Testing temporal stability of the larval digenean community in *Heleobia conexa* (Mollusca: Cochliopidae) and its possible use as an indicator of environmental fluctuations

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SUMMARY

Larval digeneans have been proposed as indicators of abundance and diversity of vertebrate and other hosts as well as environmental disturbances. To evaluate its response to environmental changes and its potential use as an indicator of environmental fluctuations, the temporal stability of the community of larval digeneans in *Heleobia conexa* was comparatively analysed in 4 separate years (1996, 1999, 2004 and 2005) in Mar Chiquita coastal lagoon (Buenos Aires province, Argentina). In total, 4579 specimens of *H. conexa* were collected and 22 digenean species were observed. Overall prevalence presented inter-annual and seasonal differences. These differences correlate with seasonal changes in composition of the vertebrate definitive host community and with the elimination of the preferred habitat of *H. conexa* in 1999. In general, the larval digenean community of *H. conexa* showed a yearly re-establishment following the annual cycle of *H. conexa* and the presence of definitive hosts. This annual restructuring allowed inferences about the effects of short-term environmental changes in the lagoon. According to these observations, the larval digenean community of *H. conexa* could be considered as a good bio-indicator with quick response to environmental disturbances.

Key words: digenean-snail system, snail intermediate host, bio-indicator.

INTRODUCTION

Parasites with complex life cycles can be useful tools for the study of biotic communities. They can be used as biological indicators, given their susceptibility to different types of impact (which act directly and/or indirectly on the population of their hosts) (Huspeni and Lafferty, 2004; Hechinger and Lafferty, 2005; Huspeni *et al.* 2005; Fredensborg *et al.* 2006; Smith, 2007; Hechinger *et al.* 2007; Vidal-Martínez *et al.* 2010). A taxon of parasites that are promising as biological indicators are the digeneans (Trematoda: Digenea) (Kuris and Lafferty, 1994; Huspeni and Lafferty, 2004; Huspeni *et al.* 2005).

The digeneans typically have complex life cycles with different larval stages that parasitize intermediate hosts to finally mature as adults in the definitive hosts. Given that food webs are used by digeneans to complete their life cycles, their larval stages in intermediate hosts are positive indicators of trophic relationships in an ecosystem. In addition, the composition of a larval digenean community is a result of the presence and distribution of definitive hosts in the area. Thus, a diverse and abundant fauna of definitive hosts should result in a diverse and abundant community of larval digeneans in a molluscan host (Lafferty, 1997; Huspeni and Lafferty, 2004; Huspeni *et al.* 2005; Marcogliese, 2005; Lafferty *et al.* 2006*a*).

A general feature of larval digenean communities is their spatial and temporal variability in species composition (e.g. Curtis and Hurd, 1983; Sousa, 1991; Esch and Fernández, 1994; Jokela and Lively, 1995; Curtis, 1997; Kube *et al.* 2002). This variation has been related to the biology of definitive hosts (density, intensity of infection and behaviour) (Curtis and Hurd, 1983; Fernandez and Esch, 1991*a*,*b*; Sousa, 1993; Jokela and Lively, 1995; Granovitch *et al.* 2000; Skirnisson *et al.* 2004), to the biology of the snail hosts (habitat preference, vagility and life-history dynamics) (Rohde, 1981; Fernandez and Esch, 1991*b*; Sousa, 1993; Sapp and Esch, 1994; Jokela and Lively, 1995; Kube *et al.* 2002; Faltýnkova *et al.* 2008), to abiotic environmental factors (temperature, salinity, pH, water level, etc) (Pietrock and Marcogliese, 2003; Fingerut *et al.* 2003; Poulin, 2006; Poulin and Mouritsen, 2006; Byers *et al.* 2008), and to interspecific interactions among parasite species (Kuris, 1991; Lafferty *et al.* 1994; Esch *et al.* 2001). However, these factors do not act independently. They operate interdependently (Faltýnkova *et al.* 2008).

The Mar Chiquita coastal lagoon (Buenos Aires province, Argentina) is designated a Man and the Biosphere Reserve by UNESCO. It is divided into a

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freshwater zone, influenced by continental water discharge, and an estuarine zone. Air temperature, rainfall, tides, water temperature, salinity and depth are subject to daily, seasonal, and annual variation (Reta *et al*. 2001). The lagoon is characterized by the presence of the exotic polychaete *Ficopomatus enigmaticus* (Fauvel, 1923) (Serpulidae). This species builds calcareous reef-like aggregates that increase the topographic complexity and benthic diversity in the Mar Chiquita lagoon (Schwindt *et al*. 2001). Similar to other structuring polychaete species, the colonies of *F. enigmaticus* serve as a refuge and as a preferred habitat for other species of invertebrates. In fact, the abundances of some invertebrates are higher among *F. enigmaticus* tubes than in the surrounding habitats. These organisms include crabs (Luppi and Bas, 2002), amphipods (Obenat *et al*. 2006; Schwindt *et al*. 2001; Bruschetti *et al*. 2009) and molluscs (Bruschetti *et al*. 2009).

The reef-like aggregates of *F. enigmaticus* serve as resting and feeding areas for shorebirds. The lagoon is an important stopover site for migratory birds and for local breeding species (Botto *et al*. 1998; Ferrero, 2001), and the existence of such areas could be considered the main factor determining the distribution and habitat use by shorebirds in the environment (Bruschetti *et al*. 2009). Because these *F. enigmaticus* areas increase the probabilities of contact between invertebrate intermediate hosts and vertebrate definitive hosts, they may serve as foci of parasite transmission.

The gastropod *Heleobia conexa* (Gaillard, 1974) (Cochliopidae), one of the invertebrate intermediate hosts associated with *F. enigmaticus*, is usually found in the middle part of the lagoon, where water and air temperatures fluctuate widely and tides are negligible. The snail lives only in the colonies of *F. enigmaticus* (De Francesco and Isla, 2003; Bruschetti *et al*. 2009). The population density of *H. conexa* can attain 11 individuals/cm^3 on hard substrate (De Francesco and Isla, 2003), and its breeding periods have been recorded in spring (September–November) and in autumn (April–May) (De Francesco and Isla, 2004). The life cycle of *H. conexa* is annual (De Francesco and Isla, 2004) and the snails can reach a maximum total length of approximately 7 mm (Etchegoin, 1997).

Heleobia conexa serves as first intermediate host in the cycles of at least 22 species of digeneans (Etchegoin, 1997, 2001). The larval digeneans found in *H. conexa* were identified and described in detail by Martorelli (1986, 1988, 1989, 1990, 1991), Martorelli and Etchegoin (1996), Etchegoin and Martorelli (1997 and 1998) and Etchegoin (1997), which makes it possible to recognize and monitor the same species in further studies into the lagoon. Only 4 cercariae were identified at the specific level, the rest were identified at the family level (Etchegoin, 1997). This level is sufficient to identify the group of vertebrates acting as definitive host (fish, amphibians, reptiles, birds or mammals) (Etchegoin, 1997; Huspeni *et al*. 2005).

As a continuation of studies initiated by Etchegoin (1997, 2001), the main objective of this study was to analyse the temporal stability of the component community of larval digeneans in the *H. conexa* population during 4 years (1996, 1999, 2004 and 2005) to evaluate its response to environmental changes and its potential use as an indicator of environmental fluctuations in the area.

MATERIALS AND METHODS

In total, 2219 specimens of *H. conexa* were collected seasonally from 2004 to 2005 in Juan y Juan, an open sport fishing area inside the freshwater zone of Mar Chiquita coastal lagoon (37° 40′S, 57°20′W). The numbers of snails collected seasonally in 2004 were 305, 310, 310 and 256 during the summer, autumn, winter and spring, respectively. In 2005, the numbers of hosts examined were 309, 305, 204 and 220 during the summer, autumn, winter and spring, respectively. Snails were collected from the reef-like aggregates of the serpulid *F. enigmaticus*, a tube-building polychaete that has invaded 80% of the main body of the lagoon (Shwindt and Iribarne, 2000). Reefs of *F. enigmaticus* can measure up to 4 m diameter and 0·5 m height (Obenat and Pezzani, 1994). In the study site, random cores (15 cm diameter × 15 cm deep) were taken and placed into plastic cups filled with water from the lagoon for transportation. In the laboratory, snails were removed from reefs and measured with a Vernier caliper (precision: 0·1 mm). Each snail was isolated in 45 ml vol. plastic cups and exposed to a 100 W incandescent lamp for 48 h to stimulate shedding of cercariae (patent infections). Finally, all gastropods were dissected under a stereomicroscope in order to detect the presence of sporocysts, rediae and immature cercariae (pre-patent infections) (Curtis and Hubbard, 1990). Shed cercariae, sporocysts, rediae and immature cercariae were identified according to Martorelli, (1986, 1988, 1989, 1990 and 1991), Martorelli and Etchegoin (1996) and Etchegoin and Martorelli (1997 and 1998).

For comparative analysis of component community structure of larval digeneans in *H. conexa* over time, data from previous studies in Juan y Juan were used. These data used the same seasonal pattern of snail collection followed in the present study and come from samples of 1430 and 930 specimens of *H. conexa* collected in 1996 and 1999, respectively (Etchegoin, 1997 and Etchegoin unpublished data). The numbers of snails collected seasonally in 1996 were 346, 375, 328 and 381 during the summer, autumn, winter and spring, respectively. In 1999, the numbers of hosts examined were 310, 200, 120 and 300 during the summer, autumn, winter and spring, respectively.

Table 1. Annual values of prevalence and detailed list of species or morphological types of larval digeneans parasitizing *Heleobia conexa* in Mar Chiquita coastal lagoon, during 4 years (1996, 1999, 2004 and 2005)

Family	Species/ Morphological type	Reference	Prevalence			
			1996	1999	2004	2005
Notocotylidae	Notocotylidae sp.1	Etchegoin and Martorelli (1998)	2·40	3·87	2·62	3·76
Heterophyidae	Pleurolophocercaria III	Martorelli and Etchegoin (1996)	3·70	3·44	2·62	2·22
	Pleurolophocercaria VI	Martorelli and Etchegoin (1996)	2·40	0	1·10	2·22
	Pleurolophocercaria IV	Martorelli and Etchegoin (1996)	1·30	0·11	0·25	0·58
	Pleurolophocercaria V	Martorelli and Etchegoin (1996)	0·07	0·43	0	2·70
	Pleurolophocercaria VII	Etchegoin (1997)	0·07	0	0	0
	Pleurolophocercaria II	Martorelli and Etchegoin (1996)	0·60	0	0·59	0
	Cercaria magnacauda I	Martorelli and Etchegoin (1996)	0·14	0	0	0
Acanthostomidae	Pleurolophocercaria I	Martorelli and Etchegoin (1996)	1·50	0·75	2·03	0·58
Microphalidae	*Microphallus simillimus*	Martorelli (1991)	8·50	0·97	8·30	8·57
	Matitrema bonaerensis	Etchegoin (1997)	0·07	0	0·17	0·48
	Microphallus szidati	Martorelli (1986) and Etchegoin (1997)	4·30	1·72	2·12	3·37
Echinostomatidae	Cercaria heleobicola IV	Martorelli (1990)	0·30	0	0	0
	Cercaria heleobicola V	Martorelli (1990)	0·60	0	0	0·10
Haploporidae	Cercaria Haploporidae sp.1	Etchegoin and Martorelli (1998)	0·30	0·97	0·42	0·29
	Cercaria Haploporidae sp.2	Etchegoin and Martorelli (1998)	0·60	1·18	0·08	1·35
	Cercaria heleobicola III	Martorelli (1989)	0·30	0·11	0·08	0·58
Cyatocothylidae	Furcocercaria sp. 1	Etchegoin and Martorelli (1998)	0·07	0	0·25	0
Homalometridae	Cercaria heleobicola II	Martorelli (1989)	0·07	0	0	0·19
Ochetosomatidae	Xiphidiocercaria sp. 2	Etchegoin and Martorelli (1998)	0·60	0·11	0·51	0·19
Psilostomatidae	Cercaria aff. *Psilochasmus oxyurus* (Creplin, 1835)	Etchegoin and Martorelli (1998)	0·40	0·86	0·34	0·29
Schistosomatidae	Cercaria heleobicola I	Martorelli (1989)	0	0	0	0·10
		Overall prevalence	28·10	17·63	21·51	27·55

To analyse and compare the composition of the community of larval digeneans in *H. conexa* over time, the following indices were used: (a) Species richness (S) which represents the total number of species in a sample (Magurran, 1988; Ludwig and Reynolds, 1988); (b) Overall prevalence = the number of parasitized snails/the number of collected snails × 100 (Lafferty *et al.* 1994); (c) Prevalence of a species = the number of snails parasitized by that species/ the number of collected snails×100; (d) Sørensen similarity index (number of species common to both communities/number of species in sample A + number of species in sample B) and Morisita-Horn index (probability that an individual drawn from sample *j* and one drawn from sample *k* will belong to the same species/ probability that 2 individuals drawn from either *j* or *k* will belong to the same species) (Krebs, 1999). Proportions of infected snails were compared between years and seasons within years with χ^2-test goodness-of-fit (Zar, 2009). Tukey test (Zar, 2009) was used for *post-hoc* comparisons.

RESULTS

Characterization of larval digenean communities in Heleobia conexa

Twenty-two digenean species, belonging to 11 families, were observed (Table 1). The greatest species richness was found in 1996 (S = 21), and the lowest species richness was found in 1999 (S = 12). Species richness values in 2004 and 2005 were 15 and 17 respectively. Furthermore, only 11 species of digeneans were observed in all sampling periods. The remaining species were recorded in 1, 2 or 3 periods. Pleurolophocercaria VII, Cercaria magnacauda I, Cercaria heleobicola IV and Cercaria heleobicola I were recorded only in 1 period; Pleurolophocercaria II, Cercaria heleobicola V, Furcocercaria sp. 1 and Cercaria heleobicola II were present in two periods and, finally, Pleurolophocercaria VI, Pleurolophocercaria V and *Maritrema bonaerensis* Etchegoin and Martorelli, 1997, were registered in 3 sampling periods. With regard to similarity between these years, the Sørensen index showed the highest values of similarity for the years 1996–2005 (Cn = 0·84) and the lowest values for the years 1996–1999 (Cn = 0·73) (Table 2). The Morisita-Horn index matches with the Sørensen index in determining that the years 1996–1999 and 1999–2004 were those with lower values of similarity (Cλ = 0·53). On the other hand, the Morisita-Horn index showed the highest values of similarity for the years 1996–2004 (Cλ = 0·97). The observed differences could be attributable to the way the Sørensen index and Morisita-Horn index calculate the similarity. The Sørensen index uses presence/ absence of species while the Morisita-Horn index uses prevalence data.

The prevalence of most larval digeneans was low (Table 1). In all sample periods only 3 species exceeded 1% (Cercaria Notocotylidae sp.1, Pleurolophocercaria III and *Microphallus szidati* Martorelli,

Table 2. Larval digeneans parasitizing *Heleobia conexa* in Mar Chiquita coastal lagoon: interannual comparisons of the parasite community similarity, using Sørensen index (Cn) and Morisita-Horn index (Cλ)

Similarity index	1996 *vs* 1999	1996 *vs* 2004	1996 *vs* 2005	1999 *vs* 2004	1999 *vs* 2005	2004 *vs* 2005
Cn	0·73	0·83	0·84	0·81	0·83	0·81
Cλ	0·53	0·97	0·96	0·53	0·54	0·94

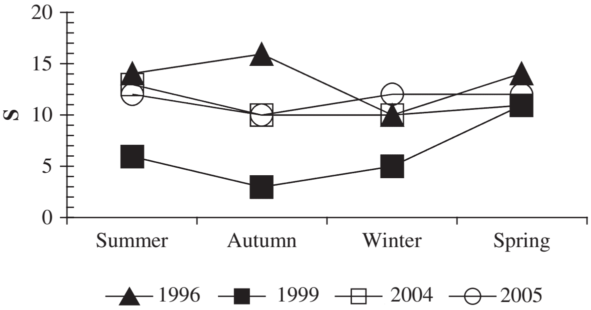

Fig. 1. Seasonal variation of species richness (S) in the larval digenean community of *Heleobia conexa* in Mar Chiquita coastal lagoon during 4 years (1996, 1999, 2004 and 2005).

1986). The most prevalent species in 1996, 2004 and 2005 was *Microphallus simillimus* (Travassos, 1920), while Notocotylidae sp.1 showed the highest values of prevalence in 1999. The annual overall prevalence never exceeded 30%, and the lowest value (17·63%) was registered in 1999 (Table 1). In χ^2 tests, proportions of infected snails were significantly different between 1996–1999 ($\chi^2_{(1)} = 8{\cdot}50$; $P < 0{\cdot}01$), 1999–2004 ($\chi^2_{(1)} = 6{\cdot}58$; $P < 0{\cdot}01$), and 1999–2005 ($\chi^2_{(1)} = 8{\cdot}88$; $P < 0{\cdot}01$).

Dynamics of larval digenean communities in Heleobia conexa

The species richness showed different seasonal patterns (Fig. 1). In 1996 it presented a decrease towards winter, and an increase towards the autumn, while 1999 showed a decrease in the autumn and an increase towards the spring. The years 2004 and 2005 showed, as in 1999, a decline towards autumn. The year 2004 presented an increase towards the summer, and in 2005 species richness remained constant through the seasons.

Overall prevalence usually peaked in spring and declined towards autumn-winter (Fig. 2). In 1996, 1999 and 2004 the proportions of infected snails were significantly different between summer-spring (1996: $\chi^2_{(1)} = 5{\cdot}04$; 1999: $\chi^2_{(1)} = 5{\cdot}82$; 2004: $\chi^2_{(1)} = 4{\cdot}50$; $P < 0{\cdot}001$ in all cases), autumn-spring (1996: $\chi^2_{(1)} = 7{\cdot}14$; 1999: $\chi^2_{(1)} = 7{\cdot}23$; 2004: $\chi^2_{(1)} = 8{\cdot}03$; $P < 0{\cdot}001$ in all cases) and winter-spring (1996: $\chi^2_{(1)} = 4{\cdot}64$; 1999: $\chi^2_{(1)} = 4{\cdot}50$; 2004: $\chi^2_{(1)} = 4{\cdot}62$; $P < 0{\cdot}001$ in all cases). In 2005 (as in 1996, 1999 and 2004) the proportions of infected snails were significantly different between summer-spring ($\chi^2_{(1)} = 9{\cdot}95$; $P < 0{\cdot}001$), autumn-spring ($\chi^2_{(1)} = 8{\cdot}55$; $P < 0{\cdot}001$) and winter-spring ($\chi^2_{(1)} = 4{\cdot}50$; $P < 0{\cdot}001$) and also between summer-winter ($\chi^2_{(1)} = 5{\cdot}19$; $P < 0{\cdot}001$) and autumn-winter ($\chi^2_{(1)} = 4{\cdot}53$; $P < 0{\cdot}001$). The most prevalent species of digenean in all seasons in the years 1996, 2004, 2005 was *M. simillimus*. In the autumn, winter and summer of 1996, Notocotylidae sp.1 was the most prevalent species, whereas in spring Pleurolophocercaria III showed the highest values of prevalence.

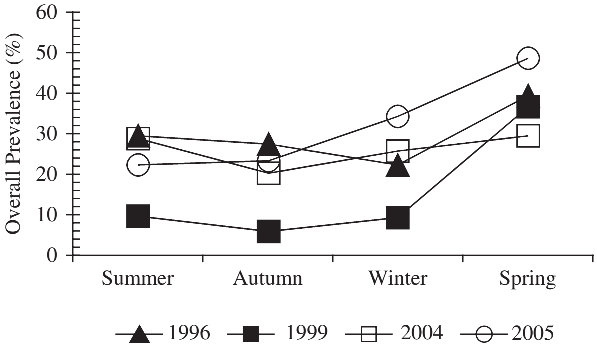

Fig. 2. Seasonal variation in the overall prevalence of larval digeneans parasitizing *Heleobia conexa* in Mar Chiquita coastal lagoon during 4 years (1996, 1999, 2004 and 2005). Values of overall prevalence: 1996 (S: 29·50, A: 27·50, W: 22·30, Sp: 39·40); 1999 (S: 9·68, A: 6·00, W: 9·17, Sp: 36·67; 2004 (S: 28·76, A: 20·32, W: 25·72, Sp:29·39); 2005 (S: 22·32, A: 23·28, W: 34·30, Sp: 48·64). S, Summer; A, Autumn; W, Winter; Sp, Spring.

DISCUSSION

The component community of larval digeneans in *H. conexa* presented a high diversity and low-medium prevalence, both features with annual and seasonal variations.

The diversity of larval digeneans in *H. conexa* varied seasonally in each year, decreasing towards autumn, except in 1996, and increasing towards the spring and summer. According to Etchegoin (1997, 2001), the diversity of this community is strongly influenced by the presence of bird definitive hosts. In the study area, 62 species of birds have been registered. From the total number of bird species censored, 28% includes only migratory birds, with migratory stopovers mainly in spring and summer (Ferrero, 2001). As the diversity of larval digeneans in the first intermediate host is directly dependent on the presence of definitive hosts (Kuris and Lafferty, 1994; Huspeni *et al.* 2005; Hechinger *et al.* 2005, 2008), increased seasonal diversity in larval digeneans of *H. conexa* should be correlated with seasonal changes in the composition of the vertebrate definitive host community.

The prevalence in each year showed 1 peak in spring, as seen in all sampling periods. According to De Francesco and Isla (2004), recruitment of juveniles to the adult population of *H. conexa* occurs in late spring. These juveniles will mature and grow in size over the year to produce new susceptible hosts for infection by digeneans. For this reason, seasonal variation in prevalence throughout the year should be, in part, associated with the population dynamics of the snail host, as was indicated by Kube *et al.* (2002) for *Hydrobia ventrosa* and Faltýnková *et al.* (2008) for *Valvata macrostoma*.

The component community in *H. conexa* showed a difference in the abundance and diversity of morphological types of cercariae between 1999 and the other sampling periods. These annual differences could be due to indirect and/or direct anthropogenic effects. Since 1999, 'Juan y Juan' has become an open fishing area with an increasing number of visitors who use propeller and rowing boats for recreational fishing. To facilitate movement of boats in this area, the reef-like aggregates of the polychaete *F. enigmaticus* were extensively eliminated (approximately 80% of the reefs) (Etchegoin, *personal communications*). Extraction of reef-like aggregates could have altered the population dynamics of *H. conexa* because it lives only in the colonies of the polychaete (De Francesco and Isla, 2003; Bruschetti *et al.* 2009). In addition, some bird species utilize areas with reefs of *F. enigmaticus* as a surface for foraging and resting (Bruschetti *et al.* 2009). As a consequence of the extraction of reef-like aggregates, a displacement to other parts of the lagoon of the bird hosts associated with the reefs may have caused an interruption of digenean life cycles in the area. Human activities can change behaviour and/or alter the distribution of birds (definitive host) (Burger, 1986; Lafferty, 2001; Burger *et al.* 2004; Lafferty *et al.* 2006*b*), and can affect directly or indirectly the distribution and abundance of larval digenean communities (Lafferty, 1997; Bustnes and Galaktionov, 1999; Lafferty and Kuris, 2004; Loot *et al.* 2005, 2008).

In the years 2004 and 2005 a gradual restoration of larval digenean diversity has been observed. As pointed out by Pezzani and Obenat (1988) and by Obenat and Pezzani (1989, 1994), physical and human erosion produces the detachment of parts of the aggregates of *F. enigmaticus* and provides new areas for larval recruitment and growth. The dispersion of these aggregates can provide new substratum, food, and shelter for invertebrates (including *H. conexa*) which are part of the diets of birds. Probably, the formation of new colonies of the polychaete could restore some links between intermediate and definitive hosts and, in consequence, produce a gradual yearly increase in the diversity of larval digeneans in the area.

Using larval digeneans as a bio-indicators of environmental disturbances is useful for detecting general changes over time but not for determining specific causes (Keas and Blankespoor, 1997). Nevertheless, the use of digeneans as bio-indicators of species diversity, abundance, and trophic functions can be applied in a monitoring project with limited financial resources because they provide a high information yield at low cost (Huspeni *et al.* 2005; Hechinger and Lafferty, 2005; Hechinger *et al.* 2007).

If we compare the richness observed in the larval digenean community of *H. conexa* with the richness of the 24 species of snails listed by Huspeni *et al.* (2005), only *Hydrobia ulvae* exceeds the number of trematode species (22 *vs* 32). Even when the environment was altered by human activity, the number of species of larval digeneans observed was 12 (year 1999). This number exceeds the minimum number of larval digeneans (3) suggested by Huspeni *et al.* (2005) to consider larval digeneans in the first intermediate host as bio-indicators of indirect information about vertebrate and invertebrate communities as well as trophic links between second intermediate and final hosts. On the other hand, the larval digenean community of *H. conexa* showed a year to year re-establishment following the annual cycle of *H. conexa* (De Francesco and Isla, 2001, 2004) and the presence of definitive hosts. A similar pattern was observed by Esch *et al.* (2002) in the snail *Helisoma anceps*. The annual restructuring of this snail-digenean system could allow inferences about environmental changes in the lagoon within a short period of time, including disturbances due to human activities and fluctuations in the diversity and in the abundance of vertebrate definitive hosts (mainly birds).

According to our results, the larval digenean community of *H. conexa* could be considered as a good bio-indicator with quick response to environmental disturbances. Future studies (at micro- and macro-scales) in the lagoon will shed light on the relationships between the abundance and diversity of larval digeneans in *H. conexa* and the distribution and abundance of definitive hosts as well as human activities in the reserve. These studies will also include the analysis of the importance of the aggregates of *F. enigmaticus* as preferred habitat of *H. conexa* and its role in facilitating the transmission of digeneans in the area.

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