Journal of Helminthology, Page 1 of 7
doi:10.1017/S0022149X16000808

Larval digenean preferences in two sympatric snail species at differing tidal levels off the Atlantic coast of Patagonia

G. Di Giorgio[1]*, C. Gilardoni[1], E. Bagnato[1], F. Cremonte[1] and C. Ituarte[2]

[1]Laboratorio de Parasitología, Instituto de Biología de Organismos Marinos (CCT CONICET-CENPAT), Bvd. Brown 2915, U9120ACF Puerto Madryn, Chubut, Argentina: [2]Museo Argentino de Ciencias Naturales, Av. Ángel Gallardo 470, C1405DJR, Buenos Aires, Argentina

(Received 7 June 2016; Accepted 4 October 2016)

Abstract

Larval stages of the trematodes *Maritrema madrynense* and Hemiuroidea fam. gen. et sp. differentially parasitized *Siphonaria lessonii* and *S. lateralis*, two sympatric pulmonate snail species on the rocky intertidal shore at Puerto Deseado, south-western Atlantic coast of Patagonia, Argentina. Snail specimens were collected at two sampling sites with contrasting physical–chemical characteristics. One site, in the upper intertidal, was exposed to sewage from fish-processing plants, greater hydrodynamic forcing and desiccation, a wider temperature range, longer exposure to ultraviolet radiation and higher abundance of birds. The second site, in the lower intertidal, was generally characterized by less stressful environmental conditions. At both sites, *S. lateralis* showed a markedly higher density than *S. lessonii* (55.13 vs. 5.87 snails/m^2, respectively). Despite this, the prevalence of both digeneans was higher in *S. lessonii* (17.37% and 3.52% for *M. madrynense* and Hemiuroidea, respectively) than in *S. lateralis* (0.09% and 0% for *M. madrynense* and Hemiuroidea, respectively). This study demonstrates high parasite specificity for the host. Low densities of *S. lessonii* are attributed to castration of parasitized hosts and reduction of their physiological condition. The prevalence and intensity of infection of both digenean parasites were higher at the more stressful, upper intertidal site, thus suggesting that a higher abundance of birds and exposure to sewage pollution may promote the transmission of trematodes.

Introduction

Trematode parasites are important faunal components in the intertidal zone, as they are capable of influencing several aspects of their host's life history, including survival and reproductive function, as well as the abundance and population structure of their hosts (e.g. Lauckner, 1980). Temporal and spatial variation in the recruitment of parasites to host populations may result from a variety of biotic factors, such as their host behaviour, population density and susceptibility to infection (e.g. Kuris & Lafferty, 1994; Studer & Poulin, 2012). Abiotic factors, such as habitat temperature, may also favour or limit parasite recruitment (Thieltges *et al*., 2009; Studer & Poulin, 2012). Previous studies reported that eutrophication from sewage pollution could have positive effects on the abundance of parasites (e.g. Lafferty, 1997; Blanar *et al*., 2009).

The intertidal zone of rocky shores supports a rich fauna of invertebrates and birds, thus promoting the development of complex digenean life cycles. Trematode life cycles typically include two intermediate hosts, where the first is usually a snail and is highly specific

*E-mail: digiorgio@cenpat-conicet.gob.ar

(Lauckner, 1980) and a vertebrate is the definitive host. The trematode *Maritrema madrynense* uses the pulmonate snails *Siphonaria lessonii* and *Siphonaria lateralis* as first intermediate hosts, the crab *Cyrtograpsus altimanus* and the isopod *Exosphaeroma* sp. as second intermediate hosts and the kelp gull *Larus dominicanus* as definitive host (Alda & Martorelli, 2009; Diaz & Cremonte, 2010; Gilardoni *et al*., 2011). Siphonariid gastropods act as the first intermediate host of two additional trematode species, a hemiuroid and a schistosomatid (Alda & Martorelli, 2009; Gilardoni *et al*., 2011), and are also hosts of a haplosporidian protozoan species, *Haplosporidium patagon* (Ituarte *et al*., 2014).

The aim of this study was to determine whether host characteristics (density and size) and environmental factors (tidal level, sewage pollution, temperature, abundance of birds) promote or restrict the transmission of trematodes that parasitize two intertidal pulmonate snails, *S. lessonii* and *S. lateralis*. The study was conducted at two contrasting intertidal sites along the Patagonian coast of Argentina.

Materials and methods

Snail collection and examination

Two sampling sites with contrasting physical characteristics were selected along the intertidal rocky shore at Puerto Deseado (47°45′S, 65°55′W). Site 1 was located in the upper level of the intertidal zonal and was characterized by a high level of stress. It was exposed to a higher degree of hydrodynamic forcing (wave action) and desiccation, and more prolonged exposure to ultraviolet (UV) radiation (about 10 h/day). This site was also exposed to a sewage outfall from fish-processing plants which discharge directly on to the rocky intertidal, thus leading to nutrient enrichment and more eutrophic conditions. Site 2 was located in the lower level of the intertidal, and is thus representative of a lower level of stress, including shorter exposure to UV radiation (~2 h/day). Although the two sites are only about 700 m apart, site 2 is located upstream from the sewage discharge point and is thus not directly exposed to sewage pollution. To determine the level of sewage pollution at site 1, and to test if site 2 is also affected by sewage pollution, water samples were collected in September 2014 at each site. Nutrient concentrations (ammonium, nitrite + nitrate, and phosphate) were measured in September 2014 in duplicate, and biological oxygen demand (BOD), salinity, dissolved oxygen and pH were determined without replication. Daily variations in seawater and air temperatures were recorded with dataloggers (Thermochron® iButton® device (DS1921G), Maxim Integrated, California, USA). Two dataloggers, protected in plastic capsules to avoid wetting during immersion, were deployed at each site and set to record temperatures every 60 min. Maximum, minimum and mean monthly temperatures were calculated at each site from records obtained between May 2013 and January 2014.

To characterize the potential availability of definitive hosts that can affect the transmission of *M. madrynense*, two or three shorebird censuses were performed at each sampling site during low tides. Observations of all shorebirds present at each sampling were made using binoculars. To date, only the kelp gull *L. dominicanus* has been confirmed as a definitive host of *M. madrynense* (Diaz & Cremonte, 2010). However, given that trematode specificity for its definitive hosts is generally low, the numbers of all types of birds were determined in this study (including grebes, cormorants, ducks, oystercatchers, plovers and other gulls). The abundances of total shorebirds and, in particular, that of kelp gulls, were calculated by averaging the number of the observations made at each site.

Specimens of *S. lessonii* and *S. lateralis* were collected bimonthly during the lowest tides at the two sites from December 2012 to November 2014. Eight 25 × 25 cm quadrats were placed randomly at each of the two sampling sites and all individuals inside each quadrat were collected manually. The density of the two host species was calculated as the number of individuals/m^2. Additionally, due to the low density of *S. lessonii*, 50 specimens of this species were collected at each site to allow assessment of the population dynamics of parasite infections. It was not necessary to collect additional specimens of *S. lateralis* because the density of this species was relatively high, and thus the number of snails collected from eight quadrats was sufficient to undertake the study of the dynamics of parasite infection.

The specimens collected were transported to the laboratory, measured (with calipers with a precision of 0.1 mm), and weighed (total body wet weight, wet weight of drained soft tissue and shell wet weight). Trematodes were detected under a stereoscopic microscope. The visceral mass of all specimens was inspected and, in dubious cases, microscopic squashes of the digestive gland and gonad tissues were used to further screen for parasites. The prevalence of infection was calculated following Bush *et al*. (1997), and the infection intensity was calculated according to Studer & Poulin (2012) as the percentage of the overall wet weight of the gastropod (including shell weight) that consisted of parasite tissue. For this purpose, gastropods of similar size (13–16 mm) infected with sporocysts of *M. madrynense* and Hemiuroidea fam. gen. et sp. were used. The proportion of hosts with sporocysts of *M. madrynense* containing cercariae, and that of hosts with sporocysts containing metacercariae, were also calculated by observing ten randomly selected sporocysts under the light microscope.

Data analysis

Several models were proposed to study the relationship of prevalence and intensity of infection in relation to gastropod characteristics (shell length and density) and environmental factors (site, season and year). For this purpose, Generalized Linear Models (GLMs) (McCullagh & Nelder, 1989) were used. In the case of over-dispersed data, the GLM was corrected following Crawley (2007). Akaike's information criterion (AIC) was used to determine the best model for the analysed data (Burnham & Anderson, 2002). The step function in the R program was used to select the best model (Venables & Ripley, 2002), a function that compares all possible models and selects the one with the lowest AIC. Additionally, a Spearman correlation test was performed to evaluate the relationship between the prevalence of *M. madrynense*

Table 1. Mean values of seawater physico-chemical characteristics at the upper and lower intertidal levels of both sampling sites together with the abundance of birds and density of the snail hosts *Siphonaria lateralis* and *S. lessonii*; ranges are given in parentheses.

		Upper level		Lower level	
Seawater quality	BOD (mg/l)	146.6		0.7	
	Salinity (ppm)	25.16		32.34	
	Dissolved oxygen (%)	51.9		106.6	
	pH	7.64		7.92	
	Nitrite + nitrate (μM)	149		13.13	
	Silicon oxide (μM)	141		3.15	
	Ammonium (μM)	149		6.61	
	Phosphate (μM)	100		1.38	
		Average	Variation	Average	Variation
Temperature (°C)	Autumn	8.24 (4.2–11.2)	7	8.9 (6–1.7)	9.75
	Winter	5.27 (−4–1.25)	13.5	5.85 (3–18)	16.67
	Spring	12.37 (0.2–25.5)	21.25	7.59 (−1.5–18.5)	14.58
	Summer	13.26 (4.7–29.2)	23.87	12.52 (7–31)	21.75
Abundance of birds	Total birds	230 (110–355)		68.7 (26–192)	
	Kelp gulls	151 (70–233)		37.57 (17–73)	
Density of snails (number/m^2)	*S. lateralis*	68.85 (32.08–107.92)		41.6 (12.07–72.08)	
	S. lessonii	4.62 (2.92–7.50)		7.12 (3.33–11.23)	

and the abundance of birds. Double infections (*M. madrynense* and Hemiuroidea fam. gen. et sp.) were examined by comparing observed frequencies and randomly expected frequencies using a heterogeneity test (*G*) (Sokal & Rohlf, 1981). All statistical analyses were performed using R (R Development Core Team, 2012).

Results

Characterization of sampling sites

Site 1 was characterized by higher values of total biological oxygen demand (BOD) and nutrients (nitrite + nitrate, silicon oxide, ammonium, phosphate), and by lower values of salinity and dissolved oxygen. At both sampling sites, the mean monthly temperatures showed a linear increase from the winter to the summer (table 1). At site 1, the amplitude of the seasonal temperature variation (maximum − minimum temperature values) showed a gradual and linear increase from the autumn to the summer. This high intertidal site is therefore characterized by more prolonged periods of air exposure (up to 10 h), and thus higher ultraviolet (UV) radiation and desiccation. At site 2, the amplitude of the seasonal temperature variation does not show such a clear pattern, it is more moderate from the winter to the spring, and temperature shows an increase in summer (table 1). More prolonged immersion likely results in more stable environmental conditions at this site. The abundance of birds was significantly different between the two sites, with a greater number of total birds and *L. dominicanus* recorded at site 1 (tables 1, 2). Seasonality in the abundance of birds was observed: at both sites, as fewer birds were recorded in winter compared to the other seasons.

Parasitological survey of the host species

The GLM analysis indicated that *S. lateralis* had a significantly higher density than *S. lessonii* at both study sites. The dominant gastropod species, *S. lateralis*, had a significantly higher density at site 1 than 2, whereas *S. lessonii* showed a higher density at site 2 (tables 1, 2).

Siphonaria lessonii was found to be parasitized by the trematode *M. madrynense* (Microphallidae) and by the Hemiuroidea fam. gen. et sp., whereas only the microphallid species was found in *S. lateralis*. Despite the fact that both pulmonate snail species co-occurred at both study sites, the identified larval trematode parasites (sporocysts with cercariae and/or metacercariae of *M. madrynense* and sporocysts with cercariae of Hemiuroidea fam. gen. et sp.) differentially affected the two host species. A total of 3422 specimens of *S. lateralis* were inspected and only three were found to be parasitized by *M. madrynense*. In contrast, a total of 1560 specimens of *S. lessonii* were inspected and 324 were found to be parasitized, 269 by *M. madrynense* and 55 by Hemiuroidea fam. gen. et sp. The GLM analysis indicated that the prevalence of *M. madrynense* was significantly lower in *S. lateralis* than in *S. lessonii* (table 2). The Hemiuroidea fam. gen. et sp. was only found in *S. lessonii* (prevalence = 3.52%). Given the low prevalence of infection in *S. lateralis*, the results that follow will deal only with findings in *S. lessonii*. The overall prevalence of *M. madrynense* and Hemiuroidea fam. gen. et sp. infecting *S. lessonii* was higher at site 1 than at site 2 (table 3). Analysis by GLM indicated that the presence/absence of *M. madrynense* did not vary significantly with shell length of the host, but this relationship was positive and significant in Hemiuroidea fam. gen. et sp. (table 2). The latter was found parasitizing *S. lessonii* specimens between 11 and 19 mm in length. The highest prevalence values (3.7%) were recorded in the larger specimens (>17 mm length).

With respect to the seasonality of infection, GLM analysis indicated that the presence/absence of *M. madrynense* varied significantly throughout the year; the austral summer was the season in which the lowest number of parasitized specimens was found (tables 2, 3). The GLM analysis also indicated that the presence/absence of Hemiuroidea fam. gen. et sp. did not vary significantly with season.

Table 2. The relationship between infection levels of *Maritrema madrynense* (Ma) and Hemiuroidea fam. gen. et sp. (He) in the snail species *Siphonaria lateralis* (*Sla*) and *S. lessonii* (*Sle*) together with selected biotic and physical factors using Generalized Linear Models (GLMs); autumn (a), spring (sp), su (summer), w (winter), sporocysts (spo), cercariae (cer), metacercariae (mer) and significant *P* values are shown in bold.

Response variables and distribution	Explanatory variables	Best model	Estimated values	Standard error	Statistic values	*P* values
Abundance of birds (quasi-poisson)	Site * Season * Year	Site $_{2–1}$	−0.84	0.31	−2.69	**<0.01**
		Season $_{a-w}$	0.46	0.21	2.72	0.03
		Season $_{sp-w}$	0.53	0.22	2.43	0.02
		Season $_{su-w}$	0.45	0.23	2.06	0.04
		Year $_{2014–2013}$	0.24	0.23	1.07	0.29
		Site $_2$ * Season $_{sp}$	−0.64	0.41	−1.54	0.13
Density of snails (gaussian)	Species * Site	Species $_{Sle-Sla}$	−28.09	3.60	−7.81	**<0.01**
		Site $_{2–1}$	−16.98	3.60	−4.72	**<0.01**
		Species * Site	8.87	2.27	3.90	**<0.01**
Presence – absence of parasites						
Maritrema madrynense (binomial)	Gastropod species	Species $_{Sle-Sla}$	5.50	0.58	9.47	**<0.01**
M. madrynense (binomial)	Host shell length + site + season + year	Site $_{2–1}$	−1.72	0.18	−3.63	**<0.01**
		Season $_{a-w}$	−0.003	0.22	−0.01	0.99
		Season $_{sp-w}$	−0.21	0.21	−0.99	0.32
		Season $_{su-w}$	−0.69	0.24	−2.82	**<0.01**
Hemiuroidea fam. gen. et sp. (quasi-binomial)	Host shell length + site + season + year	Host shell length	0.26	0.07	3.93	**<0.01**
		Site $_{2–1}$	−1.16	0.30	−3.78	**<0.01**
		Year $_{2014–2013}$	−0.75	0.30	−2.48	0.12
Intensity of infection (quasi-binomial)	Site + season + year + species	Site $_{2–1}$	−0.21	0.04	−5.17	**<0.01**
		Season $_{a-w}$	−0.02	0.05	−0.37	0.71
		Season $_{sp-w}$	−0.07	0.05	−1.45	0.15
		Season $_{su-w}$	−0.04	0.08	−0.59	0.56
		Year $_{2014–2013}$	0.008	0.03	0.23	0.82
		Species $_{Ma-He}$	0.12	0.04	3.24	**<0.01**
% of spo of *Ma* with cer and met (quasi-binomial)	Larval stage + Site	Larval stage $_{met-spo}$	2.88	0.27	10.71	**<0.01**
		Site $_{2–1}$	−1.61	0.17	−9.35	**<0.01**

*Indicates an interaction factor; + indicates an additive factor.

Table 3. Mean values of the prevalence (%) and infection intensity (I) (%) of *Maritrema madrynense* and Hemiuroidea fam. gen. et sp. in *S. lessonii* at upper and lower intertidal levels of both sampling sites; ranges are given in parentheses.

		Upper level		Lower level	
Parasite species	Season	%	I	%	I
Martrema madrynense	Summer	19.34	25.73 (19.9–29.5)	5.68	18.18 (13.1–22.3)
	Autumn	32.11	25.25 (20.3–30.4)	7.67	15.25 (15.3–21.1)
	Winter	35.24	22.92 (19.8–29.2)	6.06	15.28 (14.6–21.1)
	Spring	30.74	26.51 (21.3–29.1)	5.4	18.55 (15.9–20.1)
Hemiuroidea fam. gen. et sp.	Summer	2.21	18.13 (15.8–24.8)	1.7	9.89 (8.9–11.7)
	Autumn	7.37	19.75 (13.7–24.5)	1.34	12.13 (8.9–12.1)
	Winter	3.81	20.71 (16.1–25.9)	3.03	24.23 (9.05–19.4)
	Spring	6.67	26.66 (17.5–23.8)	1.8	9.20 (6.42–11.9)

On average, 20.29 ± 4.88% (standard error) ($n = 144$) of the total weight of parasitized specimens of *S. lessonii* corresponded to *M. madrynense* tissues (table 3). This percentage in gastropod hosts did not differ significantly among seasons, but significant differences were observed between sampling sites, higher infection intensity at site 1 (tables 2, 3). The parasite Hemiuroidea fam. gen. et sp. contributed on average 19.55 ± 6.8% ($n = 41$) of the total weight of *S. lessonii*. The percentage of tissue occupied by Hemiuroidea fam. gen. et sp. was thus significantly lower than that occupied by *M. madrynense* (tables 2, 3).

The GLM analysis indicated that a higher percentage of specimens parasitized by sporocysts of *M. madrynense* contained only metacercarie and a much lower proportion was observed housing sporocysts with cercariae (table 2). At site 1 more specimens were found parasitized by sporocysts with metacercariae than at site 2 (94% vs. 89%, respectively).

The Spearman correlation coefficient showed a positive relationship between bird abundance and prevalence of *M. madrynense* ($r_s = 0.84$, $P < 0.01$). Double infections (*M. madrynense* + Hemiuroidea fam. gen. et sp.) were observed in *S. lessonii* with a very low prevalence (0.002%). Prevalence of double infections was lower than that expected to occur randomly ($G = 4.05$, $P < 0.05$).

Discussion

The overall prevalence of *M. madrynense* was 17.37% in *S. lessonii* and only 0.09% in *S. lateralis*; the overall prevalence of Hemiuroidea fam. gen. et sp. was 3.52% in *S. lessonii* but *S. lateralis* was not found to be infected by this digenean. The same pattern was reported by Di Giorgio *et al*. (2014) for the protozoan *Haplosporidium patagon* (Haplosporida), which also exhibited a higher prevalence in *S. lessonii* (3.78%) than in *S. lateralis* (0.13%). Results of the present study indicated that the parasites *M. madrynense* and Hemiuroidea fam. gen. et sp. have a high host specificity. Strong specificity of a digenean species for its first intermediate host has been reported in a number of previous studies (Lauckner, 1980; Galaktionov & Dobrovolskij, 2003). Maintenance of strict host specificity by parasites when several closely related host species occur sympatrically is poorly understood (Donald *et al*., 2004). In the present study *M. madrynense* was found to be capable of parasitizing both sympatric host species, yet a very low prevalence was recorded in the case of *S. lateralis*. It is noteworthy that, a priori, it is expected that the encounter probability of the parasite and host is much greater in the case of *S. lateralis* because its density is markedly higher than that of *S. lessonii* (55.13 vs. 5.87 individuals/m^2). Indeed, it is possible that the low densities of *S. lessonii* observed in the present study can be explained by the fact that the parasite causes castration in this snail species (Di Giorgio *et al*., unpublished data) and also has the potential to reduce the host's physiological condition, thereby increasing the snail population's vulnerability to predation (Minchella & Loverde, 1981), or increasing mortalities by eliciting physiological stress (Lafferty, 1993; Thomas *et al*., 1995).

Based on host density data determined in the present study, *S. lessonii* occurs at a lower density at site 1, a more eutrophic site affected by sewage-derived nutrients, than site 2. This finding agrees with the results of Tablado *et al*. (1994), who reported lower densities of *S. lessonii* in populations affected by disrupted environmental conditions due to sewage pollution. Despite the lower density of *S. lessonii* at site 1, the prevalence of infection of both digeneans was higher at site 1 than at site 2. The high nutrient values measured at site 1 are associated in the present study with the higher prevalence of infection by *M. madrynense* and Hemiuroidea fam. gen. et sp. at this site. Poulin (1992), Lafferty (1997) and Blanar *et al*. (2009) reported that eutrophication had a positive effect on the abundance of parasites. At the same time, the more eutrophic site appears to attract a larger number of birds (Gandini & Frere, 1998). In the present study, the average abundance of total birds was 230 at site 1 vs. 69 at site 2. The kelp gull was also more abundant at the eutrophic site (151 vs. 38 at sites 1 and 2, respectively). This may be another possible explanation for the higher parasitic prevalence at the more eutrophic site.

Several previous studies reported that parasite populations are strongly influenced by the distribution and abundance of their definitive hosts (Smith, 2001; Hechinger & Lafferty, 2005). Hoff (1941) found that higher values of infection prevalence by *Cryptocotyle lingua* in the limpet, *Littorina littorea*, were related to the high abundance of gulls observed feeding in the area. Few studies, however, provide robust documentation of the existence of a direct link between bird abundance and parasite spatial heterogeneity (Smith, 2001; Hechinger & Lafferty, 2005; Fredensborg *et al*., 2006). In the present study, a strong positive correlation was found between bird abundance and the prevalence of *M. madrynense*. In the case of Hemiuroidea fam. gen. et sp., the definitive hosts are fish (Gibson *et al*., 2002). The highest prevalence of this parasite was found at site 1, located in the upper intertidal zone. Studies of the trematode life cycle, as well as the ecology and behaviour of the second intermediate and definitive hosts, are necessary to better understand the spatial distribution of this hemiuroid parasite.

The trematode *M. madrynense* was found parasitizing the digestive gland and the gonad of the false limpet *S. lessonii*, and showed a higher percentage of individuals with sporocysts containing only metacercariae than of those with sporocysts containing cercariae. This finding may indicate that shortening of the *M. madrynense* life cycle is possible when it uses *S. lessonii* as both a first and second intermediate host. A comparison between sites in the present study showed a higher percentage of gastropods parasitized by sporocysts containing metacercariae at site 1 than at site 2. Usually, both abbreviated and three-host life cycles are observed in a single parasite population. This is interpreted as a transmission strategy in response to a diversity of biotic factors (such as the presence or absence of a definitive host) (Holton, 1984), or abiotic factors (extreme environmental conditions, e.g. low temperatures) that could restrict transmission by affecting the free-living larval stage (Skirnisson & Galaktionov, 2002), aerial exposure, salinity extremes and hydrodynamic forcing (Lagrue & Poulin, 2007). In the present study, the higher percentage of metacercariae at site 1 could be explained by the high level of stress, including the wide temperature variation, experienced at this site. Under these stressful conditions, an abbreviated life cycle would represent a selective advantage and adaptive response for the parasite.

The prevalence of double infections (*M. madrynense* + Hemiuroidea fam. gen. et sp.) was lower than that expected to occur randomly. This could be due to the fact that the presence of a given parasite species limits the entry of another parasite species, or may be because both occupy the same tissue (gonad and digestive gland) in their host. In the present study, a positive correlation was found between the prevalence of infection of Hemiurioidea fam. gen. et sp. and the size of the host. An increase in parasite prevalence with the size of gastropod hosts was also reported by Al-kandari *et al*. (2000, 2007) and Fredensborg *et al*. (2005), possibly due to the fact that larger individuals, assumed to be older, were exposed to infective larvae (miracidia) for a longer time. Furthermore, Skirnisson *et al*. (2004) reported that large gastropods offer more space and nutrients for the parasite.

Different seasonal patterns in the prevalence and abundance of trematodes in molluscs have been documented previously (Kube *et al*., 2002; Fermer *et al*., 2009). In this study, the prevalence of infection of *M. madrynense* was minimal during summer, when temperatures recorded at both sites were the highest (29°C and 31°C at sites 1 and 2, respectively), coinciding with a wider temperature range. Prinz *et al*. (2010) reported a peak in the prevalence of trematode *Echinostephilla patellae* in the intertidal gastropod *Patella vulgata* during the austral autumn, coinciding with an increase in the abundance of birds. In our study, bird abundance showed no seasonal pattern of variation. Variation of mean monthly temperatures differed among seasons, however, as it was markedly higher in summer than during the rest of the year. This may explain the lower prevalence of infection in summer, as a wide range of monthly temperatures could negatively affect egg hatching and the survival of miracidia that infect the first host. Furthermore, a definite seasonal pattern of infection was not detected in previous studies (Cannon, 1979; Taskinen *et al*., 1994; Muñoz *et al*., 2013). Similarly, in the present study there was no evidence of a seasonal pattern in the prevalence of Hemiuroidea fam. gen. et sp. in the study area.

Parasite biomass is known to be related to host size, energy availability, season and habitat characteristics (Hechinger & Lafferty, 2005; Kuris *et al*., 2008). In this study the digenean parasites occupied a relatively large percentage of the overall weight of the gastropod host (up to 26.66%). Furthermore, the percentage of tissues occupied by each parasite species differed between sampling sites, such that infection intensity was higher in the upper intertidal than in the lower intertidal. This intraspecific variation may be associated with the more eutrophic conditions and thus the greater food availability (algal biomass) for these siphonariid gastropods at site 1. The trematode *M. madrynense* was also found to occupy a greater percentage of host tissues than Hemiuroidea fam. gen. et sp. Similarly, intra-specific variation was observed between two study sites in a parasite–host system including larval trematodes and the gastropod *C. californica* by Hechinger *et al*. (2009). These authors demonstrated that the relative body mass of parasite and host can vary inter-specifically, both with taxonomic family and the type of host tissue affected.

Acknowledgements

All authors are members of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). The authors thank Dra. Titina Zapata, from the Centro de Investigaciones, Universidad de la Patagonia Austral at Puerto Deseado, for kindly allowing access to laboratory facilities, the Prefectura Naval Argentina, Puerto Deseado, for their help with sampling and the Laboratory of Chemical Oceanography and Water Pollution for performing chemical analyses of water samples. Editing services were provided by Dr Monica Bricelj, techMar research Inc., New York, USA.

Financial support

This study was funded by Agencia Nacional de Promoción Científica y Tecnológica (PICT 2010-0730 and PICT 2013-1702) and the Professional Association of Diving Instructors (PADI) Foundation.

Conflict of interest

None.

References

Alda, P. & Martorelli, S.R. (2009) Larval digeneans of the siphonariid pulmonates *Siphonaria lessoni* and *Kerguelenella lateralis* and the flabelliferan isopod *Exosphaeroma* sp. from the intertidal zone of the Argentinean Sea. *Comparative Parasitology* **76**, 267–272.

Al-kandari, M.A., Abdul-Salam, J.M., Moussa, M.A. & Sreelatha, B.S. (2007) Seasonal dynamics of the cercarial and metacercarial stages of the microphallid trematode *Maritrema eroliae* (Digenea: Microphallidae). *Acta Zoologica Sinica* **53**, 463–469.

Al-kandari, W.Y., Abdul-Salam, J. & Meakins, R. (2000) Temporal variations in the infection of a population of *Cerithidea cingulata* by larval trematodes in Kuwait Bay. *Journal of Helminthlogy* **74**, 17–22.

Blanar, C.A., Munkittrick, K.R., Houlahan, J., Maclatchy, D.L. & Marcogliese, D.J. (2009) Pollution and parasitism in aquatic animals: a meta-analysis of effect size. *Aquatic Toxicology* **93**, 18–28.

Burnham, K.P. & Anderson, D.R. (2002) *Model selection and multimodel inference: A practical information-theoretic approach*. New York, Springer.

Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W. (1997) Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *Journal of Parasitology* **83**, 575–583.

Cannon, L.R.G. (1979) Ecological observations on *Cerithium moniliferum* Kiener (Gastropoda: Cerithiidae) and its trematode parasites at Heron Island, Great Barrier Reef. *Australian Journal of Marine and Freshwater Research* **30**, 365–374.

Crawley, M.J. (2007) *The R book*. Silwood Park, UK, Wiley, Imperial College London.

Diaz, J.I. & Cremonte, F. (2010) Development from metacercaria to adult of a new species of *Maritrema* (Digenea: Microphallidae) parasitic in the kelp gull, *Larus dominicanus*, from the Patagonian coast, Argentina. *Journal of Parasitology* **96**, 740–745.

Di Giorgio, G., Gilardoni, C. & Ituarte, C. (2014) Pathology of *Haplosporidium patagon* affecting siphonariid gastropods in Patagonia. *Diseases of Aquatic Organisms* **112**, 59–67.

Donald, K.M., Kennedy, M., Poulin, R. & Spencer, H.G. (2004) Host specificity and molecular phylogeny of larval Digenea isolated from New Zealand and Australian topshells (Gastropoda: Trochidae). *International Journal for Parasitology* **34**, 557–568.

Fermer, J., Culloty, S.C., Kelly, T.C. & O'Riordan, R.M. (2009) Intrapopulation distribution of *Meiogymnophallus minutus* (Digenea, Gymnophallidae) infections in its first and second intermediate host. *Parasitology Research* **105**, 1231–1238.

Fredensborg, B.L., Mouritsen, K.N. & Poulin, R. (2005) Impact of trematodes on host survival and population density in the intertidal gastropod *Zeacumantus subcarinatus*. *Marine Ecology Progress Series* **290**, 109–117.

Fredensborg, B.L., Mouritsen, K.N. & Poulin, R. (2006) Relating bird host distribution and spatial heterogeneity in trematode infections in an intertidal snail – from small to large scale. *Journal of Animal Ecology* **149**, 275–283.

Galaktionov, K.V. & Dobrovolskij, A.A. (2003) *The biology and evolution of trematodes*. Dordrecht, The Netherlands, Kluwer Academic.

Gandini, P.A. & Frere, E. (1998) Seabird and shorebird diversity and associated conservation problems in Puerto Deseado, Patagonia, Argentina. *Ornitología Neotropical* **9**, 13– 22.

Gibson, D.I., Jones, A. & Bray, R.A. (2002) *Keys to the Trematoda, Volume 1*. Wallingford, CABI Publishing and London, The Natural History Museum.

Gilardoni, C., Etchegoin, J.A., Diaz, J.I., Ituarte, C. & Cremonte, F. (2011) A survey of larval digeneans in the commonest intertidal snails from Northern Patagonian coast, Argentina. *Acta Parasitologica* **56**, 163–179.

Hechinger, R.F. & Lafferty, K.D. (2005) Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proceedings of the Royal Society of London* **272**, 1059–1066.

Hechinger, R.F., Lafferty, K.D., Mancini, F.T., Warner, R.R. & Kuris, A.M. (2009) How large is the hand in the puppet? Ecological and evolutionary factors affecting body mass of 15 trematode parasitic castrators in their snail host. *Evolutionary Ecology* **23**, 651–667.

Hoff, C.C. (1941) A case of correlation between infection of snail hosts with *Cryptocotyle lingua* and the habits of gulls. *Journal of Parasitology* **27**, 539.

Holton, A.L. (1984) Progenesis as a mean of abbreviating life histories in two New Zealand trematodes, *Coitocaecum parvum* Crowcroft, 1945 and *Stegodexamene anguillae* MacFarlane, 1951. *Mauri Ora* **11**, 63–70.

Ituarte, C., Bagnato, E., Siddall, M. & Cremonte, F. (2014) A new species of *Haplosporidium* Caullery & Mesnil, 1899 in the marine false limpet *Siphonaria lessonii* (Gastropoda: Siphonariidae) from Patagonia. *Systematic Parasitology* **88**, 63–73.

Kube, S., Kube, J. & Bick, A. (2002) Component community of larval trematodes in the mudsnail *Hydrobia ventrosa*: temporal variations in prevalence in relation to host life history. *Journal of Parasitology* **88**, 730–737.

Kuris, A.M. & Lafferty, K.D. (1994) Community structure: larval trematodes in snail hosts. *Annual Review of Ecological Systems*. **25**, 189–217.

Kuris, A.M., Hechinger, R.F., Shaw, J.C., Whitney, K.L., Aguirre-Macedo, L., Boch, C.A., Dobson, A.P., Dunham, E.J., Fredensborg, B.L., Huspeni, T.C., Lorda, J., Mababa, L., Mancini, F.T., Mora, A.B., Pickering, M., Talhouk, N.L., Torchin, M.E. & Lafferty, K.D. (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* **454**, 515–518.

Lafferty, K.D. (1993) Effects of parasitic castration on growth, reproduction and population dynamics of the marine snail *Cerithidea californica*. *Marine Ecology Progress Series* **96**, 229–237.

Lafferty, K.D. (1997) Environmental parasitology: what can parasites tell us about human impacts on the environment? *Parasitology Today* **13**, 251–255.

Lagrue, C. & Poulin, R. (2007) Life cycle abbreviation in the trematode *Coitocaecum parvum*: can parasites adjust to variable conditions? *Journal of Evolutionary Biology* **20**, 1189–1195.

Lauckner, G. (1980) Diseases of Mollusca: Gastropoda. pp. 311–400 *in* Kinne, O. (*Ed.*), *Diseases of marine animals. Volume 1. General aspects, Protozoa to Gastropoda*. Chichester, UK, John Wiley & Sons.

McCullagh, P. & Nelder, J.A. (1989) *Generalized linear models*. London, Chapman & Hall.

Minchella, D.J. & Loverde, P.T. (1981) A cost of increased early reproductive effort in the snail *Biomphalaria glabrata*. *American Naturalist* **118**, 876–881.

Muñoz, G., Torres, P., Valdés, J. & Rodríguez, A. (2013) Spatio-temporal variation in the prevalence of trematodes in the bivalve *Perumytilus purpuratus*. *Acta Parasitologica* **58**, 155–166.

Poulin, R. (1992) Toxic pollution and parasitism in freshwater fish. *Parasitology Today* **8**, 58–61.

Prinz, K., Kelly, T.C., O'Riordan, R.M. & Culloty, S.C. (2010) Temporal variation in prevalence and cercarial development of *Echinostephilla patellae* (Digenea, Philophthalmidae) in the intertidal gastropod *Patella vulgata*. *Acta Parasitologica* **55**, 39–44.

R Development Core Team. (2012) *R: A language and environment for statistical computing*. Vienna, Austria, R Foundation for Statistical Computing. Available at http://www.r-project.org/ (accessed 11 October 2016).

Skirnisson, K. & Galaktionov, K.V. (2002) Life cycles and transmission patterns of seabird digeneans in SW Iceland. *Sarsia* **87**, 144–151.

Skirnisson, K., Galaktionov, K.V. & Kozminsky, E.V. (2004) Factors influencing the distribution of digenetic trematode infections in a mudsnail (*Hydrobia ventrosa*) population inhabiting salt marsh ponds in Iceland. *Journal of Parasitology* **90**, 50–59.

Smith, N.F. (2001) Spatial heterogeneity in recruitment of larval trematodes to snail intermediate hosts. *Oecologia* **127**, 115–122.

Sokal, R.R. & Rohlf, F.J. (1981) *Biometry*. 2nd edn. New York, W.H. Freeman.

Studer, A. & Poulin, R. (2012) Seasonal dynamics in an intertidal mudflat: the case of a complex trematode life cycle. *Marine Ecology Progress Series* **455**, 79–93.

Tablado, A., López Gappa, J.J. & Magaldi, N.H. (1994) Growth of the pulmonate limpet *Siphonaria lessoni* (Blainville) in a rocky intertidal area affected by sewage pollution. *Journal of Experimental Marine Biology and Ecology* **175**, 211–226.

Taskinen, J., Valtonen, E.T. & Mäkelä, T. (1994) Quantity of sporocysts and seasonality of two *Rhipidocotyle* species (Digenea: Bucephalidae) in *Anodonta piscinalis* (Mollusca: Bivalvia). *International Journal for Parasitology* **24**, 877–886.

Thieltges, D.W., Fredensborg, B.L. & Poulin, R. (2009) Geographical variation in metacercarial infection levels in marine invertebrate hosts: parasite species character versus local factors. *Marine Biology* **156**, 983–990.

Thomas, F., Renaud, F., Rousset, F., Cezilly, F. & Meeus, T.D. (1995) Differential mortality of two closely related host species induced by one parasite. *Proceedings of the Royal Society B: Biological Sciences* **260**, 349–352.

Venables, W. & Ripley, B. (2002) *Modern applied statistics using S*. New York, Springer.