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Factors affecting horizontal transmission of the microsporidium *Amblyospora albifasciati* to its intermediate copepod host *Mesocyclops annulatus*

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ABSTRACT

Factors that directly impact horizontal transmission of the microsporidium *Amblyospora albifasciati* to its intermediate copepod host, *Mesocyclops annulatus* were examined in laboratory bioassays. Results were evaluated in relation to life history strategies that facilitate persistence of the parasite in natural populations of its definitive mosquito host, *Ochlerotatus albifasciatus*. A moderately high quantity of meiospores from mosquito larvae was required to infect adult female copepods; the IC50 was estimated at 3.6×10^4 meiospores/ml. Meiospore infectivity following storage at 25 °C was detected up to 30 days, while meiospores stored at 4 °C remained infectious to copepods for 17 months with virtually no decline in infectivity. Uninfected female *M. annulatus* are long-lived; no appreciable mortality was observed in field-collected individuals for 26 days, with a few individuals surviving up to 70 days. The pathological impact of *A. albifasciati* infection on *M. annulatus* resulted in a 30% reduction in survivorship after 7 days followed by gradual progressive mortality with no infected individuals surviving more than 40 days. This moderate level of pathogenicity allows for a steady continual release of spores into the environment where they may be ingested by mosquito larvae. Infected female copepods survived in sediment under conditions of desiccation up to 30 days, thus demonstrating their capacity to function as a link for maintaining *A. albifasciati* between mosquito generations following periods of desiccation. The susceptibility of late stage copepodid *M. annulatus* to meiospores of *A. albifasciati* and subsequent transstadial transmission of infection to adult females was established.

1. Introduction

Microsporidia are common parasites in natural mosquito populations. *Amblyospora* species are among the most widely distributed (Andreadis, 2007). Several species of *Amblyospora* have been described from natural populations of mosquitoes in Argentina (García, 1989; García and Becnel, 1994; Micieli and García, 1997; Micieli et al., 1998, 2000a,b) including *Amblyospora albifasciati* García and Becnel (1994), a parasite of the Neotropical floodwater mosquito, *Ochlerotatus albifasciatus* (Macquard) (=*Aedes albifasciatus*, see Reinert (2000)) and the cyclopoid copepod, *Mesocyclops annulatus* (Micieli et al., 2000a). It has a life cycle typical of most *Amblyospora* species. Meiospore stages formed in mosquito larvae are infectious *per os* to adult female stages of the intermediate copepod host. Uninucleate spores formed in the ovaries of the copepod are released into the water after death. These spores are responsible for horizontal transmission of the parasite to mosquito larvae via oral ingestion. Mosquito larvae infected by this pathway develop benign infections leading to the production of binucleate spores in adult female mosquitoes. These spores are subsequently responsible for transovarial transmission to the next generation of mosquito larvae wherein meiospores are again produced (Micieli et al., 2000a).

Micieli et al. (2001) examined seasonal prevalence rates of *A. albifasciati* infection in field populations of *Oc. albifasciatus* and *M. annulatus* and concluded that horizontal transmission was a critical pathway needed for *A. albifasciati* to complete its life cycle in nature. Knowledge of the various factors that directly impact horizontal transmission dynamics of this parasite between the mosquito and copepod hosts is presently unknown. An understanding of these factors can help to identify the specific mechanisms employed by the microsporidium that allow it to persist in nature.

The aquatic habitats in which these hosts develop are largely ephemeral or semi-permanent pools that typically exhibit wide variation in water levels throughout the year with periodic flooding and drying (Maciá et al., 1995). As a consequence, the parasite must possess strategies which reflect the transient nature of the breeding sites. The objectives of this investigation were to examine several factors that may directly impact parasite transmission between mosquito larvae and copepods including: (1) the infectivity of meiospores to copepods, (2) the viability of meiospores outside of the mosquito host, (3) the longevity of intermediate copepod

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host, (4) the capacity of meiospores to survive under conditions of desiccation, and (5) the efficiency of transstadial transmission.

2. Materials and methods

2.1. Meiospore acquisition and purification

Amblyospora albifasciati meiospores from fat body tissues of living fourth instar *Oc. albifasciatus* larvae were used as a source of inoculum. The infected larvae were collected from a transient freshwater pool located near La Plata City, Argentina (34°51′07″ S, 58°57′30″ W). Infected larvae were homogenized in a tissue grinder, passed through a syringe with cotton, and centrifuged twice at 3500 rpm. The supernatant was eliminated and the pellet containing spores was re-suspended in distilled water.

2.2. Culture of M. annulatus

Females copepods were collected from the same transient fresh water pool located near La Plata City as noted above. Samples were transported to the laboratory and gravid females were individually isolated in 8 cm diameter plastic cups containing 150 ml of dechlorinated water. Copepods were fed fish food once a week and maintained at 26 °C ± 1. Copepods were identified according to Reid (1985) and Ringuelet (1958) and reconfirmed by Dr. S. Menú Márquez, University of Buenos Aires, Argentina. Adult female stages were used in all bioassay feeding experiments except where noted in Section 2.7.

2.3. Meiospore bioassays with copepods (IC50)

The concentration of mature meiospores spores used in the bioassays was estimated with a hemocytometer and exposure rates ranged from 5×10^2 to 1×10^6 meiospores/ml. Three separate assays with two replicates were performed. For each replicate, one beaker containing five copepods in 5 ml of distilled water was used at each concentration with one untreated control. All feeding assays were conducted in an incubator at 25 °C for duration of 15 days, the normal time required for *A. albifasciati* to complete its development in the copepod host (Micieli et al., 2000a). During this period the copepods were not fed.

Living copepods that survived the 15-day exposure period were smeared, stained with a 10% Giemsa-stain solution (pH: 7, 4) and examined for microsporidia infection. Dead copepods not were examined for infection as in the majority of the assays, mortality occurred during the first few days of exposure. Overall mortality was recorded and included dead copepods that were recovered in the beakers as well as those individuals that were missing. The results of the three assays were combined and the percent infection in surviving copepods was calculated for each concentration. The data were analyzed by the Probit method (Chi, 1997).

2.4. Longevity and viability of meiospores at 4 °C and 25 °C

The survival of meiospores outside of the mosquito host was evaluated in bioassay experiments following storage in distilled water at 4 °C and 25 °C. Four degree centigrade was selected as a likely temperature that might be used for long-term storage of spores for future use in field applications, while 25 °C represented a high temperature extreme which meiospores would be typically exposed to in the vernal pools during the summer (Micieli et al., 2001).

Meiospores held at 4 °C were evaluated after storage for 8, 17, 21 and 24 months. Fresh meiospores obtained from infected individuals just prior to the feeding trials were used as a control. Two separate assays with two replicates were conducted for each storage period. Ten copepods were exposed to 4×10^4 spores/ml in 10 ml of dechlorinated water for each replicate.

For 25 °C bioassays, spores were evaluated after storage for 1 (fresh spores), 15, 30, 50 and 60 days. These bioassays were similarly conducted in Petri dishes containing an inoculum of 5×10^4 spores/ml, 10 ml of dechlorinated water, and ten copepods. Copepods were exposed for 15 days, after which they were counted, smeared, stained with a 10% Giemsa-stain solution (pH: 7, 4) and examined for microsporidia infection.

The results of the two replicated assays were combined for each temperature regime and the percent infection in survivors was determined. Data were analyzed by Chi-square analysis and multiple logistic regression (SPSS Incorporated, 2003).

2.5. Impact of infection on copepod longevity

The impact of *A. albifasciati* infection on *M. annulatus* was evaluated by comparing the longevity of uninfected with infected adult female copepods. Infected copepods used in these assays were obtained from the previously described experimental assays following 15 days of exposure to meiospores. Uninfected copepods were taken from laboratory cultures and held during the 15 days of exposure under the same conditions as the exposed copepods but without the addition of meiospores. Three separate assays with three replicates were carried out. The number of copepods used in each replicate ranged from 4 to 5. The longevity of naturally infected and uninfected adult female copepods collected from the field was similarly evaluated. Copepods were individually isolated in tissue cell vials to which 3 ml of dechlorinated water and 1 mg of fish food were added. The water was maintained at the same level during the assays but no additional food was added. The experiment was conducted in an incubator at 25 °C and copepod mortality was recorded daily for 7 days. Data were analyzed using Kruskal–Wallis one way ANOVA on ranks and Dunn's Method for pairwise multiple comparisons (SPSS Incorporated, 2003).

2.6. Copepod infection and survival under dry conditions

The ability to infect copepods under dry conditions was evaluated. Three assays with three replicates were performed. For each replicate, five adult female copepods were initially exposed to 5×10^4 spores/ml for 24 h in 5 ml of dechlorinated water, following which they were placed into 3.5 cm diameter plastic containers containing1 cm of sterilized sand as sediment and 10 ml of water. Twenty-four hours later, the standing water was removed with a pipette. One replicate, for each of the three assays in which the water was not removed, was used as a control. After a period of 15 or 30 days, the dry containers from which the water had been previously removed were flooded with dechlorinated water. Mortality and the infection status of surviving of live copepods were determined as described previously. The results of the three assays were combined and percent infection of survivors was calculated for each condition and period of time. Infection rates were compared by Chi-square analysis.

2.7. Infectivity of meiospores to copepodid stages

Twenty early (I–II) and 20 late stage (V) copepodids were exposed to meiospores of *A. albifasciati* at a concentration of 5×10^4 spores/ml in 10 ml of dechlorinated water. Two replicates were conducted under two different temperatures, 25 °C and 16 °C for both development stages. Fifteen days after exposure, copepodid and adult stages were identified and counted. Microsporidian infection was determined through dissection of each specimen and through examination of Giemsa-stained smears of whole cope-

pods. Percent infection was calculated for each developmental stage.

3. Results

3.1. Meiospore IC50 to copepods

The results of the initial bioassay experiments to determine the IC50 of meiospores to adult copepods that survived after 15 days of exposure at 25 °C are shown in Table 1. The IC50 was estimated as 3.6×10^4 meiospores/ml with 95% fiducial limits of 1.3×10^4–1.2×10^5 meiospores/ml. The regression equation was $y = 1.99 + 0.65x$ and the Chi-square value was 7.2 (df = 6; $p = 0.3$).

3.2. Longevity and viability of meiospores at 4 °C and 25 °C

The results of the bioassay experiments with meiospores stored at 4 °C are shown in Tables 2. Fifty percent of live copepods that were exposed to fresh meiospores of *A. albifasciati* (control) at a concentration of 4×10^4 meiospores/ml for 15 days became infected. This was not significantly greater than the 46.6% and 44.4% infection observed in copepods that were exposed to meiospores that had been stored for 8 or 17 months, respectively (Chi-square = 0.13; df = 2; $p = 0.935$). No infections were found in any copepods that were exposed to meiospores that had been stored more than 21 months. Multiple logististic regression analysis of the data showed a highly significant ($P < 0.001$) affect of meiospore age on infectivity, but high degree of variability resulting in a poor fit between the data and the logistic regression equation ($y = 0.49 - 0.11x$, Pearson Chi-square statistic = 95.2, $P = 0.70$; Log Likelihood statistic = 104.4).

Infectivity of *A. albifasciati* meiospores to *M. annulatus* following storage at 25 °C is shown in Table 3. The infectivity of fresh meiospores to copepods at a concentration of 5×10^4 meiospores/ml (control) was nearly identical to that achieved at a concentration of 4×10^4 meiospores/ml. However, a significant decline in infectivity was observed after 15 and 30 days (Chi-square = 7.47, df = 2; $p = 0.023$), and no infection was achieved with spores stored for 50 or 60 days at this temperature. Multiple logistic regression analysis of the data showed a highly significant ($P < 0.001$) affect of meiospore age on infectivity at 25 °C as it did at 4 °C, but with a high degree of variability, resulting in a poor fit between the data and the logistic regression equation ($y = -0.15 - 0.06x$, Pearson Chi-square statistic = 119.5, $P = 0.83$; Log Likelihood statistic = 117.9).

Table 1
Infective concentration of *Amblyospora albifasciati* meiospores to adult *Mesocyclops annulatus*.[a]

Meiospore concentration (spores/ml)	No. copepods exposed	No. copepods dead/missing	No. copepods live	Percent live copepods infected
1×10^6	30	8	22	86.3
5×10^5	30	19	11	81.8
1×10^5	30	2	28	57.1
5×10^4	30	12	18	55.5
1×10^4	30	0	30	30.0
5×10^3	30	7	23	30.4
1×10^3	30	0	30	6.6
5×10^2	30	11	19	26.3
Control	30	2	28	0

[a] Combined results of three assays.

Table 2
Infectivity of *Amblyospora albifasciati* meiospores to adult female *Mesocyclops annulatus* following storage at 4 °C for 8–24 months (15 day exposure period at a concentration of 4×10^4 spores/copepod).

Meiospore age (months)	No. copepods exposed	No. copepods dead/missing	No. copepods live	Percent live copepods infected[a]
0 (fresh)	40	22	18	50.0 a
8	40	25	15	46.6 a
17	40	13	27	44.4 a
21	40	14	26	0
24	40	20	20	0

[a] Values followed by a common letter are not significantly different by Chi-square analysis ($P > 0.05$).

Table 3
Infectivity of *Amblyospora albifasciati* meiospores to adult female *Mesocyclops annulatus* following storage at 25 °C for 15–60 days (15 day exposure period at a concentration of 5×10^4 spores/copepod).

Meiospore age (days)	No. copepods exposed	No. copepods dead/missing	No. copepods live	Percent live copepods infected[a]
0 (fresh)	40	12	28	46.4 a
15	40	2	38	21.0 b
30	40	2	38	18.4 b
50	40	23	17	0
60	40	23	17	0

[a] Values followed by a common letter are not significantly different by Chi-square analysis ($P > 0.05$).

3.3. Impact of infection on copepod longevity

No significant mortality was observed in uninfected field-collected copepods that were held in the laboratory at 25 °C up to 26 days (Fig. 1). Thereafter, a steady rate of decline was seen with the last remaining copepods surviving up to 70 days. Mortality was detected immediately in infected field-collected copepods with a 30% reduction in survivorship after only 7 days. A steady gradual decline in the number of infected copepods was recorded thereafter with 100% mortality by day 50. The mortality pattern observed in uninfected lab-reared copepods was nearly identical to the pattern observed with infected field-collected copepods except that some of the uninfected lab-reared specimens survived up to 100 days. Very high mortality rates were seen in laboratory-treated copepods with only 10% surviving after 14 days and none by day 26. Differences in the median survival times for copepods among the four treatment groups were highly significant ($H = 54.2$, df = 3, $P < 0.001$, Kruskal–Wallis one way ANOVA on ranks), and significance differences ($P < 0.05$) were revealed in pairwise multiple comparisons (Dunn's method) for all treatments except uninfected laboratory reared copepods vs. field-collected infected copepods

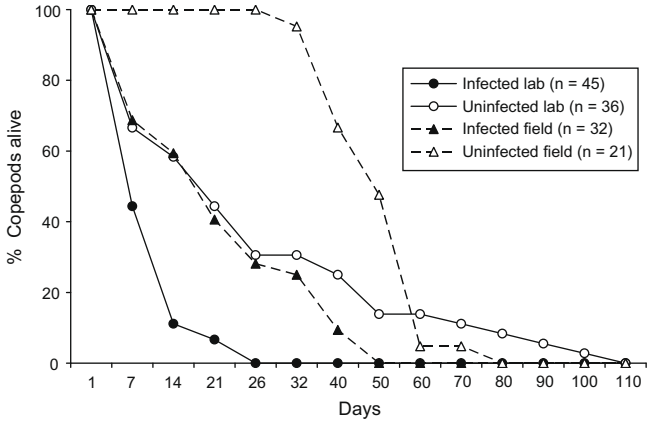

Fig. 1. Longevity of uninfected and *Amblyospora albifasciati* – infected adult female *Mesocyclops annulatus* obtained from field collections and laboratory bioassays.

both of which exhibited similar median survival times of 21 days. The median survival times for uninfected field-collected copepods was 50 days, and only 7 days for laboratory-infected copepods.

3.4. Copepod infection and survival under dry conditions

The survivorship and infection status of adult female copepods that were exposed to meiospores and then held under dry conditions wherein the water was withdrawn, compared to copepods that were held in containers that remained inundated with water up to 30 days are shown in Table 4. High survivorship was observed in both groups after 15 days (82% and 71%, respectively), and no significant difference was recorded in the percent of surviving copepods that were found to be infected with *A. albifasciati* (29.7% vs. 46.8%, respectively, Chi-square = 2.14; df = 1; $P = 0.142$) during this period. Thirty-six percent of copepods held under dry conditions were still alive after 30 days and the percent infection among the survivors was virtually the same (31.2%, Chi-square = 0.01; df = 1; $P = 0.911$). Conversely, no live copepods were recovered from the cohort that remained in water for 30 days.

3.5. Infectivity of meiospores to copepodid stages and transstadial transmission

The infectivity of meiospores of *A. albifasciati* to early (I–II) and late (V) stage copepodids is shown in Table 5. When early stage copepodids were exposed to meiospores no adult copepods were recovered at either temperature (25 °C or 16 °C), nor were any infections with *A. albifasciati* recorded in any copepodid stages. In the assays where late stage copepodids were exposed to meiospores, both male and female adults, as well as copepodids were found after 15 days at both temperatures but *A. albifasciati* infections were only detected in adult females. Copepods that did not survive the 15-day exposure period were not examined for infection.

4. Discussion

With this investigation we have obtained new knowledge on the transmission dynamics of *A. albifasciati* between *Oc. albifasciatus* and *M. annulatus*, from which certain life history strategies that facilitate persistence of this microsporidium in nature may be inferred. The initial bioassays to determine the IC50 of meiospores to copepods revealed a moderately high concentration rate of 3.6×10^4 spores/ml. We acknowledge that infection rates could have been potentially greater, since the infection status of copepods that initially died were not examined. However, since most of this copepod mortality occurred very early in the bioassays, it is unlikely that it was due to infection with the microsporidium.

Our results were comparable to the IC50 estimated by Sweeney et al. (1989) for meiospores of *Amblyospora dyxenoides* from *Culex annulirostris* to *Mesocyclops* sp. (9.9×10^3 meiospores/ml), and consistent with the concentration rate of 2×10^4 meiospores/ml reported by Andreadis (1991) to infect 75% and 43% of *Acanthocyclops vernalis* copepods with *Amblyospora connecticus* in filtered and unfiltered water, respectively from the larval habitat. Although more quantitative assessments of other mosquito/copepod-parasitic microsporidia are needed, these findings collectively suggest that meiospores in general do not appear to be highly infectious to copepods. Furthermore, if infectivity rates achieved in confined laboratory bioassays are applicable to field settings where fewer encounters between infectious spores and susceptible hosts would be expected, then it is logical to deduce that comparatively large concentrations of spores are probably required to infect a field population.

The results obtained in the bioassay experiments to evaluate the infectivity of meiospores following storage in an aqueous solution at 25 °C and 4 °C were revealing and clearly suggest that free spores of *A. albifasciati* are capable of surviving prolonged periods in the aquatic environment outside of the host. Meiospore infectivity following storage at 25 °C was detected for up to 30 days, albeit reduced, while meiospores stored at 4 °C remained infectious to copepods for nearly a year and a half (17 months) with virtually no decline in infectivity. Unfortunately, no studies are available to compare meiospore viability of *A. albifasciati* with other *Amblyospora* species at 25 °C. However, these findings support our earlier hypothesis (Micieli et al., 2001) that meiospores of *A. albifasciati* are relatively long-lived and can remain viable as long as the larval habitat remains inundated with water. Micieli et al. (2001) have found that although water levels fluctuate greatly in habitats where *Oc. albifasciatus* and *M. annulatus* develop, some standing water can be found throughout much of the year at many major production sites that rarely dry out entirely. *Oc. albifasciatus* larvae infected with *A. albifasciati* occur throughout the summer months of January and February where meiospores released from dead larvae are exposed to water temperatures that may be as high as 27 °C (Micieli, unpublished data). Enhanced meiospore survival outside of the host at 25 °C as demonstrated in the present study, would undoubtedly appear to facilitate transmission under these environmental conditions and therein serve as an effective survival strategy. Additional studies are needed to evaluate meiospore survival and infectivity at 10 °C, the mean water temperature during the winter (June) in the La Plata area, and between 15 °C and 19 °C, the mean water temperatures during the months of July to September when prevalence rates of *A. albifasciati* are greatest in copepod populations (Micieli et al., 2001).

Our demonstration of extended meiospore infectivity up to 17 months following storage at 4 °C contrasts sharply with an earlier study by Andreadis (1991) who reported a significant decline in the infectivity of meiospores of *A. connecticus* after storage for only 5 months at 4 °C and very little apparent viability after 17 months (5.4% infection). Reasons for the discrepancy in these two systems are likely inherent. However, in the present study meiospores were purified prior to storage, while Andreadis (1991) maintained meiospores in whole larval cadavers in distilled water that supported microbial growth that is usually detrimental

Table 4
Survivorship of adult female *Mesocyclops annulatus* and percent infection with *Amblyospora albifasciati* held for 15 and 30 days post-exposure under dry and flooded conditions.

No. days post-exposure	No. copepods exposed	Dry			Flooded		
		No. dead/ missing	No. live	% Live infected[a]	No. dead/ missing	No. live	% Live infected
15	45	8	37	29.7 a	13	32	46.8
30	45	29	16	31.2 a	45	0	–

[a] Values followed by a common letter are not significantly different by Chi-square analysis ($P > 0.05$).

Table 5
Infectivity of meiospores of *Amblyospora albifasciati* to early (I–II) and late (V) stage copepodids of *Mesocyclops annulatus* at 25 °C and 16 °C, 15 days post-exposure.

Temperature and copepodid stage exposed	No. exposed	No. copepods live and (% infection)		
		Copepodids (V)	Adult females	Adult males
25 °C				
Early stage (I–II)	40	35 (0)	0	0
Late stage (V)	40	8 (0)	12 (58.3)	13 (0)
16 °C				
Early stage (I–II)	40	26 (0)	0	0
Late stage (V)	40	1 (0)	22 (31.8)	6 (0)

to spore survival. Despite these differences, our observations and those of Andreadis (1991) have important practical implications for long-term storage of meiospores which can be effectively refrigerated at 4 °C and subsequently used in inoculative or inundative release programs to augment horizontal transmission rates in copepod populations at critical times (Andreadis, 2007).

Uninfected adult female *M. annulatus* appear to be comparatively long-lived as no appreciable mortality was observed in field-collected individuals that were held at 25 °C up for 26 days, with a few individuals surviving as long as 70 days. In comparison, the pathological impact of *A. albifasciati* infection on *M. annulatus* was clearly evident as approximately 70% mortality was observed in infected field-collected individuals over the same 26 day time period, with no infected individuals surviving more than 40 days. Mortality rates in uninfected and infected laboratory cultured copepods were noticeably greater for each respective cohort in comparison to field-collected specimens, but exhibited the same general pattern. However, the gradual progressive mortality observed in infected copepods over a period of several weeks indicates that *A. albifasciati* is only moderately pathogenic to *M. annulatus*. This has important implications for horizontal transmission dynamics and persistence of *A. albifasciati* in nature. Since transmission can only take place with release of spores following death of the copepod host, this moderate level of pathogenicity would allow for a steady continual release of a fresh inoculum of infectious spores into the aquatic environment where they could be ingested by developing mosquito larvae. This strategy would appear to be advantageous for persistence of *A. albifasciati* as *Oc. albifasciatus* is a multivoltine species that is omnipresent throughout much of the year producing up to eight broods (Micieli et al., 2001). However, field studies (Micieli et al., 2001) have shown that despite the apparent abundance of a steady infusion of infectious spores produced by these copepods, infection rates in mosquito larvae never exceed 20%, thus implying low levels of horizontal transmission consistent with low spore infectivity and/or limited feeding on spores by *Oc. albifasciatus* larvae.

Our demonstration that a large majority (82%) of adult *M. annulatus* can survive 15 days of desiccation, and to a lesser degree up to 30 days (36%) even when infected with *A. albifasciati*, is significant and consistent with the behavior observed in other species of *Mesocyclops* (Zhen et al., 1994). This finding also helps to explain the detection of *A. albifasciati* infection in field populations of *M. annulatus* immediately following re-flooding of the habitat as noted by Micieli et al. (2001). The ability of infected copepods to survive periods of desiccation buried in the sediment has obvious implications for short-term persistence of the microsporidium in an ephemeral habitat that is subject to periodic flooding and drying, and where egg hatch and larval development of its target host, *Oc. albifasciatus,* occur with each flooding event (Campos and Sy, 2006). *Mesocyclops annulatus* may thus function as an effective link for maintaining *A. albifasciati* between mosquito generations following periods of desiccation.

In this investigation we demonstrated susceptibility of late stage (V) copepodid *M. annulatus* to meiospores of *A. albifasciati* and subsequent transstadial transmission of infection to adult females, but were unable to demonstrate the same to any adult males. These results are consistent with the established site of *A. albifasciati* infection in the copepod host, ovarian tissue, and corroborate prior bioassays with this microsporidium in which males were refractory to infection (Micieli et al., 2000a). The susceptibility of female stages and lack of infectivity to male hosts are undoubtedly a function of tissue specificity and the propensity of this microsporidium to invade and develop in host ovarian tissue. This appears to be a common phenomenon that has been reported in all other recognized intermediate copepod hosts including: *A. vernalis* (*A. connecticus*) (Andreadis, 1988), *Apocyclops* sp. (*Amblyospora indicola*) (Sweeney et al., 1990), *Mesocyclops albicans* (*A. dyxenoides*) (Sweeney et al., 1988), *Macrocyclops albidus* (*Amblyospora californica* and *Amblyospora salinaria*) (Becnel, 1992; Becnel and Andreadis, 1998), *Metacyclops mendocinus* (*Amblyospora dolosi*) (Micieli et al., 1998), *Orthocyclops modestus* (*Hyalinocysta chapmani*) (Andreadis and Vossbrinck, 2002), and *Paracyclops fimbriatus fimbriatus* (*Amblyospora camposi*) (Micieli et al., 2000b).

The inability of *A. albifasciati* to infect early stage (I–II) copepodids remains unresolved. This may have been a consequence of the 15-day evaluation period which did not afford the time needed for the immature copepodids to develop to adulthood where infections could be detected, or it could possibly have been due to the absence of undifferentiated gonadal tissue. According to Schram (1986), recognizable gonadal tissue does appear in the first copepodid stage and progressively develops through six successive molts to the sexually mature adult, but secondary sexual characters associated with sexual dimorphism do not appear until the third copepodid stage (Dussart and Defaye, 1995).

In summary, we have detailed several life history strategies employed by *A. albifasciati* that reflect the biology of its hosts and the ephemeral aquatic environment in which they inhabit that would appear to facilitate horizontal transmission and persistence in nature. These include: (1) long-lived meiospores that can remain viable outside of the mosquito host and thus allow for less reliance on the hosts for survival and dispersal, (2) moderate pathogenicity in a long-lived copepod host resulting in an steady continuous release of infective inoculum (spores) into the environment, (3) the ability of infected hosts to survive in the sediment under conditions of desiccation thus providing an effective link for horizontal transfer of the microsporidium between mosquito generations, and (4) transstadial transmission with no apparent acute mortality.

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