# The parasitic isopod *Mothocya nana* drives dietary shifts and poorer condition of Brazilian silversides *Atherinella brasiliensis*

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ABSTRACT: Cymothoids are ectoparasites that may attach to various parts of the fish for molting and reproductive purposes, thus increasing the energetic costs of the host. This study investigated the influence of the parasitic isopod *Mothocya nana* on the physiological condition and diet of adult Brazilian silversides *Atherinella brasiliensis* at a sandy beach in southeastern Brazil. We collected 268 *A. brasiliensis* individuals, of which 230 fish were non-parasitized (mean ± SE total weight [TW] = 16.92 ± 0.38 g; total length [TL] = 127 ± 0.88 mm) and 38 were parasitized by up to 2 isopods (TW = 15.89 ± 0.79 g; TL = 126 ± 1.96 mm). Parasitic prevalence ($P$) and intensity ($I$) reached highest values in June 2015 ($P$ = 20.88 %; $I$ = 1.31) and were slightly higher on males ($P$ = 17.39 %; $I$ = 1.33) than on females ($P$ = 13.07 %; $I$ = 1.27). Parasitized fish revealed poorer condition than non-parasitized ones, among which male hosts were especially burdened by *M. nana*'s attachment. The condition factor, the eviscerated condition factor and the fullness index each showed a decreasing trend according to the parasite's development and offspring weight (i.e. increase in egg and larval weight). Parasitized and non-parasitized Brazilian silversides fed mainly on microcrustaceans, but the first group showed reduced phytoplankton intake and was associated with fewer trophic categories in comparison to non-parasitized fish. These dietary shifts revealed correlation with the poorer physiological condition reported for infected *A. brasiliensis*, whose decreased feeding efficiency is likely related to potential impairment of the filter-feeding mechanism and/or altered behaviour due to pressure atrophy and the increased energetic costs imposed by *M. nana*'s development.

KEY WORDS: Parasitism · Cymothoidae · Diet · Physiological condition · Generalized linear models · Partial redundancy analysis

## 1. INTRODUCTION

Parasitism has been reported as one of the main factors regulating fish populations (Lester 1984, Finley & Forrester 2003), since it can compromise fish fitness by affecting behavioural, morphological and physiological traits (Bunkley-Williams & Williams 1998, Fogelman & Grutter 2008, Binning et al. 2013). As components of the natural environment, parasites affect host population dynamics and community structure by influencing connectedness, number and length of food chain links and species richness (Marcogliese 2004, Hatcher et al. 2006, Lafferty et al. 2008, Welicky & Sikkel 2014). Isopods from the

family Cymothoidae are obligatory fish parasites distributed in 40 genera and 380 species, of which 41 occur in the tropical Atlantic (Brusca 1981, Bunkley-Williams & Williams 1998, Smit et al. 2014). The species *Mothocya nana* (Schioedte & Meinert 1884) inhabits the western Atlantic and was found parasitizing fish from the genus *Hyporhamphus* in the Caribbean Sea and along the US east coast (Bruce 1986, Kensley & Schotte 1989). Records of this parasite along the Brazilian coast were also found by Thatcher (2000) and Golzio et al. (2017), but neither of these authors evaluated the effects of *M. nana* on its host — a relationship that is yet to be described between any South American fish and this isopod.

*M. nana* is an easily detected, gill-inhabiting ectoparasite (Bruce 1986, Kensley & Schotte 1989). Members of this genus are protandrous hermaphrodites (Bello et al. 1997, Leonardos & Trilles 2004), changing from male to female once attached to the host. These isopods are considered to feed mainly on host blood cells, leading from minor tissue damage (Leonardos & Trilles 2003) to poor host condition (Kawanishi et al. 2016). Increased energetic costs due mainly to the cymothoids' molting process (Brusca 1978, Fogelman & Grutter 2008) have also been reported to lead to a decrease in fish metabolism, growth and reproduction (Adlard & Lester 1994, Rohde 2005, Fogelman & Grutter 2008, Fogelman et al. 2009), as well as changes in fish feeding behaviour (Meadows & Meadows 2003), movement patterns (Welicky & Sikkel 2015) and swimming ability (Binning et al. 2013, 2014).

Brazilian silversides *Atherinella brasiliensis* (Quoy & Gaimard, 1825) are widely distributed along the Brazilian coastal zone and are often caught in the surf zones of sandy beaches within bays and estuarine areas. This fish is a generalist-opportunist, as it uses a wide array of prey types such as zooplankton, insects, benthic invertebrates and plant matter (Bemvenutti 1990, Rocha et al. 2008, Contente et al. 2011, Alves et al. 2016). Consequently, *A. brasiliensis* plays a key role in the food web of coastal ecosystems, transferring energy from lower to higher trophic levels (Bervian & Fontoura 2007, Contente et al. 2011, Alves et al. 2016). The aim of this study was to analyze the influence of *M. nana* on the physiological condition and diet of adult *A. brasiliensis* at a sandy beach of southeastern Brazil. We hypothesized that parasitized *A. brasiliensis* would display worse physiological condition than non-parasitized fish. We also expected parasitation by *M. nana* to have an effect on fish diet, especially during parasite development and reproduction.

## 2. MATERIALS AND METHODS

### 2.1. Study site

The coastal region of Itaipu (22° 53′ 14″ S, 43° 22′ 48″ W; Fig. 1) is located on the oceanfront of the city of Niterói, Rio de Janeiro, Brazil (Fig. 1). Its semicircular-shaped coastline extends for 4.5 km and is bisected by the Itaipu Lagoon canal. The beach profile is mostly steep, with depths between 3 and 28 m. Itaipu beach features a narrow surf zone, with high-enegy waves breaking on the beach (i.e. reflective beach). The sediment consists predominantly of medium sand, with some areas of finer sand deposits from the Itaipu canal (Salvador & Silva 2002, Monteiro-Neto et al. 2008). Three islands (Menina Island, Mãe Island and Pai Island) protect the beach from incoming swells (Fig. 1B), which results in a habitat conducive to reproduction and growth of estuarine and marine species. As a result, the Itaipu region hosts a variety of species, including some of economic interest (Sergipense & Pinto 1995).

### 2.2. Sampling

We performed 3 sampling campaigns, each one on a single capture date — 24/06/2014 (i.e. June 2014), 06/10/2014 (i.e. October 2014) and 08/05/2015 (i.e. June 2015) — using a seine net with a cod end (20 × 2 m; 7 mm). For each sample, the seine was opened 20 m away from the shoreline (i.e. 1.5–2.0 m depth) and hauled (deeper to shallower), towards the coast), resulting in a sampling area of 400 m² per haul. Sampling transects were carefully distributed along the beach to avoid overlapping hauls and were carried out between 12:00 and 16:00 h. Sampling was authorized by the Instituto Brasileiro de Recursos Naturais Renováveis (IBAMA). Captured fish were placed in plastic bags, labeled and cooled on ice in the field before being transferred to the Laboratory of Applied Ecology–Veterinary College (UFF) for storage in a freezer.

### 2.3. Laboratory procedures

All *Atherinella brasiliensis* were identified according to Figueiredo & Menezes (1978). Samples were weighed (total weight [TW], g) using precision scales (accuracy of 0.001 g), measured (total length [TL], mm) and eviscerated. The livers, gonads and stomachs were weighed, and stomachs were stored in

Fig. 1. Geographical location of Itaipu Beach in Niterói, RJ, Brazil. Asterisk indicates the area of the beach where sampling occurred

10% formalin for dietary analysis. Data on the sex of each fish were obtained from the gonads (also preserved in 10% formalin).

The mouth, flesh, gills and internal body cavity of specimens were examined to determine the presence or absence of any parasites, which were only found in the gill chambers. Whenever present, parasites were weighed (TW, g), measured (TL, mm) and preserved in 10% formalin solution to avoid dehydration and/or any deformation of the material. The samples were identified as the cymothoid species *Mothocya nana* through morphological analysis with additional specialist assistance. For reproducing parasites, the weights of eggs and larvae in the marsupium were recorded.

The diets of non-parasitized and parasitized Brazilian silversides were assessed by macroscopic (i.e. Zeiss stereoscope) and microscopic (40×) analysis of their stomach contents. For microscopic analysis, each stomach sample was homogenized in a volume of 5 ml, and 3 aliquots each of 1 ml were sampled. All stomach contents were identified to the lowest possible taxonomic level, were weighed (precision scales; accuracy of 0.001 g) and then grouped into one of 12 trophic categories (phytoplankton, algae, plant matter, bryozoans, nematodes, annelids, molluscs, crustaceans, insects, teleostei, detritus and organic matter).

## 2.4. Data analysis

*A. brasiliensis* were categorized as parasitized or non-parasitized, according to the presence/absence of the parasite. Prevalence ($P$) and intensity ($I$) of *M. nana* were calculated using the formulas: $P$ = (number of infected hosts/total number of fish sampled) × 100 and $I$ = total number of parasites/number of infected hosts (Margolis et al. 1982, Bush et al. 1997) and were calculated for each host sex and date of capture (i.e. month and year).

To analyze the possible effects of parasitism on the physiological condition of Brazilian silversides, we calculated the condition factor ($K$) (Vazzoler 1996), $K = TW/TL^3$; the eviscerated condition factor ($K_E$) (Clark 1928), $K_E = EFW/TL^3$; the fullness index (FI) (Amaral & Cabral 2004), FI = SW/EFW; the gonado-somatic index (GSI) (Allain 2001), GSI = GW/EFW; and the hepatosomatic index (HSI) (Vazzoler 1996), HSI = LW/EFW, where EFW is eviscerated fish weight (g), SW is stomach weight (g), GW is gonad weight (g), and LW is liver weight (g). We adopted both $K$ and $K_E$ to account for the parasite's influence on fish fitness (i.e. comprising the ability of feeding, reproducing and storing energy) and somatic growth (i.e. muscle building), respectively.

The values of the indices ($K$, $K_E$, FI, GSI and HSI) were tested for normality and homoscedasticity, and

among those, FI, GSI and HSI were power transformed using a Box-Cox function ($\Lambda$ = 0.5, square root transformation; Minitab® 18 Statistical Software) to meet a linear distribution. Generalized linear models (GLMs) were used to compare the values of the indices ($K$, $K_E$, FI, GSI and HSI) in relation to parasite presence, host sex and date of capture (i.e. month and year) using the TL of fish ($\log_{10}$TL) as the covariate.

GLMs were also used to investigate possible relationships among biometrical characters of the parasite (parasite TL, parasite TW, egg and larval weight) and host TL, host TW and physiological condition (i.e. $K$, $K_E$, FI, GSI and HSI indices; tests with indices also used TL of fish as the covariate). We chose the Gaussian distribution for all analyses performed and adopted a 95% confidence interval (p ≤ 0.05). All tests were performed in IBM SPSS Statistics for Windows 20.0.

The Kawakami feeding index (IAi) was used to describe the diet of Brazilian silversides, which encompasses frequency of occurrence and volume (or weight) of dietary items and categories: IAi = $FO_i \times FW_i / \sum_{i=1}^{n} (FO_i \times FW_i)$, where $FO_i$ is the frequency of occurrence of the item or category $i$ (%) and $FW_i$ is the frequency of weight of the item or category $i$ (%). FO was calculated by dividing the number of times an item or category occurred and the total occurrence of the items or categories, multiplied by 100. FW was determined as a ratio between the weight of an item or category and the total weight of the items or categories, multiplied by 100 (Kawakami & Vazzoler 1980). Stomach samples from months with low parasite prevalence (i.e. $P$ < 10%) were removed from this analysis to avoid misrepresenting feeding patterns of parasitized and non-parasitized fish. In addition, dietary analysis was performed with data from the 3 sampling campaigns pooled together as well as per capture date.

Partial redundancy analysis (RDA) was applied on the biomass of the feeding categories consumed by *A. brasiliensis* to detect the effects of parasites on fish diet. Data were square-root transformed, and the significance of the RDA axis was tested through a Monte Carlo test (499 permutations). Physiological indices ($K$, $K_E$, FI, GSI and HSI) were also used as explanatory variables for this analysis, which was performed with CANOCO 4.5 (Leps & Smilauer 2003). Host sex was not included in this model because preliminary Monte Carlo tests revealed no significant correlation between this variable and *A. brasiliensis* diet.

# 3. RESULTS

## 3.1. Prevalence and aspects of *Mothocya nana* on Brazilian silversides

We collected 268 individuals of *Atherinella brasiliensis* (Table 1), ranging from 94 to 177 mm TL. Of this sample, 230 fish were not parasitized (mean ± SE TW = 16.92 ± 0.38 g; TL = 127 ± 0.88 mm), while the remaining 38 fish (TW = 15.89 ± 0.79 g; TL = 126 ± 1.96 mm) were found to carry 49 parasites. Parasitic prevalence and intensity shifted according to host sex, being higher on males ($P$ = 17.39%; $I$ = 1.33) than on females ($P$ = 13.07%; $I$ = 1.27), and months and years, showing the highest values in June 2015 ($P$ = 20.88%; $I$ = 1.31), followed by June 2014 ($P$ = 15.9%; $I$ = 1.29) and October 2014 ($P$ = 4.76%; $I$ = 1). Eleven hosts were infected by 2 isopods: 2 hosts were found with each parasite attached to a different branchial cavity, while 9 hosts had both parasites located on the same gill. All parasites belonged to the species *Mothocya nana*; these were attached only to adult *A. brasiliensis* and were not caught on any other species. Parasites ranged from 2.1 to 14 mm in size, with a mean (± SE) TL of 8.76 ± 0.38 mm. Mean TW of *M. nana* was 0.094 ± 0.01 g, ranging from 0.001 to 0.482 g. Among the observed isopods, 57.1% (n = 28) were ovigerous and 42.9% (n = 21) did not have any eggs or larvae.

## 3.2. Biometric characters: parasite vs. host

No association was detected between host TL and parasite TL (Wald chi-square = 12.26; df = 23; p = 0.97), parasite TW (Wald chi-square = 27.73; df = 23; p = 0.23) and weight of parasite eggs and larvae (Wald chi-square = 22.72; df = 19; p = 0.25). We also did not find a significant association between host TW and parasite TL (Wald chi-square = 11.863; df =

Table 1. Non-parasitized and parasitized *Atherinella brasiliensis* per host sex and date of capture at Itaipu Beach, Niterói, Brazil

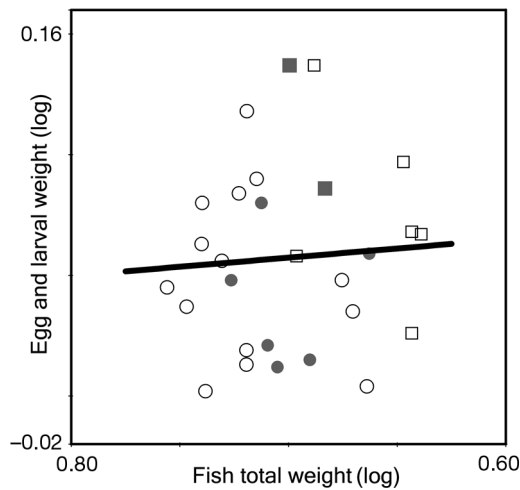|  | Jun 2014 | Oct 2014 | Jun 2015 | Total |
|---|---|---|---|---|
| **Non-parasitized** | | | | |
| Female | 26 | 35 | 112 | 173 |
| Male | 11 | 5 | 41 | 57 |
| **Parasitized** | | | | |
| Female | 5 | 2 | 19 | 26 |
| Male | 2 | 0 | 10 | 12 |

Fig. 2. Relationship between host total weight and weight of parasite eggs and larvae. Line represents the generalized linear model. Samples are coded by host sex and date of capture: gray = male; white = female. (□) June 2014, (△) October 2014, (○) June 2015

37; p = 0.99) and parasite TW (Wald chi-square = 34.854; df = 37; p = 0.57), contrary to egg and larval weight (Wald chi-square = 42.075; df = 26; p = 0.02), which revealed an increase with *A. brasiliensis* weight (Fig. 2).

### 3.3. Physiological condition of non-parasitized vs. parasitized Brazilian silversides

Non-parasitized fish showed a higher $K$ than parasitized fish (Fig. 3A). $K$ also changed among months and years and between host sex; a significant interaction between host sex and parasite presence was also detected for this index (Table 2), revealing that parasitized males had the lowest $K$ (Fig. 3B). Influ-
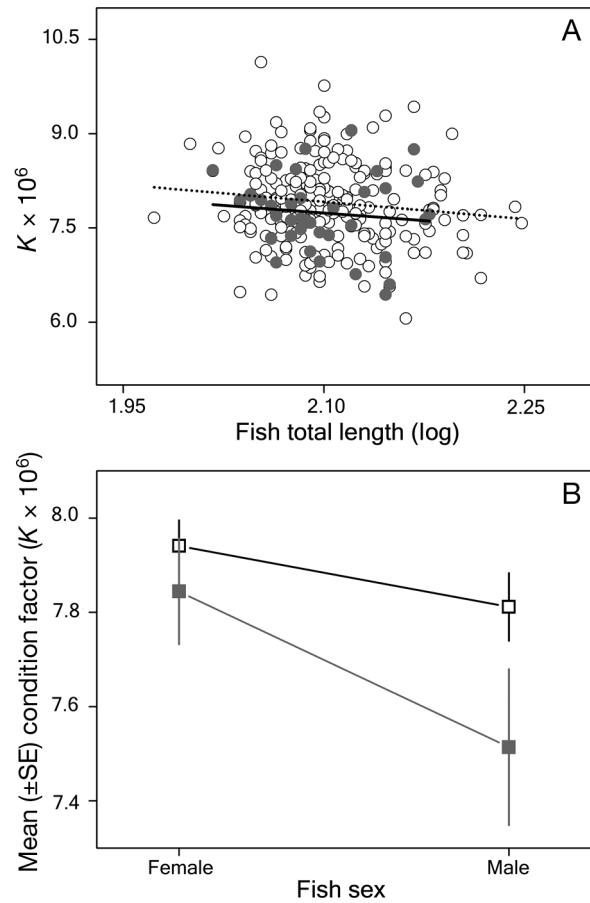


Fig. 3. (A) Relationship between the condition factor ($K$) and fish total length for parasitized (solid line; gray circles) and non-parasitized (dotted line; open circles) *Atherinella brasiliensis*. (B) Means ± SE of $K$ per sex for infected (gray) and uninfected (white) fish are also displayed

ence of covariate TL of fish was detected for this analysis (Table 2). $K_E$ did not differ according to parasite presence, host sex, months and years or the

Table 2. Results of generalized linear model analyses. $K$: condition factor; $K_E$: eviscerated condition factor; GSI: gonadosomatic index; FI: fullness index; HSI: hepatosomatic index. *$p \leq 0.05$

| Factor | $K$ | | | $K_E$ | | | GSI | | | FI | | | HSI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wald chi-square | df | p | Wald chi-square | df | p | Wald chi-square | df | p | Wald chi-square | df | p | Wald chi-square | df | p |
| Parasite presence | 3.83 | 1 | 0.05* | 0.18 | 1 | 0.67 | 0.74 | 1 | 0.39 | 2.11 | 1 | 0.15 | 0.02 | 1 | 0.90 |
| Fish sex | 5.81 | 1 | 0.02* | 0.01 | 1 | 0.93 | 50.58 | 1 | <0.001* | 7.38 | 1 | 0.01* | 1.28 | 1 | 0.26 |
| Months and years | 26.94 | 2 | <0.001* | 0.46 | 2 | 0.80 | 2.28 | 2 | 0.32 | 40.60 | 2 | <0.001* | 17.66 | 2 | <0.001* |
| Parasite × Fish sex | 4.82 | 1 | 0.03* | 0.06 | 1 | 0.80 | 0.04 | 1 | 0.85 | 1.42 | 1 | 0.23 | 0.28 | 1 | 0.60 |
| Parasite × Months and years | 0.70 | 2 | 0.71 | 0.03 | 2 | 0.99 | 1.29 | 2 | 0.53 | 3.99 | 2 | 0.14 | 2.04 | 2 | 0.36 |
| Parasite × Fish sex × Months and years | 4.92 | 3 | 0.18 | 0.34 | 3 | 0.95 | 4.68 | 3 | 0.20 | 2.85 | 3 | 0.42 | 0.66 | 3 | 0.88 |
| **Covariate** Fish total length | 3.89 | 1 | 0.05* | 0.05 | 1 | 0.83 | 31.61 | 1 | <0.001* | <0.001 | 1 | 0.99 | 8.60 | 1 | 0.003* |

Table 3. Relationship between the physiological indices of parasitized *Atherinella brasiliensis* and the biometrical characters of *Mothocya nana* (generalized linear model analyses; *p ≤ 0.05). Values of the covariate total length (TL) of fish are displayed for each of the factors analyzed. *K*: condition factor; *K*$_E$: eviscerated condition factor; GSI: gonadosomatic index; FI: fullness index; HSI: hepatosomatic index; TW: total weight

| Factor | *K* | | | *K*$_E$ | | | GSI | | | FI | | | HSI | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Wald chi-square | df | p | Wald chi-square | df | p | Wald chi-square | df | p | Wald chi-square | df | p | Wald chi-square | df | p |
| Parasite TL | 32.17 | 21 | 0.05* | 41.29 | 27 | 0.04* | 14.58 | 21 | 0.84 | 14.32 | 21 | 0.86 | 20.09 | 21 | 0.52 |
| Parasite TW | 270.58 | 45 | <0.001* | 171.61 | 45 | <0.001* | 23.97 | 45 | 0.99 | 1207.89 | 45 | <0.001* | 32.49 | 45 | 0.92 |
| Weight of parasite eggs and larvae | 80.36 | 24 | <0.001* | 35.27 | 24 | 0.06 | 12.12 | 24 | 0.98 | 129.50 | 24 | <0.001* | 8.04 | 24 | 0.99 |
| **Covariate (fish TL)** | | | | | | | | | | | | | | | |
| Parasite TL | 0.02 | 1 | 0.88 | 1.15 | 1 | 0.28 | 1.57 | 1 | 0.21 | 0.17 | 1 | 0.68 | 0.01 | 1 | 0.92 |
| Parasite TW | 12.24 | 1 | <0.001* | 2.84 | 1 | 0.09 | 0.76 | 1 | 0.38 | 5.40 | 1 | 0.02* | 0.19 | 1 | 0.66 |
| Weight of parasite eggs and larvae | 0.54 | 1 | 0.46 | 0.27 | 1 | 0.60 | 0.03 | 1 | 0.86 | 1.97 | 1 | 0.16 | 0.09 | 1 | 0.76 |

interaction between these factors. Covariate TL of fish did not influence *K*$_E$ (Table 2). The GSI, FI and HSI indices also did not differ because of parasite presence (Table 2). However, the GSI and HSI indices changed significantly according to host sex and months and years, respectively, whereas the FI index differed with both of these factors. Interactions between factors were not significant for any of these indices, and influence of the covariate TL of fish was found for GSI and HSI (Table 2).

*K* and *K*$_E$ both showed a significant association with parasite TL, parasite TW and egg and larval weight (Table 3). Both indices decreased with increasing parasite TL (Fig. 4A,B), TW (Fig. 4C,D) and weight of parasite eggs and larvae (Fig. 4E,F). However, the association between *K*$_E$ and weight of parasite eggs and larvae was found to be only marginally significant (Table 3). FI also showed a significant association with parasite TW and weight of parasite eggs and larvae but
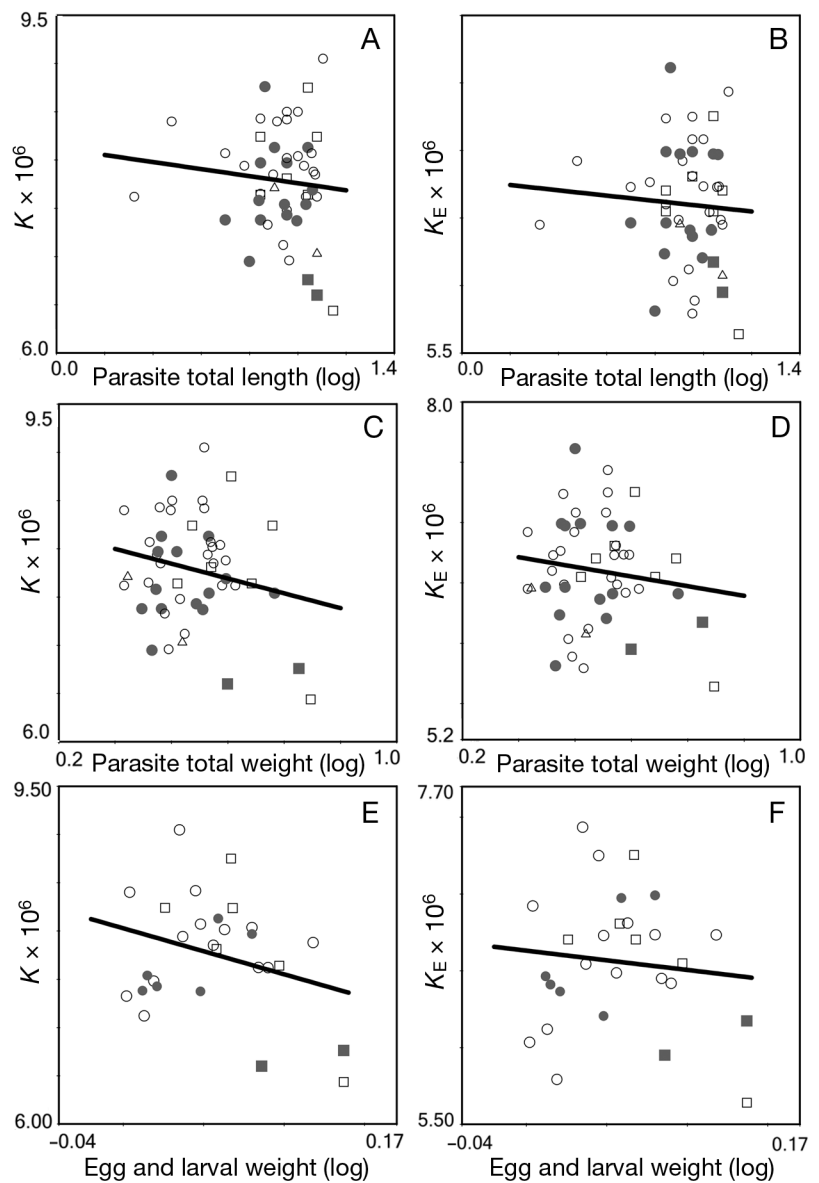


Fig. 4. Relationship between the condition factor (*K*) and the eviscerated condition factor (*K*$_E$) with (A,B) parasite total length, (C,D) parasite total weight and (E,F) weight of parasite eggs and larvae. Lines represent the generalized linear models. Samples are coded by host sex and date of capture: gray = male; white = female. (□) June 2014, (△) October 2014, (○) June 2015

Fig. 5. Relationship between the fullness index and (A) parasite total weight and (B) weight of parasite eggs and larvae. Lines represent the generalized linear models. Samples are coded by host sex and date of capture: gray = male; white = female. (□) June 2014, (△) October 2014, (○) June 2015
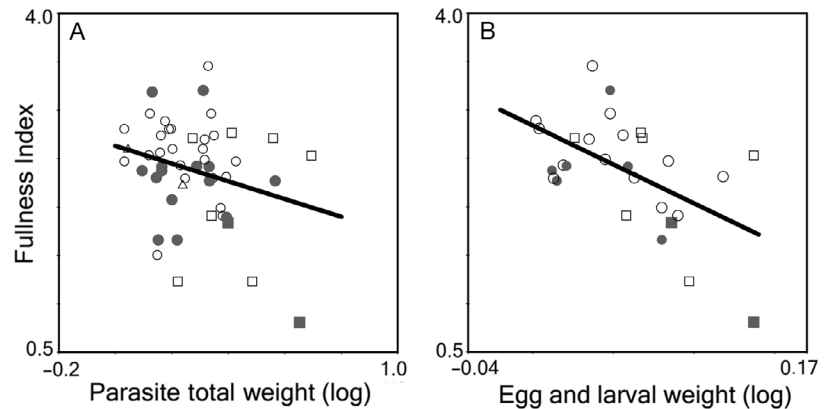
not with parasite TL (Table 3). Similar to $K$ and $K_E$, FI decreased with increasing parasite TW and egg and larval weight (Fig. 5). Influence of covariate TL of fish was detected only for $K$ and FI regarding analysis with parasite TW (Table 3). No association was found between GSI and HSI and any biometrical characters of the parasite (i.e. parasite TL, parasite TW and egg and larval weight; Table 3).

### 3.4. Diet

The stomach contents of 95 individuals were analyzed, of which 61 (64.21%) came from non-parasitized *A. brasiliensis* and 34 (35.79%) from parasitized fish. As parasite prevalence was <10% in October 2014, no samples from this date were used in the description of *A. brasiliensis* diet. Overall, non-parasitized fish fed on 20 items, while parasitized fish fed on 10 prey. Fish from both groups fed mainly on crustaceans (IAi: parasitized = 0.84, non-parasitized = 0.64) and phytoplankton (IAi: parasitized = 0.10/non- parasitized = 0.27), considering both (Table 4) and each of the capture dates (i.e. June 2014 and 2015; see Table S1 in the Supplement at www.int-res.com/articles/suppl/d132p229_supp.pdf). Phytoplankton was the main item consumed by non-parasitized fish (IAi = 0.40), followed by the microcrustacean Mysidacea (IAi = 0.31; Table 4), while parasitized fish fed mostly on microcrustacean fragments (IAi = 0.84). Analysis of IAi showed that the remaining cate-

Table 4. Frequency of occurrence (FO%), frequency of weight (FW%) and Kawakami's feeding index (IAi) for each item consumed by *Atherinella brasiliensis* at Itaipu Beach, Niterói, RJ, Brazil. Blank spaces: not present in stomach contents

| Trophic item/category | Non-parasitized (n = 61) | | | Parasitized (n = 34) | | |
|---|---|---|---|---|---|---|
| | FO% | FW% | IAi | FO% | FW% | IAi |
| **Phytoplankton** | | | | | | |
| Unidentified microalgae | 18.22 | 31.14 | 0.40 | 17.94 | 19.6 | 0.10 |
| **Algae** | | | | | | |
| Macroalgal fragments | 2.8 | 1.78 | 0 | 11.54 | 8.39 | 0.03 |
| **Plant matter** | | | | | | |
| Leaf fragments | 0.47 | 0.01 | 0 | | | |
| Seed | 0.47 | 0 | 0 | | | |
| **Bryozoans** | | | | | | |
| Bryozoan fragments | | | | 2.56 | 0.02 | 0 |
| **Nematodes** | | | | | | |
| Unidentified nematode | 0.46 | 0.04 | 0 | | | |
| **Annelids** | | | | | | |
| Polychaetes (tube) | 4.21 | 0.13 | 0 | | | |
| **Molluscs** | | | | | | |
| Shell fragments | 0.93 | 0.09 | 0 | | | |
| **Crustaceans** | | | | | | |
| Mysidacea | 12.62 | 34.73 | 0.31 | | | |
| *Livoneca* sp. | 0.47 | 0.14 | 0 | | | |
| Microcrustacean fragments | 14 | 15.70 | 0.16 | 42.31 | 66.99 | 0.84 |
| Crustacean larvae | 1.87 | 0.12 | 0 | | | |
| **Insects** | | | | | | |
| Diptera | 0.47 | 0.15 | 0 | | | |
| Formicidae | 0.47 | 0.05 | 0 | 1.28 | 0.03 | 0 |
| Insect larvae | 3.27 | 1.34 | 0 | 1.28 | 0.11 | 0 |
| Insect fragments | 0.47 | 2 | 0 | 1.28 | 0.36 | 0 |
| **Teleostei** | | | | | | |
| Fish eggs | 3.27 | 1.54 | 0 | | | |
| Fish scales | 3.74 | 0.23 | 0 | 2.56 | 0.12 | 0 |
| Fish spines | 0.93 | 0.04 | 0 | | | |
| **Detritus** | | | | | | |
| Gravel | 13.55 | 3.82 | 0.04 | | | |
| Sand | | | | 1.28 | 0.01 | 0 |
| **Organic matter** | 17.29 | 6.93 | 0.08 | 17.95 | 19.6 | 0.02 |
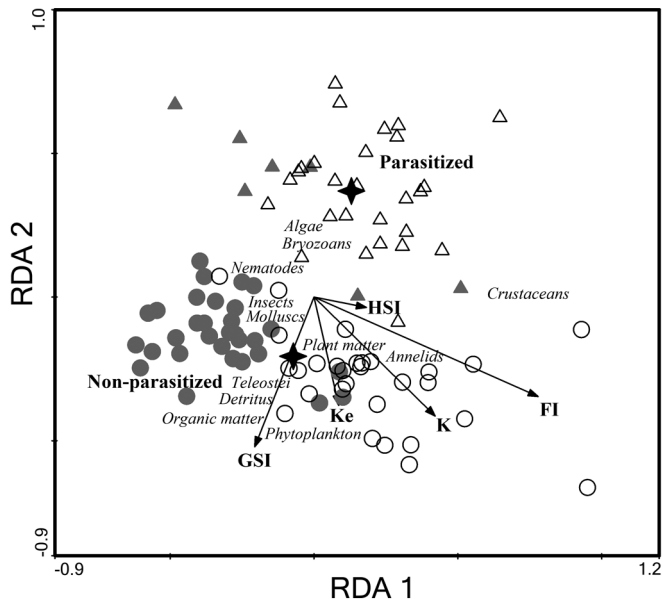
Fig. 6. Partial redundancy analysis (RDA) showing the relationship between the diet of parasitized (O) and non-parasitized (△) *Atherinella brasiliensis* with physiological indices (condition factor [$K$], eviscerated condition factor [$K_E$], fullness index [FI], gonadosomatic index [GSI] and hepatosomatic index [HSI]). Samples are coded by date of capture (dark gray = June 2014; white = June 2015)

gories and items consumed by *A. brasiliensis* had lower dietary representations (Table 4).

RDA axes 1 (eigenvalue = 0.152) and 2 (eigenvalue = 0.028) accounted for 95.7% of data variance in the diet of *A. brasiliensis* (Monte Carlo test: p = 0.002) and revealed distinct feeding patterns for parasitized and non-parasitized fish (Fig. 6). Six trophic categories (i.e. phytoplankton, organic matter, detritus, teleostei, annelids and plant matter) were correlated with non-parasitized Brazilian silversides, in contrast to only 2 categories (i.e. algae and bryozoans) associated with infected fish (Fig. 6). Non-parasitized fish were also highly correlated with GSI, $K_E$, $K$ and FI indices.

## 4. DISCUSSION

The present study revealed a negative influence of the cymothoid parasite *Mothocya nana* on the condition of Brazilian silversides *Atherinella brasiliensis*. Adverse effects of cymothoid parasites have been reported for many species of fish, especially during their juvenile stage (Adlard 1989, Adlard & Lester 1994, Fogelman & Grutter 2008, Parker & Booth 2013). Our study, however, addressed the effects of parasitism on adult *A. brasiliensis*, revealing a

decline in fish condition due to *M. nana*'s presence. The development of this parasite while attached to the host's branchial cavity seems to have influenced *A. brasiliensis* feeding by reducing food intake and promoting dietary shifts, which correlated with the poorer physiological condition of parasitized fish. In addition to parasite presence, we tested our data for differences related to fish sex, date of capture and interactions between these factors and the occurrence of parasitism. Applying this analytical procedure revealed that adult male *A. brasiliensis* were especially burdened by the energetic costs imposed by *M. nana*. However, it is still unclear if this parasite compromises the fish's life cycle on a long-term scale, as we only performed 3 sampling campaigns over 2 yr, during which time there were temporal changes in the fish's physiological indices regardless of *M. nana*'s presence.

Cymothoid isopods have been shown to shape the structure and dynamics of host populations through impaired reproduction (Adlard & Lester 1994, Fogelman et al. 2009), diminished growth (Parker & Booth 2013, Roche et al. 2013), changes in fish behaviour (Binning et al. 2014, Welicky & Sikkel 2015) and increased energetic costs (Östlund-Nilsson et al. 2005, Binning et al. 2013) that ultimately can cause host death (Adlard & Lester 1994, Fogelman & Grutter 2008). Our sample of parasitized fish revealed poorer $K$ than non-parasitized ones; however, *M. nana*'s presence did not affect $K_E$, FI, GSI and HSI, but $K$, $K_E$ and FI decreased with parasite development (i.e. increase in length, weight and egg and larval weight), revealing that *M. nana*'s growth and reproduction compromises fish feeding and condition — a pattern that was supported by dietary analysis.

Previous studies reported the influence of cymothoid parasites on host feeding (Meadows & Meadows 2003, Parker & Booth 2013, Vigneshwaran et al. 2018). However, contrary to our results, these authors did not evaluate or find any differences regarding the dietary categories consumed by infected and non-infected fish. Overall, we recorded a high consumption of microcrustacean prey by both groups of Brazilian silversides, followed by unequal contributions of phytoplankton. While non-parasitized fish fed largely on the latter resource, infected *A. brasiliensis* showed a smaller contribution of phytoplankton to their diet, which may be related to potential mechanical damage on fish gills due to *M. nana*'s attachment. Tissue injury due to pressure atrophy (Leonardos & Trilles 2003) and/or continuous feeding of cymothoid parasites (Brusca & Gilligan 1983, Colorni et al. 1997) may lead to morphological alter-

ations on the gills such as pale colouration and the absence of some filaments (Monfort et al. 2009), which were observed in samples from our study (J. S. Souza pers. obs.). Such deterioration of branchial tissue seems to further influence the feeding of parasitized Brazilian silversides. As a planktivorous fish (Bemvenutti 1990, Rocha et al. 2008, Contente et al. 2011), *A. brasiliensis* has long and narrowly spaced gill rakers that aid filter feeding on both phyto- and zooplankton from the water column (Figueiredo & Menezes 1978). However, alterations and loss of gill tissue driven by *M. nana*'s development into large and ovigerous females may compromise the fish's ability to retain small particles such as the microalgae comprising phytoplankton and thus could account for the lower phytoplankton intake recorded in our sample of parasitized Brazilian silversides.

Parasitized *A. brasiliensis* also showed an overall decrease in FI as the parasite developed in weight and in egg and larval weight, which may suggest a shift in host feeding behaviour. The filter-feeding strategy adopted by Brazilian silversides has great energetic expenditure, as it demands continuous swimming and thus may be unaffordable for parasitized fish, which are burdened by cymothoid parasites' feeding, molting and breeding (Brusca 1978, Fogelman & Grutter 2008). Moreover, non-parasitized fish fed on bottom-associated items such as nematodes, annelids and molluscs, revealing benthic–pelagic shifts in feeding behaviour, contrary to parasitized fish. Reduced foraging efforts have been previously reported for infected fish (Meadows & Meadows 2003) and may lead to poor condition (Vigneshwaran et al. 2018), as the parasite compromises the host's food intake while increasing its energetic requirements. Our sample of parasitized *A. brasiliensis* revealed that this pattern was enhanced as the parasite developed and reproduced, due not only to increased energetic costs but also to potential impairment of the fish's filter-feeding mechanisms, thus leading to a decrease in host feeding efficiency and condition. Moreover, the tendencies detected for $K$ and FI were also reported for $K_E$, revealing that the detrimental effect caused by *M. nana*'s development may impact the somatic growth of adult Brazilian silversides, a response that could be stronger in juvenile fish.

*A. brasiliensis*' diet also comprised an unidentified nematode and a *Livoneca* sp. isopod; however, the 2 individuals that fed on these items were not considered parasitized fish. Members of the *Livoneca* genus are gill-inhabiting ectoparasites when in their adult form, whereas the juveniles are zooplankton (Brusca 1981), and thus may have been accidentally preyed upon by the Brazilian silversides. As the specimen found was dislodged from the fish's gills, we did not consider it to be actively parasitizing *A. brasiliensis*, and thus its occurrence as just a dietary item. The unidentified nematode found in 1 stomach sample was also not considered to be effectively parasitizing *A. brasiliensis* as it was not attached to the fish's intestinal vessels. Hence, both *A. brasiliensis* individuals were pooled together with other non-parasitized fish, leaving the results of statistical analyses strictly related to *M. nana*'s presence and development, supporting our hypothesis that this parasite is mainly responsible for the detrimental effect on host health.

The parasitic load imposed by *M. nana* had a greater impact on the condition of male than of female *A. brasiliensis*, which could also be related to feeding. Male fish showed lower values of FI in comparison to females, regardless of *M. nana*'s presence (mean ± SE FI: females = 2.33 ± 0.05, males = 2.17 ± 0.07; Table 2), which may explain, at least partially, the greater impact this parasite had on male hosts. Food intake was found to be an important factor influencing the condition of *Cheilodipterus quinquelineatus* parasitized by *Anilocra apogonae* during laboratory trials performed by Östlund-Nilsson et al. (2005), revealing that the effects of parasitism on host condition were only detected under limited food supply. Those authors then suggested parasitized fish in the wild may have to forage harder to maintain their condition, in contrast to what was observed in our sample of male Brazilian silversides, which showed lower food intake than females, thus leading to poorer condition. Additional research on sex-related differences in fish feeding behaviour and/or energetic expenditure may help understand the male's higher susceptibility to this parasite's load.

Contrary to host sex, *M. nana* did not seem to influence the temporal shifts detected for $K$, FI and HSI (Table 2). We expected parasitized fish to show lowest values of these indices during months with highest parasitic prevalence and/or intensity (i.e. June 2015), or no variation at all, as reported by Gomiero et al. (2012). Those authors recorded poor condition of *Astyanax intermedius* parasitized by *Paracymothoa astyanaxi*, revealing that infected hosts did not go through the annual stages of their life cycle, in contrast to non-parasitized individuals. Despite our findings not fully agreeing with Gomiero's et al. (2012), we cannot conclude with certainty that *M. nana* does not affect *A. brasiliensis*' life cycle, since we only performed 3 sampling campaigns over 2 yr.

In conclusion, our results showed a negative relationship between the parasite *M. nana* and the condition of adult *A. brasiliensis*. The development and reproduction of this parasite likely imposes a high energetic cost on Brazilian silversides while also compromising feed intake, thus leading to poor physiological condition. Our work contributes to the advancement of knowledge on interactions between gill-cymothoid parasites and fish and represents an important step towards unraveling the mechanisms through which a parasite may affect host health. This research also raised novel questions on patterns of infection according to host sex and throughout the annual cycle, which needs to be addressed by further studies.

LITERATURE CITED

Adlard RD (1989) The effects of the parasitic isopod *Anilocra pomacentri* Bruce, 1987 (Cymothoidae) on the population dynamics of the reef fish *Chromis nitida* Whitley (Pomacentridae). PhD thesis, University of Queensland

Adlard RD, Lester RJG (1994) Dynamics of the interaction between the parasitic isopod, *Anilocra pomacentri*, and the coral reef fish, *Chromis nitida.* Parasitology 109: 311–324

Allain V (2001) Reproductive strategies of three deep-water benthopelagic fishes from the northeast Atlantic Ocean. Fish Res 51:165–176

Alves VEM, Patrício J, Dolbeth M, Pessanha A, Palma ART, Dantas EW, Vendel AL (2016) Do different degrees of human activity affect the diet of Brazilian silverside *Atherinella brasiliensis?* J Fish Biol 89:1239–1257

Amaral V, Cabral HN (2004) Ecology of the whiskered sole in the Sado Estuary, Portugal. J Fish Biol 64:460–474

Bello G, Vaglio A, Piscitelli G (1997) The reproductive cycle of *Mothocya epimerica* (Isopoda: Cymothoidae) a parasite of the sand smelt, *Atherina boyeri* (Osteichthyes: Atherinidae), in the Lesina Lagoon, Italy. J Nat Hist 31: 1055–1066

Bemvenutti MDA (1990) Hábitos alimentares de peixes-rei (Atherinidae) na região estuarina da lagoa dos Patos, Rio Grande do Sul, Brasil. Atlântica 12:79–102

Bervian G, Fontoura NF (2007) Growth of the silverside *Atherinella brasiliensis* in Tramandaí Estuary, southern Brazil (Actinopterygii: Atherinopsidae). Neotrop Ichthyol 5:485–490

Binning SA, Roche DG, Layton C (2013) Ectoparasites increase swimming costs in a coral reef fish. Biol Lett 9: 20120927

Binning SA, Barnes JI, Davies JN, Backwell PR, Keogh JS, Roche DG (2014) Ectoparasites modify escape behaviour, but not performance, in a coral reef fish. Anim Behav 93: 1–7

Bruce NL (1986) Revision of the isopod crustacean genus *Mothocya* Costa, in Hope, 1851 (Cymothoidae: Flabellifera), parasitic on marine fishes. J Nat Hist 20:1089–1192

Brusca RC (1978) Studies on the cymothoid fish symbionts of the eastern Pacific (Crustacea: Isopoda: Cymothoidae) II. Biology and systematics of *Lironeca vulgaris.* Allan Hancock Found Occas Pap 2:1–19

Brusca RC (1981) A monograph on the isopods Cymothoidae (Crustacea) of the eastern Pacific. Zool J Linn Soc 73: 117–199

Brusca RC, Gilligan MR (1983) Tongue replacement in a marine fish (*Lutjanus guttatus*) by a parasitic isopod (Crustacea: Isopoda). Copeia 1983:813–816

Bunkley-Williams L, Williams EH (1998) Isopods associated with fishes: a synopsis and corrections. J Parasitol 84: 893–896

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583

Clark F (1928) The weight–length relationship of the Californian sardine (*Sardina coerulea*) at San Pedro. Fish Bull 12:22–44

Colorni A, Trilles JP, Golani D (1997) *Livoneca* sp. (Flabellifera: Cymothoidae), an isopod parasite in the oral and branchial cavities of the Red Sea silverside *Atherinomorus lacunosus* (Perciformes, Atherinidae). Dis Aquat Org 31:65–71

Contente RF, Stefanoni MF, Spach HL (2011) Feeding ecology of the Brazilian silverside *Atherinella brasiliensis* (Atherinopsidae) in a sub-tropical estuarine ecosystem. J Mar Biol Assoc UK 91:1197–1205

Figueiredo LL, Menezes NA (1978) Manual de peixes marinhos do sudeste do Brasil. II. Teleostei. Museu de Zoologia de São Paulo, São Paulo

Finley RJ, Forrester GE (2003) Impact of ectoparasites on the demography of a small reef fish. Mar Ecol Prog Ser 248: 305–309

Fogelman RM, Grutter AS (2008) Mancae of the parasitic cymothoid isopod, *Anilocra apogonae*: early life history, host-specificity, and effect on growth and survival of preferred young cardinal fishes. Coral Reefs 27:685

Fogelman RM, Kuris AM, Grutter AS (2009) Parasitic castration of a vertebrate: effect of the cymothoid isopod, *Anilocra apogonae*, on the five-lined cardinalfish, *Cheilodipterus quinquelineatus.* Int J Parasitol 39:577–583

Golzio JE, Falkenberg JM, Praxedes RC, Coutinho AS and others (2017) Gill parasites of fish from two estuaries in northeastern Brazil: new hosts and geographical records. An Acad Bras Cienc 89:2281–2291

Gomiero LM, Souza UP, Braga FMDS (2012) Condition factor of *Astyanax intermedius* Eigenmann, 1908 (Osteichthyes, Characidae) parasitised by *Paracymothoa astyanaxi* Lemos de Castro, 1955 (Crustacea, Cymothoidae) in the Grande River, Serra do Mar State Park-Santa Virgínia Unit, São Paulo, Brazil. Braz J Biol 72:379–388

Hatcher MJ, Dick JTA, Dunn AM (2006) How parasites affect interactions between competitors and predators. Ecol Lett 9:1253–1271

Kawakami E, Vazzoler G (1980) Método gráfico e estimativa de Índice alimentar aplicado no estudo de alimentação de peixes. Bol Inst Oceanogr 29:205–207

Kawanishi R, Sogabe A, Nishimoto R, Hata H (2016) Spatial variation in the parasitic isopod load of the Japanese halfbeak in western Japan. Dis Aquat Org 122:13–19

Kensley B, Schotte M (1989) Marine isopod crustaceans of the Caribbean. Smithsonian Institution Press, Washington, DC

Lafferty KD, Allesina S, Arim M, Briggs CJ and others (2008) Parasites in food webs: the ultimate missing links. Ecol Lett 11:533–546

Leonardos I, Trilles JP (2003) Host–parasite relationships: occurrence and effect of the parasitic isopod *Mothocya epimerica* on sand smelt *Atherina boyeri* in the Mesolongi and Etolikon Lagoons (W. Greece). Dis Aquat Org 54:243–251

Leonardos I, Trilles JP (2004) Reproduction of *Mothocya epimerica* (Crustacea: Isopoda: Cymothoidae), parasitic on the sand smelt *Atherina boyeri* (Osteichthyes: Atherinidae) in Greek lagoons. Dis Aquat Org 62: 249–253

Leps J, Smilauer P (2003) Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge

Lester RJG (1984) A review of methods for estimating mortality due to parasites in wild fish populations. Helgol Mar Res 37:53–64

Marcogliese DJ (2004) Parasite: small players with crucial roles in the ecological theater. EcoHealth 1:151–164

Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA (1982) The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). J Parasitol 68:131–133

Meadows DW, Meadows CM (2003) Behavioral and ecological correlates of foureye butterflyfish, *Chaetodon capistratus*, (Perciformes: Chaetodontidae) infected with *Anilocra chaetodontis* (Isopoda: Cymothoidae). Rev Biol Trop 51:77–81

Monfort KCF, Fujimoto RY, Varella JEA, Guimarães MDF, Santos AFL, Diniz DG (2009) Ocorrência de *Livoneca desterroensis* (Isopoda Cymothoidae), parasita de sardinha — *Cetengraulis edentulus* (Cuvier, 1828) (Engraulidae, Clupeiforme), capturadas na região do nordeste Paraense, Brasil. Bol Inst Pesca Sao Paulo 34: 657–662

Monteiro-Neto C, Tubino RA, Moraes LES, Mendonça Neto JP, Esteves GV, Fortes WL (2008) Associações de peixes na região costeira de Itaipu, Niterói, RJ. Iheringia (Zool) 98:50–59

Östlund-Nilsson S, Curtis L, Nilsson GE, Grutter AS (2005) Parasitic isopod *Anilocra apogonae*, a drag for the cardinal fish *Cheilodipterus quinquelineatus.* Mar Ecol Prog Ser 287:209–216

Parker D, Booth AJ (2013) The tongue-replacing isopod *Cymothoa borbonica* reduces the growth of largespot pompano *Trachinotus botla.* Mar Biol 160:2943–2950

Rocha AAF, da Silva-Falcão EC, Severi W (2008) Alimentação das fases iniciais do peixe-rei *Atherinella brasiliensis* (Atherinopsidae) no estuário do rio Jaguaribe, Itamaracá–PE. Agraria 3:365–370

Roche DG, Strong LE, Binning SA (2013) Prevalence of the parasitic cymothoid isopod *Anilocra nemipteri* on its fish host at Lizard Island, Great Barrier Reef. Aust J Zool 60: 330–333

Rohde K (ed) (2005) Marine parasitology. CSIRO Publishing, Collingwood

Salvador MVS, Silva MAM (2002) Morphology and sedimentology of Itaipu embayament–Niterói/RJ. An Acad Bras Cienc 74:127–134

Schioedte JC, Meinert F (1884) Symbolae ad monographium Cymothoarum Crustaceorum Isopodum familiae. IV. Cymothoidae. Trib. II Cymothoinae. Trib. III. Livonecinae. Naturhistorisk Tidsskrift 14:221–454

Sergipense S, Pinto DG (1995) Aspectos de ocorrência e distribuição espacial da ictiofauna da Lagoa de Itaipu, Niterói-Rio de Janeiro. Publ Espec Inst Oceanogr São Paulo 11:179–186

Smit NJ, Bruce NL, Hadfield KA (2014) Global diversity of fish parasitic isopod crustaceans of the family Cymothoidae. Int J Parasitol Parasites Wildl 3:188–197

Thatcher VE (2000) The isopod parasites of South American Fishes. In: Salgado-Maldonado G, García Aldrete AN, Vidal-Martínez VM (eds) Metazoan parasites in the Neotropics: a systematic and ecological perspective. Instituto de Biologia UNAM, México

Vazzoler AEAM (1996) Biologia da reprodução de peixes teleósteos: teoria e prática. EDUEM, Maringá

Vigneshwaran P, Ravichandran S, Prema M (2018) Parasitic isopod *Cymothoa eremita* (Brünnich 1783) (Isopoda: Cymothoidae) affects the growth of black pomfret *Parastromateus niger* (Bloch 1795) in the southeast coast of India. Thalassas. doi:10.1007/s41208-018-0097-7

Welicky RL, Sikkel PC (2014) Variation in occurrence of the fish-parasitic cymothoid isopod, *Anilocra haemuli*, infecting French grunt (*Haemulon flavolineatum*) in the north-eastern Caribbean. Aust J Mar Freshwater Res 65: 1018–1026

Welicky RL, Sikkel PC (2015) Decreased movement related to parasite infection in a diel migratory coral reef fish. Behav Ecol Sociobiol 69:1437–1446