monogenean *P. heterocotyle* was the most prevalent species (Table 1).

Larval endoparasites represented 55.32% of all parasites collected, adult endoparasites amounted to 22.45%, and ectoparasites made up 22.22%. All parasites of *C. edentulus* had the typically aggregated distribution pattern observed in many parasite systems (Table 3). The nematode *Hysterothylacium* sp. showed positively correlation between host's total length and parasite abundance ( $r_s = 0.346$ ,  $p < 0.001$ ) and prevalence

Table 1. Prevalence, mean intensity, mean abundance, and site of infection/infestation of metazoan parasites of *Cetengraulis edentulus* from the Sepetiba Bay, Rio de Janeiro, Brazil.

Parasites	Voucher number	Prevalence (%)	Mean intensity	Mean abundance	Site of infection/infestation
<b>Digenea</b>					
<i>Monorchis</i> sp.	CHIOC # 39591	19	2.42 ± 2.01	0.46 ± 1.28	Stomach
<i>Parahemiurus merus</i>	CHIOC # 39593	19	1.52 ± 0.77	0.29 ± 0.68	Stomach
<i>Rhipidocotyle</i> sp. (metacercariae)	CHIOC # 39592	12	1.25 ± 0.45	0.15 ± 0.43	Mesenteries
<i>Parahemiurus</i> sp.	CHIOC # 39589	8	1.62 ± 0.74	0.13 ± 0.48	Stomach
<i>Lecithochirium microstomum</i>	CHIOC # 39588	6	1.51 ± 0.83	0.09 ± 0.40	Stomach
<b>Monogenea</b>					
<i>Pseudanthocotylodes heterocotyle</i>	CHIOC # 39594	48	1.79 ± 1.39	0.86 ± 1.31	Gills
<i>Cribomazocraea travassosi</i>	CHIOC # 39590	2	1	0.02 ± 0.14	Gills
<b>Nematoda</b>					
<i>Hysterothylacium</i> sp. (larval)	CHIOC # 39103	40	5.61 ± 7.69	2.24 ± 5.55	Mesenteries
<b>Copepoda</b>					
<i>Neobomolochus elongatus</i>	MZUSP # 42134	3	1	0.03 ± 0.17	Opercular cavity
<i>Nothobomolochus cresseyi</i>	MZUSP # 42135	2	1	0.02 ± 0.14	Opercular cavity
<b>Isopoda</b>					
<i>Livoneca desterroensis</i>	MZUSP # 42133	3	1.50 ± 0.70	0.03 ± 0.22	Opercular cavity

 Table 2. Frequency of dominance and mean relative dominance of metazoan parasites of *Cetengraulis edentulus* from the Sepetiba Bay, Rio de Janeiro, Brazil.

Parasites	Frequency of dominance	Frequency of dominance shared with one or more species	Mean relative dominance
<i>Hysterothylacium</i> sp. (larval)	27	5	0.280 ± 0.398
<i>Pseudanthocotylodes heterocotyle</i>	24	8	0.273 ± 0.374
<i>Parahemiurus merus</i>	9	6	0.076 ± 0.196
<i>Monorchis</i> sp.	8	2	0.086 ± 0.219
<i>Parahemiurus</i> sp.	2	3	0.031 ± 0.127
<i>Rhipidocotyle</i> sp. (metacercariae)	2	2	0.038 ± 0.156

 Table 3. Values of variance to mean ratio of parasite abundance (ID) and index of Discrepancy (D) of metazoan parasites of *Cetengraulis edentulus* from the Sepetiba Bay, Rio de Janeiro, Brazil.

Parasites	ID	D
<i>Hysterothylacium</i> sp. (larval)	14.183	0.830
<i>Monorchis</i> sp.	3.576	0.873
<i>Pseudanthocotylodes heterocotyle</i>	2.021	0.667
<i>Parahemiurus merus</i>	1.623	0.830
<i>Rhipidocotyle</i> sp. (metacercariae)	1.263	0.889

( $r = 0.292$ ,  $p = 0.003$ ). The mean abundance and prevalence of *Hysterothylacium* sp. were significantly higher in the female (4.16 and 62%) than in the male (0.84 and 24.1%) hosts ( $Z_c = -3.406$ ,  $p < 0.001$ ;  $F = 0.007$ ).

#### Infracommunities

Eighty-five specimens (85%) of *C. edentulus* were parasitized by at least one parasite species. A total of 432 individual parasites were collected, with a mean of  $4.32 \pm 6.12$  parasites/fish. Total host length and parasite abundance were not correlated ( $r_s = 0.096$ ,  $p = 0.340$ ). The mean parasite species richness  $1.63 \pm 1.29$ , was not correlated with the total body length of the fish ( $r_s = -0.028$ ,  $p = 0.779$ ). Forty-three specimens (43%) showed infection with one parasite species, and 19 (19%), 15 (15%), 5

(5%), 1 (1%) and 2 (2%), had multiple infections with 2, 3, 4, 5, 6 parasite species, respectively. Mean parasite species diversity ( $H = 0.101 \pm 0.151$ ) was not correlated with the total length of the host ( $r_s = -0.038$ ,  $p = 0.707$ ) and no significant differences in parasite diversity were observed between male ( $H = 0.114 \pm 0.164$ ) and female ( $H = 0.082 \pm 0.130$ ) fish ( $Z_c = 0.719$ ,  $p = 0.236$ ).

The endoparasites were separated into two groups – helminth larval stages (digeneans and nematodes) and adult endoparasites (digeneans) – and were used to determine possible interspecific associations. Ectoparasites were not included in this analysis because only one ectoparasitic species showed a prevalence higher than 10% (see Table 1). The helminth larval stages pair, *Rhipidocotyle* sp. – *Hysterothylacium* sp., did not share significant association ( $\chi^2 = 1.910$ ,  $p = 0.167$ ), but showed positive covariation ( $r_s = 0.234$ ,  $p = 0.018$ ). The adult endoparasites pair, *Monorchis* sp. – *Parahemiurus merus* (Linton, 1910), did not share significant association and covariation ( $\chi^2 = 2.412$ ,  $p = 0.120$ ;  $r_s = 0.138$ ,  $p = 0.170$ ).

## DISCUSSION

The present study showed that the parasitic fauna of *C. edentulus* was dominated by endoparasites in the larval stage. Sergipense et al. (1999) studied the feeding habits of *C. eden-*

*tulus* from the Sepetiba Bay and concluded that this species is a planktivorous filter-feeder fish. However, the dominance of endoparasitic larval stages found in our study may indicate that they consume zooplanktonic organisms, since they putatively act as intermediate and/or paratenic hosts for parasites of fish, birds and marine mammals. The occurrence of copepods in the diet of *C. edentulus* had been observed before at the mangrove of Itacorubi, Santa Catarina by Clezar et al. (1993) and at the Itaipu lagoon, Niterói, Rio de Janeiro, by Gay et al. (2002), who concluded that *C. edentulus* is a primarily phytoplanktophagus species that has no selective feeding habits, ingesting whatever is available in the environment. This may explain why copepods were found in the diet of *C. edentulus*. The presence of larval stages in engraulid fish had already reported by Timi (2003) and Tavares et al. (2005) (see introduction for localities and hosts). According to them, this is not surprising, because the anchovies feed mainly on zooplanktonic organisms, which are known to act as intermediate and/or paratenic hosts for parasites of several piscivorous vertebrates. In addition, the dominance of larval endoparasites has been described for some parasite communities of marine fish from the coastal zone of southeastern Brazil (Paraguassú et al. 2002, Luque et al. 2002, 2003, Sabas and Luque 2003, Alves and Luque 2006, Soares et al. 2014).

The nematode *Hysterothylacium* sp. was the most abundant and dominant species of the parasitic fauna of *C. edentulus*. The larval stages of *Hysterothylacium* have been reported infecting more than 30 fish species off the coast of Brazil (Luque and Poulin 2004, Tavares and Luque 2006, Knoff et al. 2007, Luque et al. 2011, Moreira et al. 2015, Pantoja et al. 2016), including *Anchoa marinii* Hildebrand, 1943 and *A. tricolor* (Tavares et al. 2005, Tavares 2006) (Engraulidae). The prevalence and abundance of larvae was low (less than 10%) in the last two, in comparison to *C. edentulus* (40%). The significant presence of ascaridoid nematodes is a major concern to public health and fish marketing, because the third larval stage (L3) can be accidentally consumed by humans when they eat raw or poorly cooked fish and that may cause anisakiasis disease (Limbery and Cheah 2007). However, according to some authors, the pathogenic potential *Hysterothylacium* nematodes is still controversial, and their involvement in human pathologies is questionable (Yagi et al. 1996, Cavallero et al. 2020). Based on morphological examination, *Hysterothylacium* sp. in the present study is similar to the third stage of *Hysterothylacium* type V of Pantoja et al. (2016).

Five species of digenetic trematodes were found in the gastrointestinal system of *C. edentulus* in the present study. Two, *Lecithochirium microstomum* Chandler, 1935 and *P. merus*, are cosmopolitan species found in several species of actinopterygian fish around the world (Bray 1990, Braicovich and Timi 2008). In the South Atlantic Ocean, *P. merus* can be found in five engraulid fish, namely, *A. tricolor*, *Anchoa argentivittata* (Regan, 1904), *E. anchoita*, *Engraulis ringens* Jenyns, 1842 and *Lycengraulis grossidens* (Spix & Agassiz, 1829), while *L. microstomum* was found only in *E. anchoita* (Timi et al. 1999a, Kohn et al. 2007). However, only *P.*

*merus* has been reported in other representatives of Engraulidae in different oceans, i.e., *Engraulis australis* (White, 1790) from Australia, *Engraulis capensis* Gilchrist, 1913 from South Africa, *Engraulis encrasicolus* (Linnaeus, 1758) from Europe and Africa, *Engraulis japonicus* Temminck & Schlegel, 1846 from Japan, *Engraulis mordax* Girard, 1854 from the United States and *Thryssa setirostris* (Broussonet, 1782) from China (Bray 1990, Timi et al. 1999b, Sailaja and Madhavi 2012). Thus, the present findings reaffirm the presence of hemiuroid digeneans in engraulid fish, now including *C. edentulus*.

In the present study two species of bomolochid copepods are recorded by the first-time parasitizing *C. edentulus*. One of these, *Nothobomolochus cresseyi* Timi & Sardella, 1997, was previously reported on two engraulid fish in the South Atlantic, i.e., *E. anchoita* from the littoral of Argentina and *A. marinii* from the littoral of Brazil (Timi 2003, Luque and Tavares 2007). On the other hand, *Neobomolochus elongatus* Cressey, 1981 has been reported only on the clupeid fish *Opisthonema oglinum* (Lesueur, 1818) from two different localities, first off the coast of Florida, littoral of USA and recently in the Sepetiba Bay, littoral of Brazil (Cressey 1981, Chaves and Paschoal 2021). Clupeiformes is one of the major orders of fish, with seven families, 92 genera, and about 405 species (Nelson et al. 2016, Froese and Pauly 2021). In the Sepetiba Bay this order is represented by the families Clupeidae and Eugraulidae, each with four species. They form large schools that overlap spatially and can be mixed schools (Araujo et al. 1998, Silva and Araujo 2000, Paiva and Pereira 2003). According to Tavares and Luque (2004), the aggregated habits and high population density of some hosts may facilitate the transmission of some ectoparasites with a direct life cycle, such as copepods and monogeneans. Thus, it is reasonable to consider the possible flux of ectoparasites between these clupeiform host species. The present study records for the first time the copepod *N. elongatus* parasitizing a representative of Engraulidae.

The monogenean *Pseudanthocotyloides heterocotyle* presented the highest prevalence among the metazoan parasites collected in *C. edentulus* in this study. This parasite has been previously recorded in Brazil parasitizing the gills of *C. edentulus*. One specimen was found on the carangid fish *Decapterus punctatus* (Cuvier, 1829). Both records are from the coast of the state of Rio de Janeiro (Kohn et al. 1992). Despite the record in a representative of the Carangidae, this species apparently prefers Clupeiform fish, e.g. the clupeids *Clupea harengus* Linnaeus, 1758 and *Sprattus sprattus* (Linnaeus, 1758) (type host), and the engraulids *A. marini*, *C. edentulus*, *E. anchoita*, *E. encrasicolus* and *E. ringens* being distributed in the Mediterranean Sea; North Sea, east and west coast of Scotland, west coast of Sweden; coasts of Argentina, Brazil, Chile and Uruguay (Euzet and Prost 1969, Mamaev 1982, Kohn et al. 1992, Groenewald et al. 1996, Longshaw 1996, Timi et al. 1999b, Rahimian 1999, Valdivia et al. 2007). *Cribomazocraes travassosi* Santos & Kohn, 1992 was also collected from the gills of *C. edentulus* in the present study, but it was much less prevalent (2%) than *P. heterocotyle*. This species

was first described by Santos and Kohn (1992) from the gills of *Harengula clupeiola* (Cuvier, 1829) and later it was reported by Moreira et al. (2015) in *Sardinella brasiliensis* (Steindachner, 1879), both Clupeid fish from from the coast of Rio de Janeiro. Species of monogeneans are known to be specific to a single host species, a genus, a family, or even an order (see Whittington et al. 2000). Considering that the clupeiform fish occur in mixed schools at Sepetiba Bay (see the previous paragraph), the presence of *C. travassosi* on the studied fish is possible. This study records *C. travassosi* for the first time parasitizing a representative of Engraulidae.

Studies on the biology of *C. edentulus* revealed that sexual maturation in both sexes usually occurs when individuals reach 11 to 20 cm (Silva et al. 2003, Souza-Conceição et al. 2005). For Fréon and Misund (1999), the approximation of growth percentages in certain fish species is an adaptive strategy, especially in fish that form large schools. In the present study, the correlation between the sex of *C. edentulus* and the abundance and prevalence of the larval nematode *Hysterothylacium* sp. were unexpected because the specimens of the Atlantic anchoveta studied were adults, and no significant differences were detected between the size of males and females. According to Poulin (1996), factors such as female gonadal hormones, immune system competence and even pregnancy can increase the female's susceptibility to parasites and pathogens. Thus, territoriality, movement patterns, social interactions and diet can influence the exposure to infectious stages of the parasites (Poulin 1996). Positive correlations between the sex of the host and the infection levels of some components of the parasite communities were also detected in other Brazilian marine fish and suggest ecological and/or behavioral differences between host sexes (Luque et al. 1996, Knoff et al. 1997, Alves and Luque 2001, Tavares et al. 2005).

Comparing the parasite species richness (at component community level) of the engraulid fish studied so far in the southern Atlantic Ocean, *E. anchoita* (n = 13) (Timi 2003) has a greater number of parasite species than *C. edentulus* (N=11) (present study), and *A. tricolor* (n = 10) (Tavares et al. 2005). The greater number of parasite species in *E. anchoita* may result from the fact that this host has been extensively studied in Argentina. There have been studies of population stocks using parasites as indicators, and studies to model predictability for other parasite communities (Timi 2003, Timi and Poulin 2003). In contrast, the focus of the studies on *A. marini* and *C. edentulus* had a regional focus. Some characteristics, such as the presence of larval stages, mean infracommunity richness and total parasite prevalence, seem to be similar in these engraulid hosts, although their infracommunities vary in species composition, since only the digenean *P. merus*, a parasite with a wide spectrum of fish hosts in the South Atlantic, has been recorded in the three hosts. Among the ectoparasites, the copepod *N. cresseyi* and the monogenean *P. heterocotyle* have been recorded in *E. anchoita* and *C. edentulus*.

All metazoan parasites found in *C. edentulus* had the aggregated spatial distribution pattern, which is a common

distribution pattern for parasites in marine fish (Rohde 2005, Iannaccone et al. 2010, Amarante et al. 2015). According to Poulin (2013), aggregated distributions among individual hosts are a defining feature of metazoan parasite populations and the main explanations for that are the variations in how much individual hosts are exposed, and their susceptibility to infection.

*Cetengraulis edentulus* showed a lack of pairs of associated parasite species, a pattern that according to Rohde et al. (1995), is common to most marine fish studied. According to Poulin (2001), positive and negative associations between helminth species can provide strong evidence that species interactions exist and act on community structure. But it is also discussed that interspecific relationships can only be considered valid when tested under experimental conditions (Rohde et al. 1995, Poulin 2001, Chaves and Paschoal 2021).

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