

METAZOAN PARASITES OF NEEDLEFISH *Tylosurus acus* (LACÉPÈDE, 1803) (OSTEICHTHYES: BELONIDAE) FROM THE COASTAL ZONE OF THE STATE OF RIO DE JANEIRO, BRAZIL

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ABSTRACT:- TAVARES, L.E.R.; BICUDO, A.J.A.; LUQUE, J.L. **Metazoan parasites of the needlefish *Tylosurus acus* (Lacépède, 1803) (Osteichthyes: Belonidae) from the coastal zone of the state of Rio de Janeiro, Brazil.** [Metazoários parasitos do agulhão *Tylosurus acus* (Lacépède, 1803) (Osteichthyes: Belonidae) do litoral do estado do Rio de Janeiro, Brazil]. *Revista Brasileira de Parasitologia Veterinária*, v. 13, n. 1, p. 36-40, 2004. Departamento de Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Caixa Postal 74508, Seropédica, RJ 23851-970, Brazil. E-mail: jlluque@ufrj

Between November 2002 and February 2003, thirty-one specimens of *T. acus* were examined from Angra dos Reis (23°01'S, 44°19'W), coastal zone of the State of Rio de Janeiro, Brazil, to study their infracommunities of metazoan parasites. Fifteen species of metazoan parasites were collected: 4 digeneans, 1 cestode, 3 monogeneans, 2 nematodes, and 5 copepods, and all of fishes were parasitized by two or more metazoan, with mean of 23.0 ± 32.0 parasite/fish. Digenean were the most dominant with four species and they accounted for 49.2% of the total parasites collected, and *Rhipidocotyle* sp. was the most abundant species. Abundance of *Lernanthropus tylosuri* (Richiardi, 1880) and *Scolex pleuronectis* (Müller, 1758) were positively correlated with the host total length. Relationships between the total parasite abundance and the mean parasite species richness with total body length of fish were not observed. Two pairs of ectoparasites, two pairs of adult endoparasites and one pair of endoparasite larval stages shared significant positive association and/or covariation. The metazoan parasites infracommunities of *T. acus* showed dominance of endoparasites, scarcity of significant correlation of parasitic abundance and species richness with the size of the host and low number of parasite interspecific relationships.

KEY WORDS: Parasite ecology, community structure, Belonidae, *Tylosurus acus*, Brazil.

RESUMO

Entre novembro de 2002 e fevereiro de 2003, foram examinados 31 espécimes de *T. acus* provenientes de Angra dos Reis (23°01'S, 44°19'W), litoral do estado do Rio de Janeiro, Brasil, para o estudo de suas infracomunidades de metazoários parasitos. Foram coletadas quinze espécies de metazoários parasitos: 4 digenéticos, 1 cestóide, 3 monogenéticos, 2 nematóides e 5 copépodes e todos os peixes estavam parasitados por 2 ou mais metazoários, com média $23,0 \pm 32,0$ parasitos/peixe. Os digenéticos foram dominantes, re-

presentando 49,2% do total de parasitos coletados e *Rhipidocotyle* sp. a espécie mais abundante. A abundância de *Lernanthropus tylosuri* (Richiardi, 1880) e *Scolex pleuronectis* (Müller, 1758) foram positivamente correlacionadas com o comprimento total dos hospedeiros. Relações entre a abundância parasitária total e a riqueza parasitária média com o comprimento total dos hospedeiros não foram observadas. Dois pares de espécies de ectoparasitos, dois pares de endoparasitos adultos e um par de larvas de endoparasitos apresentaram associação e/ou covariação positiva significativa. As infracomunidades de metazoários parasitos de *T. acus* demonstraram dominância de endoparasitos, escassez de correlação entre a abundância parasitária, e riqueza de espécies e o comprimento total dos hospedeiros e baixo número de associações interespecíficas parasitárias.

PALAVRAS-CHAVE: Ecologia parasitária, estrutura comunitária, Belonidae, *Tylosurus acus*, Brasil.

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INTRODUCTION

The needlefish, *Tylosurus acus* (Lacépède, 1803), is a worldwide species which occurs in Western Atlantic Ocean from Massachusetts, USA to Santa Catarina, Brazil (COLLETE; BERRY, 1965; COLLETE; PARIN, 1970; CARVALHO-FILHO, 1999). Pelagic species, inhabit near offshore islands where feed mainly on clupeid and engraulid fishes (FIGUEIREDO; MENEZES, 1978).

Some taxonomic studies included records of metazoan parasites of *T. acus*: Linton (1907) on Cestoda; Collete and Parin (1970) and Cressey and Collete (1970) on Copepoda. In addition to the taxonomical contribution, Cressey and Collete (1970) introduced to some ecological aspects in host-parasite relationships in belonids. Studies on parasites of *T. acus* from Brazil are unknown.

In this report, we studied the metazoan parasite community of *T. acus* from the coastal zone of the State of Rio de Janeiro, at the component and infracommunity levels.

MATERIALS AND METHODS

Between November 2002 and February 2003, thirty-one specimens of *T. acus* measuring 72.5 ± 10.8 (58 – 105.5) cm of total length, were examined from Angra dos Reis, coastal zone of the State of Rio de Janeiro (23°01'S, 44°19'W), Brazil. Fishes were identified according to Figueiredo and Menezes (1978). The average total length of male (70.3 ± 9.1 cm, $n = 15$) and female (74.6 ± 12.2 cm, $n = 16$) fishes in the study sample were significantly different ($t = 1.46$, $P = 0.17$).

The analysis included only parasite species with prevalence higher than 10% (BUSH et al., 1990). The index of dispersion (quotient between variance and mean of parasite abundance) was used to determine distributions patterns and its significance tested using d statistical test (LUDWIG; REYNOLDS, 1988). The frequency of dominance 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). Spearman's rank correlation coefficient r_s was calculate to determine possible correlations between the total length of hosts and abundance of parasites. Pearson's correlation coefficient r was used as an indication of the relationship between the host's total length and the prevalence of parasites, with previous arcsine transformation of the prevalence data (ZAR, 1999) and partition of host samples into three 16 cm length intervals. The effect of host sex on abundance and prevalence of parasites was tested using the Z_c normal approximation to the Mann-Whitney test and the Fisher exact test, respectively. The probable variation of parasite species richness in relation to host sex (Mann-Whitney test) and to host total length (Spearman's rank correlation coefficient) was tested. The 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. Ecological terminology follows Bush et al. (1997). Statistical significance level was evaluated at $P=0.05$. Voucher specimens of helminths were deposited in the Coleção Helminológica do Instituto Oswaldo Cruz (CHIOC), Rio de Janeiro, Brazil; copepods were deposited in the Coleção de Crustacea do Museu Nacional (MNRJ), Rio de Janeiro, RJ, Brazil.

RESULTS

Component community. Fifteen species of metazoan parasites were determined (Table 1). Digeneans were the most dominant and they accounted for 49.2% of the total parasites collected, with the most prevalent and dominant species (Table 2). *Rhipidocotyle* sp. was the most abundant species, with 251 specimens collected (35.2% of all parasites). Larval endoparasites species represent 10.1% of all parasites collected, adult endoparasites 60.6% and ectoparasites 29.3%. All parasites of *T. acus* had the typical aggregated pattern of distribution observed in many parasite systems (Table 3). Abundance of *Lernanthropus tylosuri* (Richiardi, 1880) and *Scolex pleuronectis* (Müller, 1758) were positively correlated with the host total length (Table 4). The mean abundance and prevalence of all parasite species were not significantly different in female and male hosts.

Infracommunities. All fishes were parasitizing by two or more species of metazoan and a total of 714 specimens of metazoan parasites was collected, with mean 23.03 ± 31.97 parasite/fish (2-185). Relationships between the total parasite abundance and host total length and sex were not observed ($r_s=0.346$, $P=0.57$; $Z_c=-0.416$, $P=0.68$). The mean parasite species richness 6 ± 2.1 (2-11) was not correlated with total body length of fish ($r_s=0.291$, $P=0.11$). Two hosts (6.5%) showed infection with two parasite species and 1 (3.2%), 3 (9.7%), 7 (22.6%), 7 (22.6%), 3 (9.7%), 5 (16%), 2 (6.5%) and 1 (3.2%) had multiple infections with 3, 4, 5, 6, 7, 8, 9 and 11 species, respectively. The parasite species richness had not significant differences between male and female fishes ($Z_c=-0.06$, $P=0.95$).

Two pairs of ectoparasite species shared significant positive association and covariation: *Caligodes laciniatus* (Kroyer, 1863) and *Lernanthropus tylosuri* ($r_s=0.484$, $P<0.01$; $\chi^2=5.56$, $P=0.02$); *Chlamydxine* sp. and *Nudaciraxine* sp. ($r_s=0.493$, $P<0.01$; $\chi^2=6.97$, $P<0.01$). Two pairs of endoparasite species shared significant association, although only one pair showed significant positive covariation: *Parahemiurus merus* and *Rhipidocotyle* sp. ($r_s=0.435$, $P=0.02$; $\chi^2=6.36$, $P=0.01$); *Rhipidocotyle* sp. and *Schikobalotrema acuta* (Linton, 1910) ($\chi^2=9.64$, $P<0.01$). Only one pair of endoparasite larval stages showed significant positive covariation: *Hysterothylacium* sp. and *Scolex pleuronectis* ($r_s=0.467$, $P<0.01$).

Table 1. Prevalence, intensity, mean intensity, mean abundance, and site of infection/infestation of the metazoan parasites of *Tylosurus acus* from the coastal zone of the State of Rio de Janeiro, Brazil.

Parasites	Prevalence (%)	Intensity	Mean intensity	Mean abundance	Site of infection
Digenea					
Didymozoid not identified (metacercaria)	6.5	-	1	<0.1	Intestine
CHIOC 36408					
<i>Parahemiurus merus</i>	58.1	1-33	5.4±7.4	3.16±6.2	Stomach
CHIOC 36409					
<i>Rhipidocotyle</i> sp.	87.1	1-113	9.3±21.5	8.1±20.3	Intestine
CHIOC 36410					
<i>Schikhobalotrema acuta</i>	67.7	1-10	4±2.8	2.7±2.9	Intestine
CHIOC 36411					
Cestoda					
<i>Scolex pleuronectis</i>	16.13	2-6	3.4±1.5	0.6±1.4	Intestine
CHIOC 36414					
Monogenea					
<i>Chlamydxine</i> sp.	35.5	1-6	2.5±1.6	0.9±1.5	Gills
CHIOC 36412					
Mazocraeid not identified	3.2	-	-	<0.1	Gills
<i>Nudaciraxine</i> sp.	74.2	1-9	1.7±1.8	1.3±1.7	Gills
CHIOC 36413					
Nematoda					
<i>Hysterothylacium</i> sp. (larval)	64.5	1-14	2.5±2.9	1.6±2.6	Mesenteries
CHIOC 35287					
<i>Pseudoterranova</i> sp. (larval)	9.7	1-2	1.3±0.6	0.1±0.4	Mesenteries
CHIOC 35288					
Copepoda					
<i>Caligodes laciniatus</i>	74.2	1-11	3.3±2.4	2.4±2.5	Oral valve
MNRJ 19315					
<i>Caligus</i> sp. D	3.2	-	-	<0.1	Gills
MNRJ 19316					
<i>Caligus malabaricus</i>	51.6	1-5	1.7±1.3	0.9±1.2	Gills
MNRJ 19317					
<i>Lernanthropus tylosuri</i>	48.4	1-6	2.5±1.6	1.2±1.7	Gills
MNRJ 19319					
<i>Metacaligus</i> sp.	3.2	-	-	<0.1	Gills
MNRJ 19318					

Table 2. Frequency of dominance and mean relative dominance of the metazoan parasites of *Tylosurus acus* from the coastal zone of the State of Rio de Janeiro, Brazil.

Parasite	Frequency of Dominance (%)	Frequency of dominance shared with one or more species	Mean relative dominance
<i>Parahemiurus merus</i>	3.23	0	0.103±0.129
<i>Rhipidocotyle</i> sp.	35.48	1	0.229±0.2
<i>Schikhobalotrema acuta</i>	19.36	1	0.138±0.145
<i>Scolex pleuronectis</i>	0	0	0.016±0.045
<i>Chlamydxine</i> sp.	6.45	0	0.11±0.15
<i>Nudaciraxine</i> sp.	3.23	1	0.06±0.11
<i>Hysterothylacium</i> sp.	0	2	0.089±0.114
<i>Caligodes laciniatus</i>	12.9	1	0.132±0.121
<i>Caligus malabaricus</i>	0	1	0.05±0.065
<i>Lernanthropus tylosuri</i>	3.23	1	0.06±0.09

Table 3. Dispersion index (DI) and *d* test of the metazoan parasites of *Tylosurus acus* from the coastal zone of the State of Rio de Janeiro, Brazil.

Parasites	DI	<i>d</i>
<i>Parahemiurus merus</i>	12.16	19.33
<i>Rhipidocotyle</i> sp.	50.85	47.56
<i>Schikhobalotrema acuta</i>	3.28	6.35
<i>Scolex pleuronectis</i>	3.51	6.83
<i>Chlamydxine</i> sp.	2.66	4.95
<i>Nudaciraxine</i> sp.	2.28	4.02
<i>Hysterothylacium</i> sp.	4.21	8.21
<i>Caligodes laciniatus</i>	2.64	4.91
<i>Caligus malabaricus</i>	1.74	2.54
<i>Lernanthropus tylosuri</i>	2.31	4.09

Table 4. Spearman's rank correlation coefficient (r_s) and Pearson's correlation coefficient (r) values used to evaluate possible relationships among the total length of *Tylosurus acus*, abundance and prevalence of the components of its parasite community from the coastal zone of the State of Rio de Janeiro, Brazil.

Parasites	r_s	P	r	P
<i>Parahemius merus</i>	0.252	0.171	0.959	0.129
<i>Rhipidocotyle</i> sp.	0.269	0.142	0.356	0.593
<i>Schikhalotrema acuta</i>	-0.045	0.812	0.328	0.612
<i>Scolex pleuronectis</i>	0.499*	<0.01	0.916	0.188
<i>Chlamydxine</i> sp.	-0.011	0.555	0.755	0.329
<i>Nudaciraxine</i> sp.	-0.082	0.661	0.809	0.288
<i>Hysterothylacium</i> sp.	0.154	0.408	0.755	0.329
<i>Caligodes laciniatus</i>	0.108	0.565	0.929	0.171
<i>Caligus malabaricus</i>	0.138	0.458	0.816	0.282
<i>Lernanthropus tylosuri</i>	0.354*	0.051	0.948	0.147

* significant values.

DISCUSSION

Some patterns in the structure and composition of the community of metazoan parasites of *T. acus* from Brazil were detected: (1) endoparasite dominance; (2) low influence of the size of the host on parasite species abundance; (3) low number of parasitic interspecific relationships.

The dominance of endoparasites (Digenea) has been described for several parasite communities of marine fishes from the coastal zone of the southeastern Brazil (LUQUE et al. 2002; TAVARES; LUQUE, 2004a). The piscivorous feeding habits of this species might increase the presence of trophically transmitted endoparasites. According to Figueiredo and Menezes (1978) clupeid and engraulid fishes are the main prey item of *T. acus* diet. Previous parasitological works on its small pelagic fishes, mainly engraulid, revealed a great number of larval endoparasites stages including digenean metacercariae (TIMI et al., 1999, 2001; TIMI, 2003).

Cressey and Collete (1970) studied copepods of needlefishes around the world and reported five species parasitic on *T. acus* from Western Atlantic ocean: *Caligodes laciniatus*, *Colobomatus goodingi* Cressey and Collete, 1970, *Lernanthropus tylosuri*, *Parabomolochus bellones* (Burmeister, 1835) and *Lernaeolophus sultanus* (Nordmann, 1864). At the present study were determined five species of copepods parasites *C. laciniatus* and, *Caligus* sp. D Cressey and Collete, 1970, *Caligus malabaricus* Pillai, 1961 and *L. tylosuri*, recorded by Cressey and Collete (1970) in other belonid fishes, and an unidentified species of *Metacaligus*. The high values of prevalence of these copepods might be explained by the schooling behavior of this host in some life cycle stage. Luque et al. (2004) reported the schooling behavior as a determinant for parasite species richness in fishes from the coastal zone of the State of Rio de Janeiro. Nevertheless, Figueiredo and Menezes (1978) mentioned that *T. acus* adult specimens tending to be solitary and this situation

could influence on the abundance and prevalence of the ectoparasites.

The scarcity of significant correlations of parasite abundance, prevalence and species richness with host total body length might be associated to the homogeneity of the sample because only adults were examined. Recent studies detected significant correlation between parasite quantitative descriptors and the total body length of some fishes from the coastal zone of the state of Rio de Janeiro (ALVES; LUQUE, 2001; LUQUE; ALVES, 2001; ALVES et al., 2002; TAVARES; LUQUE, 2004b).

Despite the low number of interspecific relationships, one pair of co-occurring species *C. laciniatus* and *L. tylosuri* was previous reported as one of the more frequent combination of copepod species found in large belonid species and this combination was found when involved copepod species parasitic on different sites of infestation, *C. laciniatus* under oral valves and *L. tylosuri* on gills (CRESSEY; COLLETE, 1970). These authors suggested the occurrence of taxonomic distinct belonid population based on differences in the composition of its copepod fauna. Our results are in accordance with the proposed by Cressey and Collete (1970) for the *T. acus* population stock from Western Atlantic Ocean.

Parasite communities are generally believed to lie somewhere along the interactive-to-isolationist continuum (HOLMES; PRICE, 1986; STOCK; HOLMES, 1988). Poulin and Luque (2003) proposed a index of interactivity based on the general likelihood of species co-occurrence, reflecting the potential for interactions, and this index was applied to component communities of gastrointestinal helminth parasites from 37 species of marine fish from Rio de Janeiro. Poulin and Luque (2003) results showed *T. acus* as more proximate of the interactive extreme of the continuum. As this index is correlated with the total parasite abundance per host, as well as the total prevalence of infection and the mean infracommunity richness, this result might indicate the potential of the parasite communities of belonid fishes for evaluate patterns of interactivity in future comparative studies.

REFERENCES

- ALVES, D.R.; LUQUE, J.L. Community ecology of the metazoan parasites of white croaker, *Micropogonias furnieri* (Osteichthyes: Sciaenidae), from the coastal zone of the State of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, v. 96, n. 2, p. 145-153, 2001.
- ALVES, D.R.; LUQUE, J.L.; PARAGUASSÚ, A.R. Community Ecology of the Metazoan Parasites of Pink Cusk-eel, *Genypterus brasiliensis* (Osteichthyes: Ophidiidae), from the Coastal Zone of the State of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, v.97, n. 5, p. 683-689, 2002.
- BUSH, A.O.; AHO, J.M.; KENNEDY, C.R. Ecological versus phylogenetic determinants of helminth parasite community richness. *Evolutionary Ecology*, v. 4, n. 1, p. 1-20, 1990.
- BUSH, A. O.; LAFFERTY, K.D.; LOTZ, J.M.; SHOSTAK,

- A.W. Parasitology meets ecology on its own terms: Margolis et al. Revisited. *Journal of Parasitology*, v. 83, n. 4, p. 575-583, 1997.
- CARVALHO FILHO, A. *Peixes: Costa Brasileira*. São Paulo: Melro Publisher, 1999. 347p.
- COLLETE, B.B.; BERRY, F.H. Recent studies on the needlefishes (Belonidae): an evaluation. *Copeia*, v. 1965, n. 1, p. 386-392, 1965.
- COLLETE, B.B.; PARIN, N.V. Needlefishes (Belonidae) of the Eastern Atlantic Ocean. *Atlantide Report*, v. 1970, n. 11, p. 7-60, 1970.
- CRESSEY, R.F.; COLLETE, B.B. Copepods and needlefishes: a study in host-parasite relationships. *Fishery Bulletin*, v. 68, n. 3, p. 347-432, 1970.
- FIGUEIREDO, J.L.; MENEZES, N.A. *Manual de Peixes Marinhos do Sudeste do Brasil. II. Teleostei (1)*. São Paulo: Universidade de São Paulo, 1978. 110 p.
- HOLMES, J.C.; PRICE, P.W. Communities of parasites. In: ANDERSON, D.J.; KIKKAWA, J. (Eds.). *Community Ecology: Pattern and Process*, Oxford: Blackwell Scientific Publications, 1986. p. 187-213.
- LINTON, E. Notes on parasites of Bermuda fishes. *Proceedings of the United States National Museum*, v. 33, p. 85-126, 1907.
- LUDWIG, J.A.; REYNOLDS, J.F. *Statistical Ecology: A Primer on Methods and Computing*, New York: Wiley-Interscience Publications, 1988. 337 p.
- LUQUE, J.L.; ALVES, D.R. Ecologia das comunidades de metazoários parasitos do xaréu, *Caranx hippos* (Linnaeus) e do xerelete, *Caranx latus* Agassiz (Osteichthyes, Carangidae) do litoral do estado do Rio de Janeiro, Brasil. *Revista Brasileira de Zoologia*, v. 18, n. 2, p. 399-410, 2001.
- LUQUE, J.L.; MOUILLOT, D.; POULIN, R. Parasite biodiversity and its determinants in coastal marine teleost fishes of Brazil. *Parasitology*, v. 128, n. 6, p. 671-682, 2004.
- LUQUE, J.L.; PORROZZI, F.P.; ALVES, D.R. Community ecology of the metazoan parasites of Argentine goatfish *Mullus argentineus* (Osteichthyes: Mullidae) from the coastal zone of the State of Rio de Janeiro, Brazil. *Revista Brasileira de Parasitologia Veterinária*, v. 11, n. 1, p. 33-38, 2002.
- POULIN, R.; LUQUE, J.L. A general test of the interactive-isolationist continuum in gastrointestinal parasite communities of fish. *International Journal for Parasitology*, v. 33, n. 14, p. 1623-1630, 2003.
- ROHDE, K.; HAYWARD, C.; HEAP, M. Aspects of the ecology of metazoan ectoparasites of marine fishes. *International Journal for Parasitology*, v. 25, n. 8, p. 945-970, 1995.
- STOCK, T.M.; HOLMES, J.C. Functional relationships and microhabitat distributions of enteric helminths of grebes (Podicipedidae): the evidence for interactive communities. *Journal of Parasitology*, v. 74, n. 1, p. 214-227, 1988.
- TAVARES, L.E.R.; LUQUE, J.L. Community ecology of the metazoan parasites of the later juvenile common snook *Centropomus undecimalis* (Osteichthyes: Centropomidae) from the coastal zone of the state of Rio de Janeiro, Brazil. *Brazilian Journal of Biology*, v. 64, n. 3, 2004a. In press.
- TAVARES, L.E.R.; LUQUE, J.L. Community ecology of the metazoan parasites of the white sea catfish *Netuma barba* (Osteichthyes: Ariidae) from the coastal zone of the state of Rio de Janeiro, Brazil. *Brazilian Journal of Biology*, v. 64, n. 1, p. 169-176, 2004b.
- TIMI, J.T. Parasites of Argentine anchovy in the south-west Atlantic: latitudinal patterns and their use for discrimination of host populations. *Journal of Fish Biology*, v. 63, n. 1, p. 90-107, 2003.
- TIMI, J.T.; MARTORELLI, S.R.; SARDELLA, N. Digenetic trematodes parasitic on *Engraulis anchoita* (Pisces: Engraulidae) from Argentina and Uruguay. *Folia Parasitológica* v. 46, p. n. 2, 132-138, 1999.
- TIMI, J.T., SARDELLA, N.H.; NAVONE, G.T. Parasitic nematodes of *Engraulis anchoita* Hubbs et Marini, 1935 (Pisces, Engraulidae) off the Argentine and Uruguayan coasts, South West Atlantic. *Acta Parasitologica*, v. 46, n. 2, p. 186-193, 2001.
- ZAR, J.H. *Biostatistical Analysis*. New Jersey: Prentice-Hall Press, 1999. 663 p.

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