

## Variation in worm assemblages associated with *Pomacea canaliculata* (Caenogastropoda, Ampullariidae) in sites near the Río de la Plata estuary, Argentina

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**Key words:** apple-snails, Platyhelminthes, Trematoda, Annelida, Hirudinea, symbiosis, commensalism, parasitism.

**ABSTRACT:** *Pomacea canaliculata* is a common gastropod in freshwater habitats from Central and Northern Argentina, extending northwards into the Amazon basin. Several Platyhelminthes have been reported associated to *P. canaliculata*, sharing an intimate relationship with this gastropod host. The objectives of this study were to describe the symbiotic species assemblages associated to *P. canaliculata* in the study area, and to disclose differences among them. Samples were taken in three typical small streams and one artificial lentic lagoon, all connected with the Río de la Plata estuary. The 81.53% were infested with different symbiotic (*sensu lato*) species. Among the Platyhelminthes, the commensal *Temnocephala iheringi* Haswell, 1893 was highly prevalent in all samples, always in the mantle cavity. Four trematode taxa were recognized: (a) metacercariae of *Echinostoma parcespinosum* Lutz, 1924 in the mantle cavity and sporocysts in the digestive gland; (b) metacercariae of *Dietziella egregia* (Dietz, 1909) in the pericardial cavity; (c) unidentified xiphidiocercariae and (d) unidentified sporocysts and furcocercariae in the digestive gland. Nematode larvae and oligochaetes were found in two localities in the mantle cavity. Among the Annelida, *Helobdella ampullariae* Ringuelet, 1945 was found in the mantle cavity and lung of snails only from one locality. Our results show that although some of the symbionts are present in all localities, others are restricted to some particular ones, whether in their absolute numbers or in their relative abundance. Thus, each hosting population at the studied localities may be defined by the particular combination of symbionts that bears.

### Introduction

*Pomacea canaliculata* (Lamarck, 1822) is a common gastropod in freshwater habitats in both Central and Northern Argentina, extending also northwards into the Amazon basin (Hylton Scott, 1958; Martín *et al.*,

2001). Most reports of *P. canaliculata* deal with its distribution, population dynamics, growth, and behavior. However, there are few studies on the relationship between *P. canaliculata* as a host and the symbiotic species living associated to it, either in its native (*e.g.* Di Persia and Radici de Cura, 1973; Martorelli, 1987; Ho and Thatcher, 1989; Hamann, 1992; Damborenea, 1996; Damborenea and Gullo, 1996; Gamarra-Luques *et al.*, 2004; Thiengo *et al.*, 2004; Martín *et al.*, 2005; Vega *et al.*, 2006) or its invasive area (Keawjam *et al.*, 1993). Factors determining the presence and/or prevalence of symbiotic species are poorly understood.

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These gastropods provide an adequate environment for hosting a variety of both prokaryotic and eukaryotic symbionts (Vega *et al.*, 2006). Among them, several Platyhelminthes have been reported associated to *P. canaliculata*, sharing an intimate relationship with the gastropod host (Lockyer *et al.*, 2004), which acts either as the unique, the primary, or secondary intermediate host.

The main objective of this study was to describe the symbiotic species assemblages associated with *P. canaliculata* in the study area. The results suggest that the localities may be defined and differentiated by means of the relative presence of the symbionts associated with *P. canaliculata*.

### Material and Methods

The sampled localities are three small streams and one artificial lentic lagoon, all connected with the Río de la Plata estuary. Samples were taken between December 15, 1996 and May 25, 1997. The four sampled sites differed in environmental variables such as water temperature (°C), conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ), dissolved oxygen ( $\text{mg}\cdot\text{l}^{-1}$ ), and pH (Table 1). Zapata stream (34°59'S – 57°42'W) and Bagliardi beach (35°55'S-57°49'W) have similar conductivity and pH values, but samples from the second site were taken very near its mouth on

the Río de la Plata estuary, a factor influencing height and salinity values. Los Talas lagoon (34°53'S - 57°50'W) is a deep artificial lentic habitat with scarce vegetation. El Pescado stream (34°57'S – 57°46'W) is a small and shallow stream and differs strongly from all other sampled sites, as it rendered high conductivity values. The characteristic and dominant substrate was mud and the riparian vegetation included mainly *Scirpus* sp., and to a lesser extent *Ludwigia* sp. and Lemnaceae.

Most samples were taken during the reproductive season of *Pomacea canaliculata* (i.e., between October and April, Martín *et al.*, 2001), with the exception of that taken at Los Talas lagoon (May 3, 1997) and the third sample taken from Zapata stream (May 25, 1997).

Living snails were collected in the submerged aquatic vegetation, other submerged substrates and from the bottom. Live snails were transported to the laboratory, where the total shell length was measured. The snails were dissected; the mantle cavity, the associated structures, the pericardial cavity, digestive gland, stomach, intestine, gonad and albumen gland were examined. The symbionts that snails were carrying were identified under a stereoscopic microscope. The temnocephalids were counted to determine their abundance. Sex of the host was determined in the dissected snails according to the presence of a well developed copulatory apparatus (males), a colored albumen gland (females) or none of them (undifferentiated juveniles).

TABLE 1.

Sampled sites, dates and selected environmental variables.

| Locality          | Date         | Temperature<br>(°C) | Conductivity<br>( $\mu\text{S}/\text{cm}$ ) | Dissolved oxygen<br>(mg/l) | pH  |
|-------------------|--------------|---------------------|---------------------------------------------|----------------------------|-----|
| El Pescado stream | Dec 15, 1996 | 25.5                | 1295                                        | 2.8                        | 7.9 |
| Zapata stream     | Jan 31, 1997 | 25.1                | 407                                         | 3.5                        | 9.1 |
|                   | Feb 11, 1997 | 26.8                | 137                                         | 4.0                        | 7.0 |
|                   | May 25, 1997 | 18.0                | 365                                         | 4.5                        | 8.0 |
| Bagliardi beach   | Apr 8, 1997  | 20.6                | 567                                         | 4.0                        | 8.5 |
| Los Talas lagoon  | May 3, 1997  | 18.1                | 483                                         | 1.7                        | 8.0 |

The environmental variables and the prevalence of symbiotic species were standardized and a canonical correlation analysis (partial least-square) was performed to find pairs of linear combinations that could account for any observed correlations between the two sets of variables (Quinn and Keough, 2002).

A principal component analysis included all host snails and the presence of symbionts after log. (x+1) transformation of their values was performed to reveal symbiotic species patterns among the sampled sites. Simple linear regression analysis was plotted to explore the relationship between temnocephalid abundance and host size (total length in mm).

**Results**

*Host size and sex ratio*

Table 2 shows total length (mean ± standard deviation) and percentage of males, females and undifferentiated juveniles of *P. canaliculata* that were present in each sample. Small-sized specimens predominated at Bagliardi beach and Los Talas lagoon, therefore rendering undifferentiated specimens dominant. In other sampled sites females were always more abundant than males.

*Symbiotic worms' assemblages*

A total of 287 snails were collected at the four localities, 81.53% were infested with different symbiotic (*sensu lato*) species. Among the Platyhelminthes, the commensal *Temnocephala iheringi* Haswell, 1893 was highly prevalent in all samples, always in the mantle cavity. Four trematode taxa were recognized: (a) metacercariae and sporocysts of *Echinostoma parcespinosum* Lutz, 1924, in both the mantle cavity and the digestive gland, respectively; (b) metacercariae of *Dietziella egregia* (Dietz, 1909) in the pericardial cavity; (c) some unidentified xiphidiocercariae in the digestive gland and (d) some unidentified sporocysts and furcocercariae in the digestive gland also. Nematode larvae were found in the mantle cavity of snails from El Pescado and Zapata streams. Among the Annelida, *Helobdella ampullariae* Ringuet, 1945 was found in the mantle cavity and lung of snails from Bagliardi beach only (Table 3).

Turbellaria Temnocephalidae: *Temnocephala iheringi*

Prevalence of *T. iheringi* was high in all samples except in that from Los Talas lagoon (Table 3). Total abundance of temnocephalids was higher at both El Pescado and Zapata streams than at Bagliardi beach and Los Talas lagoon. In all samples considered, correlation between

**TABLE 2.**

**Number (N), total length (mean ± standard deviation), and percentage of males, females and undifferentiated juveniles of *Pomacea canaliculata* from each sampled site.**

| Locality                 | N  | Total length<br>X ± SD | %<br>males | %<br>females | % undifferentiated<br>juveniles |
|--------------------------|----|------------------------|------------|--------------|---------------------------------|
| El Pescado stream        | 62 | 33.33 ± 8.07           | 33.87      | 62.90        | 3.22                            |
| (sample 1)               | 33 | 32.66 ± 9.63           | 30.30      | 63.64        | 6.06                            |
| Zapata stream (sample 2) | 47 | 30.14 ± 5.42           | 42.55      | 55.32        | 2.12                            |
| (sample 3)               | 19 | 28.79 ± 6.74           | 42.10      | 57.90        | 0                               |
| Bagliardi beach          | 71 | 18.12 ± 9.30           | 7.04       | 29.58        | 63.38                           |
| Los Talas lagoon         | 54 | 12.28 ± 7.05           | 5.55       | 24.07        | 62.96                           |

TABLE 3.

List of symbionts, prevalences and localities where they were found.

| Locality                 | Symbiont                         | Stage             | Localization           | % Prevalence |
|--------------------------|----------------------------------|-------------------|------------------------|--------------|
| El Pescado stream        | <i>Temnocephala iheringi</i>     | adults            | mantle cavity          | 98.38        |
|                          | Unidentified trematode           | xiphidiocercariae | digestive gland        | 1.61         |
|                          | Unidentified nematode            | larvae            | mantle cavity          | 1.61         |
|                          | Unidentified oligochaete         | adults            | mantle cavity          | 1.61         |
| Zapata stream (sample 1) | <i>Temnocephala iheringi</i>     | adults            | mantle cavity          | 81.81        |
|                          | <i>Dietziella egregia</i>        | metacercariae     | pericardial cavity     | 12.12        |
|                          | Unidentified trematode           | xiphidiocercariae | digestive gland        | 3.03         |
| Zapata stream (sample 2) | <i>Temnocephala iheringi</i>     | adults            | mantle cavity          | 100.00       |
|                          | <i>Dietziella egregia</i>        | metacercariae     | pericardial cavity     | 88.46        |
|                          | Unidentified trematode           | xiphidiocercariae | digestive gland        | 6.38         |
|                          | Unidentified nematode            | larvae            | mantle cavity          | 2.12         |
| Zapata stream (sample 3) | <i>Temnocephala iheringi</i>     | adults            | mantle cavity          | 89.47        |
|                          | <i>Dietziella egregia</i>        | metacercariae     | pericardial cavity     | 68.42        |
|                          | Unidentified trematode           | xiphidiocercariae | digestive gland        | 5.26         |
|                          | Unidentified trematode           | furcocercariae    | digestive gland        | 5.26         |
|                          | Unidentified nematode            | larvae            | mantle cavity          | 5.26         |
| Bagliardi beach          | <i>Temnocephala iheringi</i>     | adults            | mantle cavity          | 91.55        |
|                          | <i>Echinostoma parcespinosum</i> | metacercariae     | mantle cavity          | 8.45         |
|                          | <i>Helobdella ampullariae</i>    | adults            | mantle cavity and lung | 7.04         |
|                          | Unidentified oligochaete         | adults            | mantle cavity          | 4.22         |
| Los Talas lagoon         | <i>Temnocephala iheringi</i>     | adults            | mantle cavity          | 3.70         |
|                          | <i>Echinostoma parcespinosum</i> | metacercariae     | mantle cavity          | 25.92        |

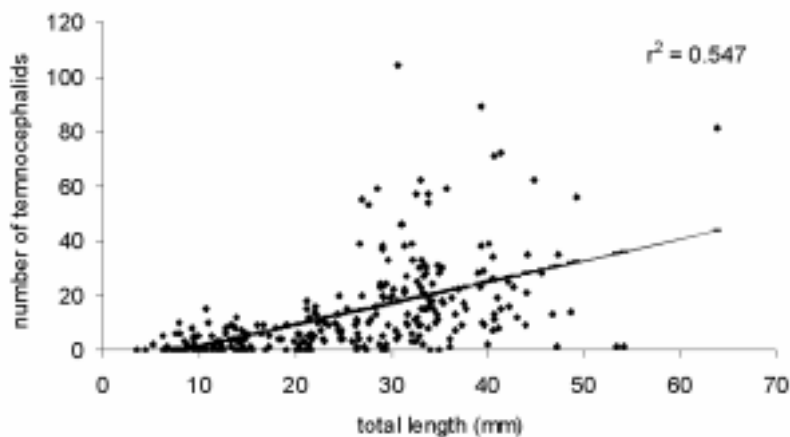


FIGURE 1. Abundance of *Temnocephala iheringi* (number of worms per snail) as a function of snail size.

abundance and size of host was 0.547 (Fig. 1). This values decreased when each locality was considered separately, except for Los Talas lagoon (Table 4). The chi square test showed no significant differences between the observed

and expected prevalence values on snails of different sexes except for the sample from Los Talas lagoon (Table 5). Among different size groups of hosts, prevalence was higher in the larger snails (Figs. 2, 3 and 4).

TABLE 4.

Abundance of *Temnocephala ihering* (mean number of specimens per host, for males, females and undifferentiated juveniles) and the correlation value between temnocephalid abundance and host size for each locality.

| Locality          | r <sup>2</sup> | Mean abundance |            |          |                  |      |
|-------------------|----------------|----------------|------------|----------|------------------|------|
|                   |                | Total          | In females | In males | Undifferentiated |      |
| El Pescado stream | 0.323          | 24.25          | 25.51      | 24.09    | 1.5              |      |
| Zapata stream     | (sample 1)     | 0.377          | 19.24      | 12.95    | 36.10            | 1.0  |
|                   | (sample 2)     | 0.265          | 18.81      | 14.88    | 23.10            | 20.0 |
|                   | (sample 3)     | 0.396          | 12.10      | 9.00     | 16.87            | ---  |
| Bagliardi beach   | 0.150          | 5.77           | 6.57       | 5.80     | 5.4              |      |
| Los Talas lagoon  | 0.607          | 0.04           | 0.06       | 0.33     | 0                |      |

TABLE 5.

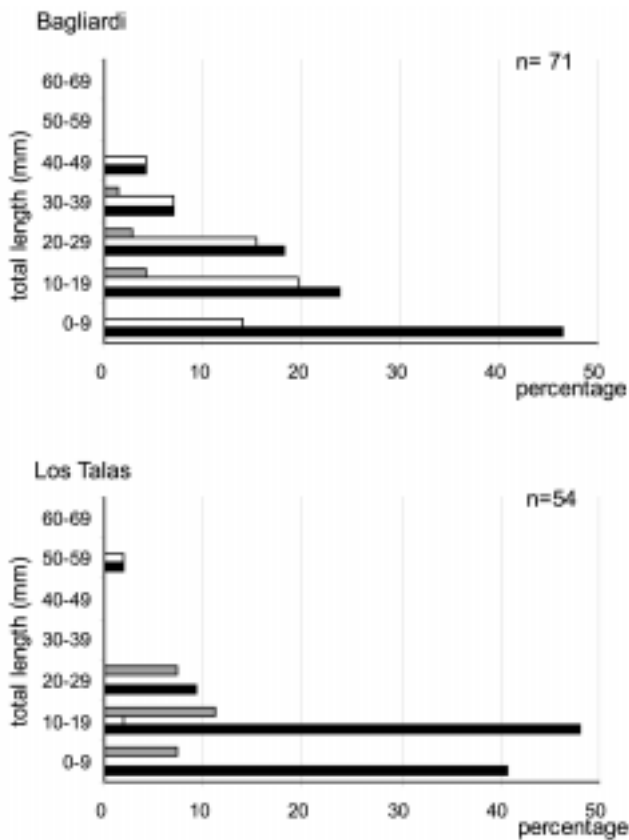
Prevalence of *Temnocephala iheringi* (T) and *Helobdella ampullariae* (L) in males, females and undifferentiated juveniles of *Pomacea canaliculata* from each sampling site (Chi square test, \*\*\*P<0.001; NS, non significant).

| Locality          |            | In females | In males | Undifferentiated juveniles | Chi square |    |
|-------------------|------------|------------|----------|----------------------------|------------|----|
| El Pescado stream | T          | 100        | 95.24    | 100                        | NS         |    |
| Zapata stream     | (sample 1) | T          | 80.95    | 90.00                      | 50.00      | NS |
|                   | (sample 2) | T          | 100      | 100                        | 100        | NS |
|                   | (sample 3) | T          | 100      | 87.50                      | -          | NS |
| Bagliardi beach   | T          | 80.95      | 100      | 95.55                      | NS         |    |
|                   | L          | 60.00      | 9.62     | -                          | ***        |    |
| Los Talas lagoon  | T          | 7.69       | 33.33    | 0                          | ***        |    |

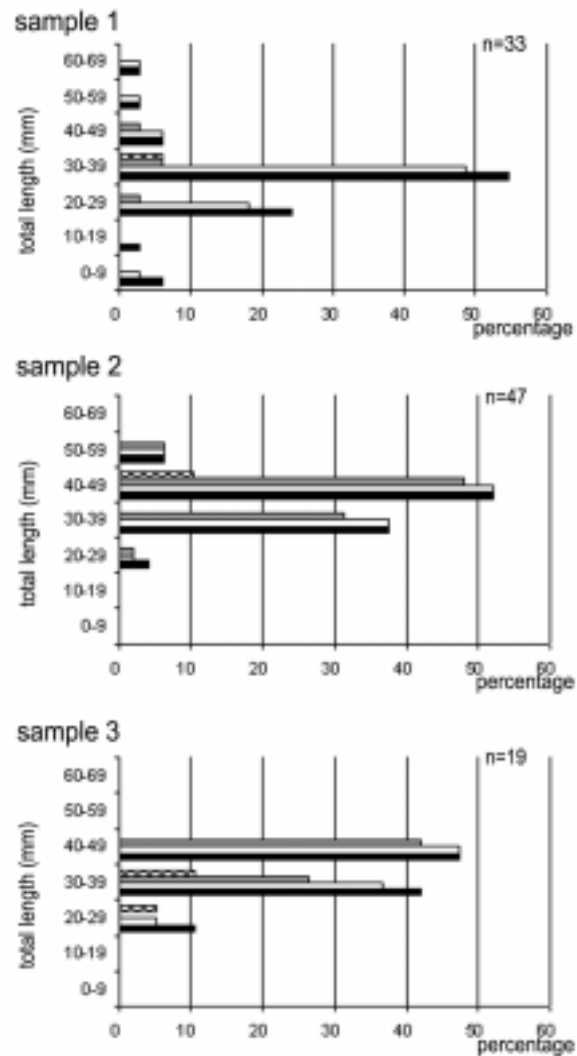
*Trematoda larvae*

Larval stages of different species of trematodes were found in all sampled localities (Table 3). Table 6 shows the total prevalence of these larval phases and their particular prevalence according to host sex. These values do not agree with the expected prevalence, according to the sex distribution pattern in each sample. A chi square test showed significant differences for all localities except for sample 2 from Zapata stream (Table 6).

Metacercariae of *Echinostoma parcespinosum* were found associated with snails from two localities, Bagliardi beach and Los Talas lagoon (Table 3). They were found in the mantle cavity arranged in a sheet. The number of metacercariae was variable. Prevalence at Bagliardi beach was 8.45 and it was found in the mantle cavity of snails between 10.38 and 33.10 mm of total length. Prevalence of metacercariae was higher in the sample from Los Talas lagoon (25.92) despite the fact that the mean host size



**FIGURE 2.** Snail size frequency (black bars) and prevalence of *E. parcespinosum* (gray bars) and *Temnocephala iheringi* (white bars) at both Bagliardi beach and Los Talas lagoon.



**FIGURE 3.** Snail size frequency (black bars) and prevalence of *Dietziella egregia* (gray bars), other trematode taxa (furcocercariae and xiphidiocercariae, banded bars) and *Temnocephala iheringi* (white bars) at Zapata stream. Samples were taken in January, February and May, see Table 1).

was smaller (Table 2). Metacercariae were found in snails ranging between 7.65 and 21.34 mm of total length. Fig. 2 shows the distribution of metacercariae in *E. parcespinosum* according to host size.

Metacercariae of *Dietziella egregia* were found exclusively in the samples from Zapata stream, within the pericardic cavity of the snails. Prevalence was very high, 88.46% (Table 3); metacercariae were found in snails ranging between 21.12 and 44.12 mm of total

length. Distribution of these metacercariae according to host size is depicted in figure 3.

Other larval forms of trematodes were found. Non-identified xiphidiocercariae appeared in the digestive gland of snails from El Pescado stream and Zapata stream, but with low prevalence values (Table 3). Furcocercariae were also found sporadically in sporo-

cysts in the digestive glands from snails at Zapata stream (Fig. 3); prevalence was 5.26%.

In samples 2 and 3 from Zapata stream, there were snails infested with metacercariae of *D. egregia* in their pericardic cavity; parasitizing the same specimens, (a) rediae with unidentified cercariae, (b) xiphidiocercariae, and (c) sporocysts were observed.

*Nematoda*

Unidentified nematode larvae were found at El Pescado stream and Zapata stream, always with low prevalence values.

Hirudinea Glossiphonidae: *Helobdella ampullariae*

This species has been found only at Bagliardi beach (Table 3). The total prevalence was 7.04%, and the chi square test showed that the distribution of this symbiont differed significantly from those predicted from the null hypothesis (Table 5, Fig. 4). Despite the scarce number of males recognized in the sample (only 6.76% were males, 28.38% females and the rest were undifferentiated juveniles), leeches were more abundant on males. Chi square value ( $\chi^2 = 1.08 \cdot 10^{-53}$ ) was significant.

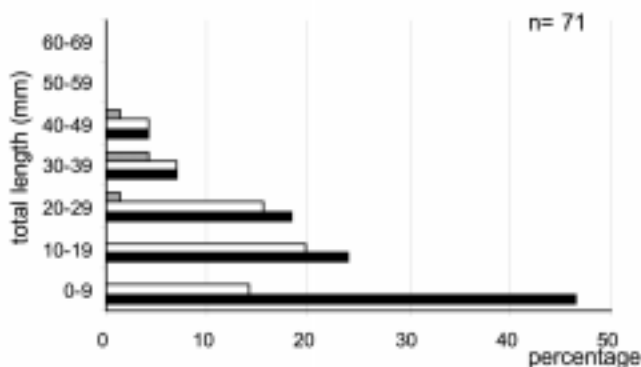


FIGURE 4. Snail size frequency (black bars) and prevalence of *Helobdella ampullariae* (gray bars) and *Temnocephala iheringi* (white bars) at Bagliardi beach.

TABLE 6.

Prevalence of trematode larvae associated with *Pomacea canaliculata* in each sampling site (Chi square test, \*\*\*P<0.001; \*\*P<0.005 and P>0.001; NS, non significant)

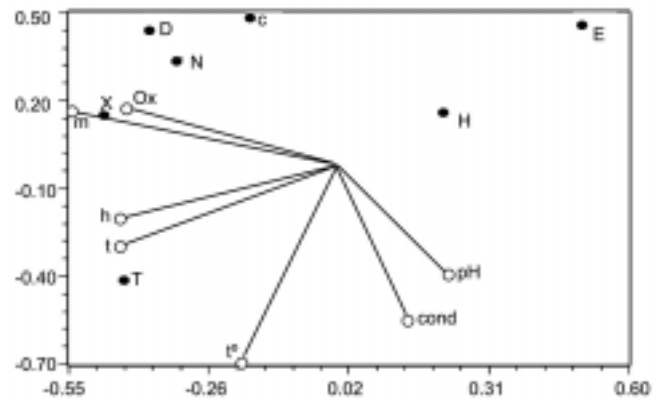
| Locality          | Prevalence |            |          |                            | Chi square |     |
|-------------------|------------|------------|----------|----------------------------|------------|-----|
|                   | Total      | In females | In males | Undifferentiated juveniles |            |     |
| El Pescado stream | 1.61       | 2.50       | 0        | 0                          | ***        |     |
| Zapata stream     | (sample1)  | 18.18      | 23.81    | 10.00                      | 0          | *** |
|                   | (sample 2) | 87.94      | 85.18    | 90.00                      | 100        | NS  |
|                   | (sample 3) | 68.42      | 63.63    | 87.50                      | 0          | **  |
| Bagliardi beach   | 8.45       | 9.52       | 20.00    | 6.66                       | ***        |     |
| Los Talas lagoon  | 25.93      | 23.52      | 100      | 20.58                      | ***        |     |

*Oligochaeta*

Unidentified oligochaetes were found at El Pescado stream and Bagliardi beach, always with low prevalence values (Table 3); they were in the mantle cavity of snails ranging from 21.19 to 28.05 mm length.

*Statistical evaluation of symbiotic assemblages*

Table 7 and Fig. 5 show results of a least-squares analysis performed using the environmental characteristics of the sampled sites, the characteristics of the host population and the presence of symbionts. These results show that the presence of *E. parcespinosum* and xiphidiocercariae, and the abundance of temnocephalids are the variables better correlated with the characteristics of the host population (size and sex), and O<sub>2</sub> concentration.



**FIGURE 5.** Canonical correlation between two sets of variables. White points: t°, temperature; cond, conductivity; Ox, oxygen; pH; t, host size; h, female proportion; m, male proportion. Black points: T, *T. iheringi*; D, *D. egregia*; E, *E. parcespinosum*; H, *Helobdella ampullariae*; X, xiphidiocercariae; c, cercariae; N, nematodes.

**TABLE 7.**

**Canonical correlation values for the two sets of variables from the samples sites.**

| Canonical variate     | 1      | 2      | 3      |
|-----------------------|--------|--------|--------|
| Canonical correlation | 0.9446 | 0.8527 | 0.8838 |

| Variable          | Variate 1 | Variate 2 | Variate 3 |
|-------------------|-----------|-----------|-----------|
| Temperature       | -0.1990   | -0.6559   | -0.3200   |
| Conductivity      | 0.1451    | -0.5137   | 0.3196    |
| Oxygen            | -0.4357   | 0.1836    | 0.7505    |
| pH                | 0.2242    | -0.3669   | 0.4478    |
| Host size         | -0.4463   | -0.2726   | 0.0075    |
| Female proportion | -0.4478   | -0.1800   | -0.0659   |
| Male proportion   | -0.5473   | 0.1755    | -0.1651   |

| Variable                | Variate 1 | Variate 2 | Variate 3 |
|-------------------------|-----------|-----------|-----------|
| <i>H. ampullariae</i>   | 0.2203    | 0.1666    | 0.6788    |
| <i>T. iheringi</i>      | -0.4335   | -0.4004   | 0.3183    |
| <i>D. egregia</i>       | -0.3826   | 0.4495    | -0.2753   |
| <i>E. parcespinosum</i> | 0.5043    | 0.4690    | -0.2219   |
| Xiphidiocercariae       | -0.4753   | 0.1590    | -0.2595   |
| Cercariae               | -0.1748   | 0.4954    | 0.4757    |
| Nematoda                | -0.3261   | 0.3453    | 0.1385    |



Principal component analysis (Table 8 and Fig. 6), considering all hosts from all localities, was used for inter-locality characterization. Variability due to the three first components was 41.80%. Although delimitation among snails from the different localities was not complete, there was a certain separation among them. The presence of temnocephalids, host characteristics, and the presence of *Dietziella egregia*, were the variables that introduced the greatest amount of variability into the first component, allowing differentiation of hosts from Los Talas lagoon (with small host size, scarce number of temnocephalids and absence of *D. egregia* metacercariae), from those coming from Zapata stream and El Pescado stream (larger, with equilibrate sex proportions, high prevalence of *T. iheringi*, and presence of xiphidiocercariae). The latter two localities can be separated when the first and third components were compared, which refer to the presence of *D. egregia*. Hosts from Bagliardi beach were in an intermediate position. The relation among the different localities observed corre-

sponds to the geographic location. Bagliardi beach is placed between Los Talas lagoon on one side, and Zapata stream and El Pescado stream on the other.

This analysis suggests that, despite the proximity among localities sampled and that many of the symbionts were found in all or most of them, they can still be characterized and differentiated by means of the relative presence of the symbionts they carry.

**Discussion**

Two of the symbiotic worms found in this study complete their entire life cycle in association with *Pomacea canaliculata*. They inhabit the mantle cavity and lung; they reproduce within the snail. These species are *Temnocephala iheringi* – which was the most abundant and was present in all localities – and *Helobdella ampullariae*, found only at Bagliardi beach. Despite the fact that both species remain associated to

**TABLE 8.**

**Principal component analysis.**

|   | Eigen value | Percent | Cumulative |
|---|-------------|---------|------------|
| 1 | 2.33450589  | 19.4542 | 19.4542    |
| 2 | 1.42340878  | 11.8617 | 31.3160    |
| 3 | 1.25843059  | 10.4869 | 41.8029    |

| Components              | 1       | 2       | 3       |
|-------------------------|---------|---------|---------|
| Size                    | 1.3360  | 0.0749  | 0.2045  |
| Sex                     | -0.9325 | -0.0400 | -0.0707 |
| <i>T. iheringi</i>      | 1.1596  | -0.0263 | 0.2376  |
| Oligochaeta             | -0.0000 | -0.0785 | 0.2270  |
| Xiphidiocercariae       | 0.3381  | 0.4411  | -0.0187 |
| Nematoda                | 0.3558  | -0.3201 | -0.7412 |
| <i>D. egregia</i>       | 0.8686  | -0.0741 | -0.2045 |
| Sporocysts              | 0.3233  | -0.3252 | -0.8030 |
| Rediae                  | 0.2857  | 0.8545  | -0.2149 |
| Furcocercariae          | -0.0101 | -0.0391 | 0.0818  |
| <i>E. parcespinosum</i> | -0.5125 | 0.5425  | -0.3296 |
| <i>H. ampullariae</i>   | 0.0800  | 0.7601  | -0.1759 |

the host mollusk during their entire life cycle, their relationship with it differs in each case. *Temnocephala iheringi* takes advantage of the breathing current generated by the host to probably feed on small crustaceans or algae that may enter with them. The results presented herein suggest that although the presence of this symbiont in males, females and undifferentiated juveniles coincides with their presence in different populations studied, the host size was determinant of the number of temnocephalids carried. In small hosts, the number of temnocephalids was small, increasing as the size of the host does so. This occurred at all localities analyzed.

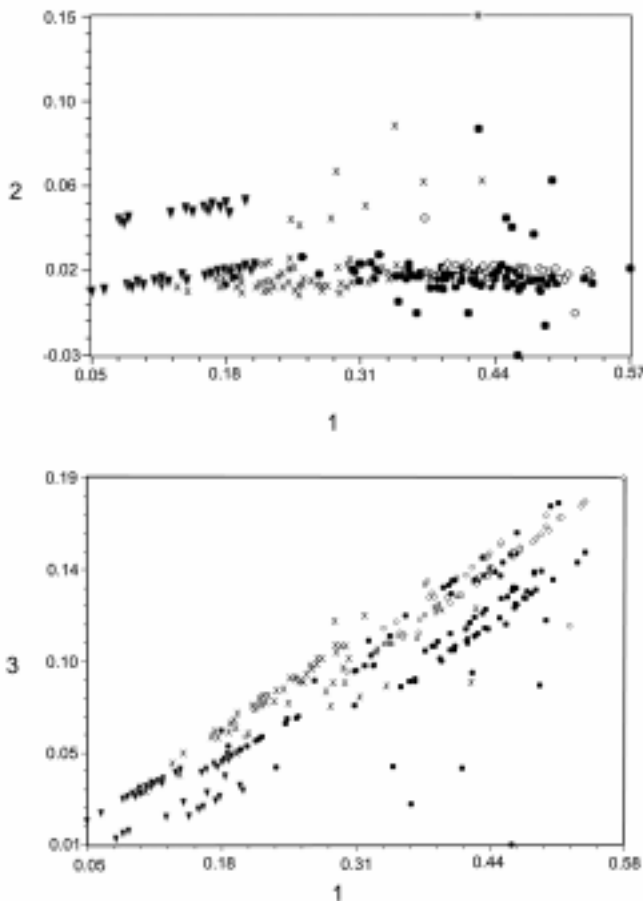
Three species of *Temnocephala* are known as symbionts of *Pomacea canaliculata* (Vega *et al.*, 2006). Among them, *Temnocephala iheringi* is the most widely ranging, as it is frequently found in the mantle cavity of *P. canaliculata* and other species of *Pomacea* and *Asolene* (Damborenea and Cannon, 2001), on the shell

of which it deposits its eggs (in the contact area between the channeled suture and the aperture, and in the umbilicus; Damborenea, 1996). Martin *et al.* (2005), in a study performed to understand the factors that affect the distribution and abundance of *T. iheringi* among populations of *P. canaliculata* in the southern part of the Pampas, found higher frequency of occurrence of temnocephalids on lentic sites with concentrations of bicarbonate lower than 6.6 meq l<sup>-1</sup>. The lentic locality analyzed in this study, Los Talas lagoon, had the lowest densities. This might be explained because of the small size of the snails in this locality.

Ringuelet (1945) considered that due to the peculiar features of *Helobdella ampullariae* (e.g. form and size of the posterior sucker, segmented and annulated appearance) and the permanent relationship that it shows with its host, the trophic relationship between them should be considered parasitism. Damborenea and Gullo (1996) studied the population of *H. ampullariae* from Bagliardi beach during 15 months and established that, contrarily to other hirudinean species associated to *P. canaliculata*, at this locality, *H. ampullariae* does not abandon its host, that its life cycle takes place entirely within it and that it shows a very long reproductive season (December to June), beyond the period in which the snail buries. The geographic distribution of this hirudinean is more restricted than that of the temnocephalids, as it was found only at one of the sampled localities in this study. However, its presence was not strongly associated to any particular environmental parameter or feature. Prevalence of Hirudinea on the hosts was low (7.04%), different from that reported previously at the same locality, when it varied between 23.33 and 68.18% (Damborenea and Gullo, 1996).

Populations of these two symbionts are adapted to the life-habits of the hosting species, taking advantage of the favorable moments (either of host activity or climate) for reproduction and transmission among hosts. The long copulation that occurs in *Pomacea canaliculata* (Albrecht *et al.*, 1996) may facilitate transmission of both temnocephalids and leeches from one individual host to another. This way of transmission was shown for a copepod species associated to this snail, *Ozmana huarpium* Gamarra-Luques and Castro Vazquez, 2004 (Gamarra-Luques *et al.*, 2004).

The other symbionts found in this study correspond to larval stages of trematodes that use *P. canaliculata* as an intermediate host. The gastropod acts as vehicle for the development and transmission of these trematode parasites, which share an intimate relationship with their gastropod hosts (Lockyer *et al.*, 2004). Embedded in



**FIGURE 6.** Principal component analysis. Host from: El Pescado stream, white circle; Zapata stream, black circle; Bagliardi beach, X; Los Talas lagoon, black triangle.

the trematode life cycle, the gastropod acts as the primary or secondary intermediate host. The mollusk provides an environment that the parasite exploits to grow and reproduce and is also the means through which the parasite can move on to the next host (either a definitive or yet another intermediate one). Despite the importance of *P. canaliculata* in freshwater environments in the region, records of the parasite species associated to it are sparse. Natural infestations with trematode larvae rarely surpass 5% (Anderson and May, 1979). *P. canaliculata* from Zapata stream shows the highest prevalence of parasite forms (reaching 90%) among the studied forms, which are represented mainly by metacercariae of *Dietziella egregia* lodged in the pericardial cavity. However, this prevalence is variable according to the sample considered; it was considerably lower during the first sampling carried out on October 31, 1997. The available information is not sufficient for establishing differences among these samples; therefore, the variation in prevalence could be due to the time of sampling. Ostrowski de Núñez *et al.* (1991) found important seasonal variations in the presence and prevalence of different larval forms of trematodes associated to *Biomphalaria occidentalis* collected in the province of Corrientes, in Northeastern Argentina.

Digiani and Ostrowski de Núñez (2000) mentioned *Dietziella egregia* for Punta Blanca, Magdalena (province of Buenos Aires), as a parasite of the “cuervillo de cañada” (*Plegadis chihi*, Threskiornithidae) and found metacercariae in the renal cavity of *P. canaliculata* from the same area. It remains unknown which is the first intermediate host for this species, as there is no information available whether the rediae and metacercariae grow in the same or different intermediate hosts (Digiani and Ostrowski de Núñez, 2000).

Los Talas lagoon also showed high prevalence of other metacercariae. In this case the trematode species is *Echinostoma parcespinosum*. Martorelli (1987) studied the vital cycle of this species, a parasite of the intestine of malacophagous rallids (*Pardirallus maculatus* and *Pardirallus sanguinolentus*). Miracidia penetrate *P. canaliculata*, reaching the gonad and digestive gland, where the rediae and cercariae develop. Cercariae may become encysted within the rediae or emerge and penetrate a second intermediate host such as *Pomacea canaliculata* or *Stenophysa marmorata* (Martorelli, 1987).

Other larvae found in this study were unidentified as rediae and cercariae (furcocercariae and xiphidiocercariae). Xiphidiocercariae were the most abundant. Life cycles and definitive hosts remain unknown for these cercariae, but it may be possible that

the hosts for the xiphidiocercariae are amphibians and for the furcocercariae are birds. Thiengo *et al.* (2004 and references therein) have carried out an intensive survey of freshwater snails from the State of Rio de Janeiro. They found four *Pomacea* species in the area, but they only found xiphidiocercariae and echinostome cercariae at four localities.

Studies on *P. canaliculata* as a host for symbionts are scattered and responding to local or particular problems, while more integral studies on the host forms are still missing. This study is an initial attempt to undertake further studies on *P. canaliculata* from different localities that, although geographically near, show different environmental features. Our results show that although some of the symbionts are present at all localities, others are restricted to particular sites, whether in absolute numbers or else in relative abundance. Thus, each hosting population at the studied localities may be defined by the particular combination of species it shows.

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