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Norma A. López-Téllez

Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional Unidad Mérida

Victor M. Vidal-Martínez

Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional Unidad Mérida

Robin M. Overstreet

Gulf Coast Research Laboratory, robin.overstreet@usm.edu

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Seasonal Variation of Ectosymbiotic Ciliates on Farmed and Wild Shrimps from Coastal Yucatan, Mexico

Norma A. López-Téllez,^{1,2} Victor M. Vidal-Martínez,¹
and Robin M. Overstreet³

1. Laboratorio de Parasitología, Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN) Unidad Mérida, Antigua Carretera a Progreso km. 6, AP 73 “Cordemex,” 97310 Mérida, Yucatán, México
2. Centro Regional de Investigación Pesquera de Lerma, Campeche, Instituto Nacional de la Pesca (CRIP Lerma-INP), km. 5 Carretera Campeche-Lerma s/n, 24030 Campeche, Campeche, México
3. Gulf Coast Research Laboratory, University of Southern Mississippi, 703 East Beach Drive, Ocean Springs, Mississippi 39564, USA

Corresponding author – Victor M. Vidal-Martínez, email vvidal@mda.cinvestav.mx

Abstract

High levels of ciliate infestation can affect respiration, feed intake, and locomotion in farmed shrimps in the tropics. Information on seasonal variation of the infestation parameters of ciliates is scarce, but it would be useful for determining the suitability of preventive measures or therapeutic treatment if necessary. This study aimed to describe the prevalence and mean intensity of infestation (MII) of ciliates on wild native shrimps *Penaeus (Farfantepenaeus) brasiliensis* and *P. (Farfantepenaeus) duorarum* and farm-cultured juvenile shrimp *Penaeus (Litopenaeus) vannamei* over an annual cycle and to determine whether an association existed among physicochemical factors and infestation on cultured shrimp. The ectosymbiotic ciliates *Acineta tuberosa*, *Epistylis* sp., and *Zoothamnium* sp. infested both farmed and wild shrimps. Based on examination of 360 farmed specimens of *P. vannamei* (30 examined each month), the species with highest infestation were *Epistylis* sp., (prevalence range = 22 – 100%; MII range = 13 ± 28 – 126 ± 124) and *Zoothamnium* sp. (0–87%; 0 – 144 ± 132). For both species, the highest values occurred during the rainy season. A total of 288 wild specimens of *P. brasiliensis*

plus 120 of *P. duorarum* obtained during three recognized seasons—dry (February to May), rainy (June to September), and north-wind (October to January)—had ciliate infestations that were significantly lower than those from farmed shrimp. For *P. brasiliensis*, the ciliate species with the highest infestation values were *Zoothamnium* sp. (0–66%; $0.03 \pm 0.2 - 66 \pm 92$), while on *P. duorarum*, were for *Epistylis* sp. (0–46%; $0 - 8 \pm 16$). Using multiple linear regression, we found significant relationships between stocking density, survival, temperature, turbidity, and intensity of infestation of the ciliates. However, the regression accounted for only 11–26% of the variance (R^2), meaning that there are still other environmental and biological variables that should be included to increase the reliability of the model. Thus, the physicochemical conditions of the farm increase the probability of ciliate transmission, especially during the rainy season.

1. Introduction

In farmed shrimp, heavy infestations of ectosymbionts are expected because transmission is enhanced due to high stocking densities and variations of environmental parameters (e.g., ammonium, nitrate, and dissolved oxygen levels), the consequence of pond fertilization (Kautsky et al., 2000; Gualteros-Rodríguez, 2003). This enhancement is important because high levels of ectosymbiont infestation in cultured shrimps can affect host respiration by lowering tolerance for low dissolved oxygen content, which reduces feed intake and, consequently, shrimp growth. Ectosymbionts can also alter locomotion, causing abnormal swimming, and even death (Brock and Kevan, 1994; Kautsky et al., 2000; Bauer, 2002), especially of larval shrimp. Decapod crustaceans eliminate ectosymbionts during molting, sloughing, grooming, hiding, burying, and nocturnal activity, but none of these mechanisms are 100% effective (Becker and Wahl, 1996; Bauer, 2002; Itani et al., 2002). Furthermore, ectosymbionts have short life cycles, reproduction synchronized with host ecdysis, fast recolonization of recently molted hosts, and ability to transfer among hosts during intermolt periods (Itani et al., 2002; Utz, 2003). Of these strategies, the most common for ciliate ectosymbionts is a combination of a short life cycle with rapid recolonization of recently molted individuals (Itani et al., 2002).

Transmission in ciliates increases during summer in tropical or subtropical climates, probably because of increased metabolism and molting caused by a higher amount of solar energy (Rhode, 1992; Jayasree et al., 2001). This is the case for *Acineta* sp., *Epistylis* sp., and *Zoothamnium* spp. infesting mud crabs *Scylla serrata* in Australia, where the ciliates had high prevalence in summer (Hudson and Lester, 1992, 1994). Independently from the molting stage of the host, recolonization of new hosts by ectosymbionts is a consequence of the environmental conditions prevailing during the year (Utz, 2003).

In Mexico, as in most countries where shrimp culture occurs, shrimp health issues are generally addressed with a reactive approach—i.e., problems are solved as they arise—rather than with a continual proactive approach. The reactive approach does not include collection of biological data essential to understanding the nature of diseases at a population level caused by different pathogens, including ectosymbiotic ciliates. Very few studies have been conducted in southeast Mexico on parasites and diseases affecting native and introduced shrimps (e.g., *Penaeus [Litopenaeus] vannamei*) in the Gulf of Mexico. To date, studies in Yucatan (a tropical region) have focused on detection and identification of ectosymbionts, metazoan parasites, and viruses. Vidal-Martínez et al. (2002) described the

prevalence of helminths and ectosymbionts in three wild shrimp species (*Penaeus* [*Farfantepenaeus*] *aztecus*, *P.* [*Farfantepenaeus*] *duorarum*, and *P.* [*Farfantepenaeus*] *brasiliensis*) and one cultured shrimp (*P. vannamei*). They reported the presence of *Apiosoma* sp. (6–57% prevalence), *Epistylis* sp. (2–29%), *Zoothamnium* sp. (2–65%), and the microsporidian *Agmasoma penaei* (2%) in cultured *P. vannamei* at Sisal. Chávez-Sánchez et al. (2002) studied both wild and cultivated shrimp in the Gulf of Mexico, reporting histopathological alterations resulting from metazoan parasites (*Prochristianella* spp.). No virus was reported in either publication.

Due to scarcity in published information, data are lacking on the seasonal dynamics of the infestation parameters (prevalence and infestation level) of ciliates on wild or cultured shrimps in Yucatan. In response, our study was undertaken with the assumption that, during the hottest season of the year (rainy season: May to September), infestations will increase on both wild and cultivated shrimps. Therefore, the objectives of this work are to describe the prevalence and infestation level of ciliates on wild native shrimps, *P. brasiliensis* and *P. duorarum*, and farm-cultured juvenile shrimp, *P. vannamei*, over an annual cycle and to determine whether an association existed among physicochemical factors and the infestations on cultured shrimp.

2. Materials and methods

Samples of *P. vannamei* were collected from December 2001 to November 2002 from an intensive culture farm at Sisal, Yucatan, Mexico (PECIS S.A. de C.V.) (21°10'N and 89°99'W), 50 km from CINVESTAV-Mérida. Native *P. brasiliensis* were collected from five localities: Celestún (20°45'N and 90°15'W), Sisal (21°10'N and 90°02'W), Chabihau (21°21'N and 89°06'W), Ría Lagartos (21°26'N and 88°06'W), and Yalahau (21°36'N and 88°09'W). Samples of *P. duorarum* were collected from Celestún and Sisal only (Fig. 1). Both native shrimp species were collected during the same period as the farmed shrimp. Sisal and Chabihau do not contain shrimp during the dry season; consequently, sampling there was restricted to the north-wind season.

At the farm, the juvenile shrimp remained in the ponds for 3–4 months. We examined 149–191-day-old juveniles. Thus, each monthly pond sample contained cohorts in the same age range. The stocking density of shrimp in these ponds was between 98 and 136 shrimp/m², with each pond harvested three times a year. Each month, 30 shrimp were collected randomly from 2.5-ha nursery ponds using a 2-m diameter cast net with a 2.5-cm mesh. For wild shrimp, we used a 3.8-cm mesh. Shrimps were transported alive to the laboratory and examined externally using a stereoscopic microscope to record cephalothorax length (CL) and sex (Pérez-Farfante, 1988). Cephalothorax length was taken using a Digimatic vernier with 0.05-mm accuracy. Molting stage was restricted to soft and hard, and 98% examined in this study were hard, or in the intermolting stage.

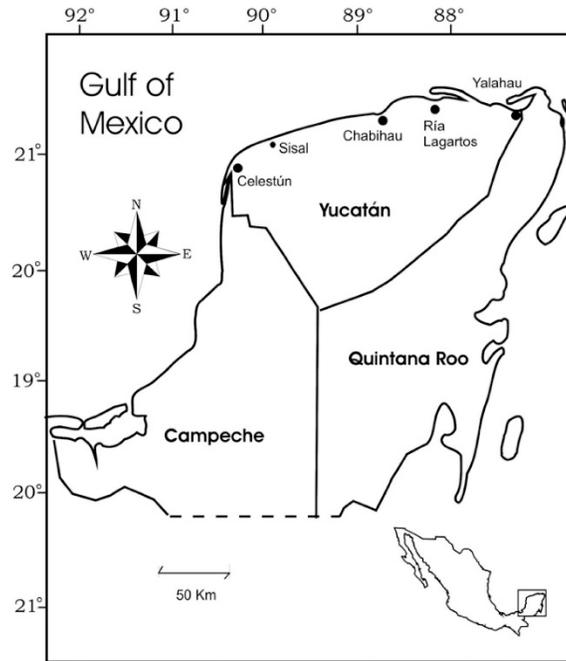


Figure 1. Sampling site locations on the Peninsula of Yucatan, Mexico.

The physicochemical data from the ponds were provided by staff of the shrimp farm. Data were collected three times a day (dawn, midday, and dusk) from the ponds. Salinity was measured with an ATAGO refractometer, dissolved oxygen (mg/l), temperature ($^{\circ}\text{C}$) with an YSI 50 multiparametric probe, and water transparency with a Sechi disk (an indirect measurement of turbidity and referred to as "turbidity"). Survival was estimated based on harvest density as compared with the original stocking density (number of organisms/ m^2) of a specific pond and expressed as a percentage (%) (Teichert-Coddington and Rodriguez, 1995). For example, if in a specific month we had a harvest density of 76 shrimp/ m^2 and the original stocking density of that pond was 98 shrimp/ m^2 , then the percentage of survival would be indicated as 77.5%.

Shrimp were dissected by separating the pleopods, periopods, exoskeleton, and gills that were placed between two $10 \times 10\text{-cm}$ glass slides with 0.70% saline solution and examined under a stereoscopic microscope. Once the colonies of ciliates were localized, we counted the number of individual zooids in 10 fields per organ or body part with a light microscope at $40\times$. The area covered by a field at $40\times$ was 0.237 mm^2 , so 10 fields per organ covered 2.37 mm^2 . Two parameters were used to describe the infestation levels of the ectosymbionts. Prevalence (P) was the number of hosts infested by a specific ectosymbiont species divided by the total number of shrimp examined in a sample (Bush et al., 1997). Intensity of infestation (II) was the total number of individual zooids of a ciliate species recorded in 50 fields (10 fields per organ) per individual shrimp. Thus, mean intensity of infestation (MII) was the number of zooids of a ciliate species in all individual shrimp in a sample divided by number of infested individual shrimp in that sample.

To describe the raw patterns of the infestation parameters P and MII, we grouped monthly samples on a bimonthly basis through the whole year sampled. This procedure was chosen because it retains the temporal trends of the infestation parameters while using a proper sample size to detect the presence of a parasite at the 5% level, in this case 57 shrimp following the recommendations of Munro et al. (1983). Since the sample size per month was 30 shrimp, grouping the data bimonthly gave us 60 shrimp, enough to fulfill the requirement described above. However, for statistical comparison of the values of infestation parameters through time, we grouped the data into three recognized seasons: “north-wind” season (October to January), “dry” season (February to May), and “rainy” season (June to September). The MII data were transformed to a base 10 logarithmic scale ($\log+1.5$) or some other suitable transformation to normalize distribution and homogenize variance. Furthermore, since there were significant differences in shrimp size among months (one-way ANOVA; $F_{1,11} = 29.56$; $P < 0.0001$), the Fulton’s condition factor ($K = W/CL^3$, where W is weight in grams and CL is the cephalothorax length in cm) was used to compensate for the effects of shrimp length and weight. This condition factor is frequently used in shrimp aquaculture (e.g., Enin, 1994). Consequently, the number of zooids of each ciliate species on each shrimp was divided by its individual K value. To determine possible significant differences within and between species among seasons, we used a two-way ANOVA ($P < 0.05$), in which prevalence of infestation was the dependent variable. We built a mean value of prevalence for each season, obtaining the average of the monthly data of this infestation parameter for each ciliate species. Each ciliate species was the first factor and the “north-wind,” “dry,” and “rainy” seasons constituted the second factor. For intensity of infestation, we produced a mean value for all the shrimp infested for each ciliate species on each season. Thus, each ciliate species was the first factor and the “north-wind,” “dry,” and “rainy” seasons constituted the second factor.

To determine possible associations between the physicochemical parameters and the intensity of infestation of ciliates on farmed shrimp, we applied backward stepwise multiple regression analysis (Pearson) ($P < 0.05$) using STATISTICA software (ver. 9.0). The independent variables were temperature ($^{\circ}\text{C}$ at 4:30 a.m.), salinity (practical salinity units, psu), dissolved oxygen (mg/l at 4:30 a.m.), survival (%), turbidity (using Secchi disk), density (number of shrimp/m²), pond number, and juvenile age (days). The dependent variable was the intensity of infestation (II) of each ciliate species.

3. Results

A total of 360 juvenile (148–191 day old) specimens of *P. vannamei* were examined, with CL ranging from 1.47 ± 0.03 cm in July to 2.21 ± 0.30 cm in February. There was no significant difference according to sex (Kruskall-Wallis test; $H_{1,300} = 2.15$; $P = 0.14$). Three species of ciliates were recognized: *A. tuberosa*, *Epistylis* sp., and *Zoothamnium* sp. on *P. vannamei*.

3.1. Differences in prevalence and intensity of infestation within ciliate species

Figure 2a shows the range in prevalence for the three ciliates during the annual cycle, December 2001 to November 2002, in farmed *P. vannamei*. The prevalence of *A. tuberosa* ranged from 3% in December/January to 35% in October/November (Fig. 2a). Prevalence was not

significantly different among seasons (Fig. 3; Tukey HSD test; $P > 0.05$). The peak of the MII for *A. tuberosa* occurred in October/November with 3 ± 7 ectosymbionts per shrimp parasitized (Fig. 2b). This peak in the rainy season was significantly higher with respect to the two other seasons (Fig. 4; Tukey HSD test; $P < 0.05$).

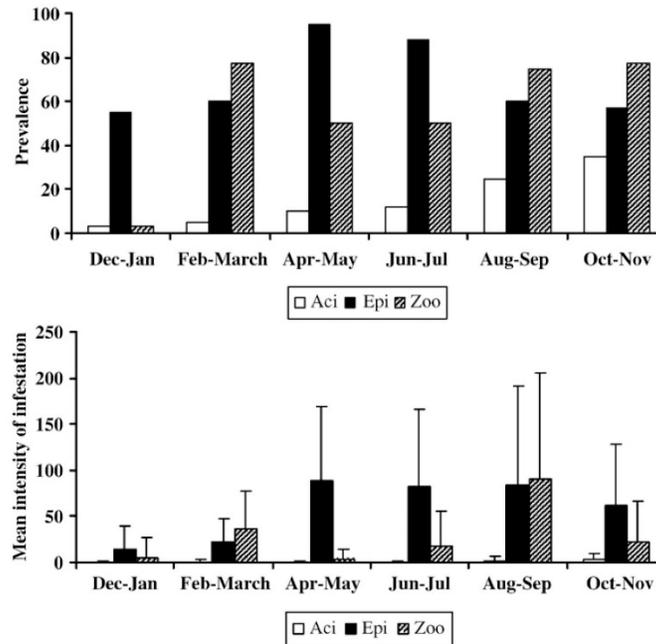
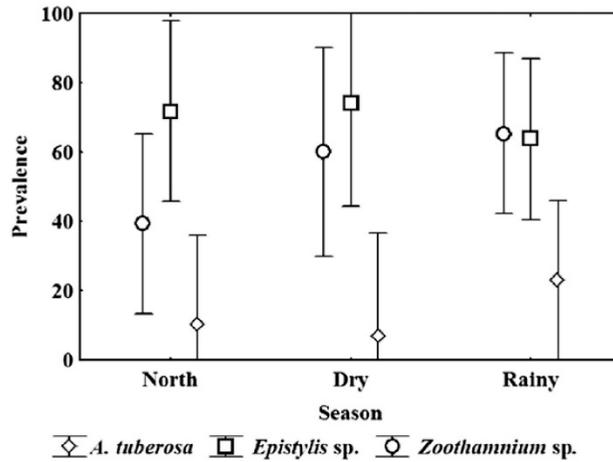


Figure 2. Bimonthly variation of the infestation parameters of ectosymbiont ciliates of cultured Pacific white shrimp *Litopenaeus vannamei* from PECIS shrimp farm, Sisal, Yucatan. (a) prevalence and (b) mean intensity of infestation \pm standard deviation. Aci = *A. tuberosa*, Epi = *Epistylis* sp. and Zoo = *Zoothamnium* sp.

The prevalence of *Epistylis* sp. ranged from 55% in December/January to 95% in April/May (Fig. 2a). There was no significant difference in prevalence for *Epistylis* sp. among seasons (Fig. 3; Tukey HSD test; $P > 0.05$). Mean intensity of infestation for *Epistylis* sp. varied considerably from 14 ± 26 zooids per shrimp infested in December/January to 83 ± 108 zooids in August/September (Fig. 2b). There were significant differences in MII for *Epistylis* sp. among all three seasons, with the highest MII occurring in the rainy season (Fig. 4, Tukey HSD test; $P < 0.05$).

The prevalence of *Zoothamnium* sp. ranged from 3% in December/January to 75% in August/September (Fig. 2a), even though there was no significant difference among seasons (Fig. 3; Tukey HSD test; $P > 0.05$). The lowest MII value for *Zoothamnium* sp. was 4 ± 22 in December/January, while the highest value was 89 ± 116 in August/September (Fig. 2b). There was no significant difference in MII for *Zoothamnium* sp. between the north-wind and dry seasons (Fig. 4; Tukey HSD test; $P > 0.05$). However, there was a significantly higher number of this ciliate during the rainy season (Fig. 4; Tukey HSD test; $P < 0.05$).

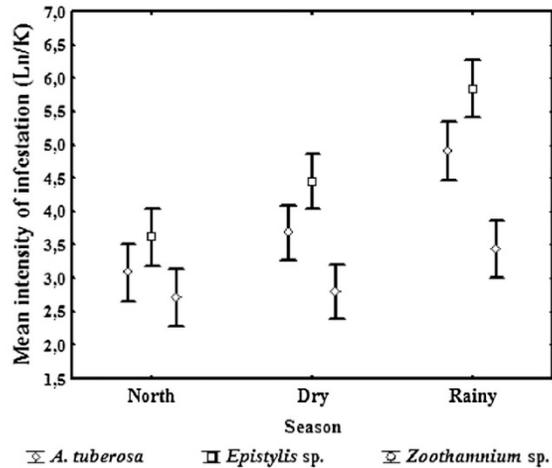


Effect	SS	df	MS	F	P
Intercept	72934.47	1	72934.47	113.34	> 0.0001
Season	717.64	2	358.82	0.56	0.58
Species	19897.35	2	9948.67	15.46	> 0.0001
Season*Species	1749.31	4	437.33	0.68	0.61
Error	17373.63	27	643.47		

Figure 3. Two-way ANOVA of the prevalence of ciliate species among seasons on the Pacific white shrimp *Litopenaeus vannamei*. North-wind season = October to January, dry season = February to May, and rainy season = June to September. Vertical bars denote 0.95 confidence intervals.

3.2. Differences in prevalence and intensity of infestation between species

Both prevalence and MII of *A. tuberosa* were significantly lower during all three seasons in comparison with those values for *Epistylis sp.* and *Zoothamnium sp.* (Figs. 3 and 4; Tukey HSD test; $P < 0.05$). There was no significant difference in prevalence for *Epistylis sp.* and *Zoothamnium sp.* among seasons (Fig. 3; Tukey HSD test; $P > 0.05$). However, the MII of *Epistylis sp.* was significantly higher than that of *Zoothamnium sp.* during the dry and rainy seasons (Fig. 4; Tukey HSD test; $P < 0.05$).



	SS	df	MS	F	P
Intercept	13210.93	1	13210.93	2828.19	< 0.0001
Season	383.57	2	191.79	41.05	< 0.0001
Species	407.39	2	203.69	43.60	< 0.0001
Season*Species	58.45	4	14.61	3.13	0.014
Error	4162.01	891	4.67		

Figure 4. Two-way ANOVA of the mean intensity of infestation of ciliate species among seasons on the Pacific white shrimp *Litopenaeus vannamei*. North-wind season = October to January, dry season = February to May, and rainy season = June to September. Vertical bars denote 0.95 confidence intervals.

3.3. The microhabitat (sites) of the ciliates on shrimp through time

Figure 5 shows the percentage of shrimp infested with each ciliate species from four different microhabitats (sites) on shrimp through time. *A. tuberosa* presented its highest value of percentage of infested shrimp for the exoskeleton (22%) in October/November (Fig. 5). The preferential microhabitats infested by *Epistylis sp.* were the pereopods and pleopods through the entire year, reaching a peak of 80 and 93% of the shrimp infested in April/May (Fig. 5). For *Zoothamnium sp.*, the preferential microhabitats were also the pereopods and pleopods, reaching values around 50% of the shrimp infested in February/March and in August/November.

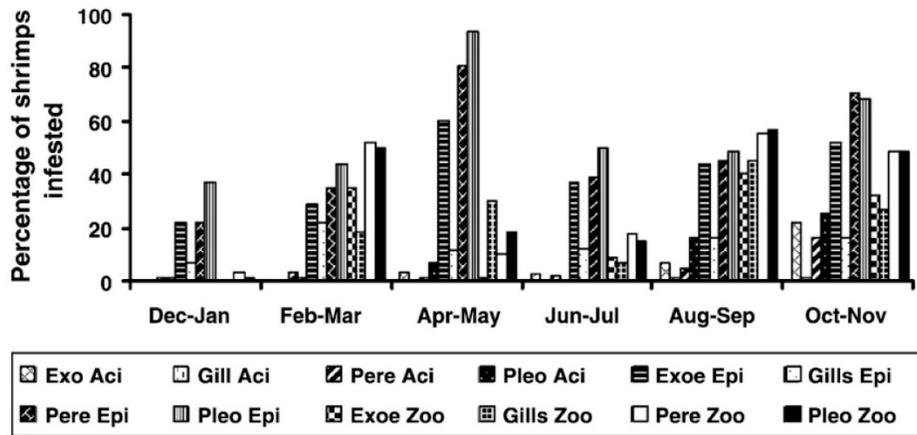


Figure 5. Bimonthly variation of the percentage of cultured shrimp *Litopenaeus vannamei* infested with ectosymbiont ciliates in different body structures. Acronyms are as follows: Exo Aci = exoskeleton with *Acineta tuberosa*, Gill Aci = gill with *A. tuberosa*, Pere Aci = pereiopod with *A. tuberosa*, Pleo Aci = pleopod with *A. tuberosa* and so on; Epi = *Epistylis* sp., Zoo = *Zoothamnium* sp.

3.4. Association between infestation parameters of ciliates and environmental variables

The backward stepwise regression for the intensity of infestation of *A. tuberosa* as a dependent variable and the physicochemical factors and shrimp biological variables as independent variables was significant and retained stocking density, survival, and temperature. Including these three variables, the model explained 26% of the variance (Table 1).

The regression analysis dealing with the intensity of infestation of *Epistylis* sp. retained three of the eight independent variables used (Table 1). These variables were survival, temperature, and turbidity, which together explained 26% of the variance. The similar backward stepwise regression analysis for *Zoothamnium* sp. showed that the only two variables retained in the model were stocking density and temperature. Including these independent variables, the model explained 11% of the variance (Table 1).

Table 1. Backward stepwise multiple regression using biological characteristics of *L. vannamei* and physicochemical parameters of the ponds as independent variables and intensity of infestation of the ciliates as dependent variables

Independent variable retained in the model	Dependent variable			
	Log (<i>Acineta tuberosa</i> intensity of infestation) + 1.5/TL			
	(n = 300; R ² = 0.26; F _{3,296} = 35.38; P < 0.0001)			
	β	SE	t(296)	P
Stocking density	0.18	0.05	3.54	< 0.001
Survival	0.37	0.05	7.10	< 0.0001
Temperature	0.18	0.05	3.39	< 0.001
	Log (<i>Epistylis</i> sp. intensity of infestation) + 1.5/TL			
	(n = 300; R ² = 0.26; F _{3,292} = 23.48; P < 0.0001)			
	β	SE	t(292)	P
Survival	0.22	0.07	3.24	< 0.001
Temperature	0.33	0.06	5.22	< 0.0001
Turbidity	0.13	0.06	2.24	< 0.001
	Log (<i>Zoothamnium</i> sp. intensity of infestation) + 1.5/TL			
	(n = 300; R ² = 0.11; F _{3,296} = 12.39; P < 0.0001)			
	β	SE	t(296)	P
Stocking density	0.16	0.06	2.91	< 0.01
Temperature	0.26	0.06	4.52	< 0.0001

The independent variables were age of juvenile (days), dissolved oxygen (mg/l), pond number, stocking density (number of organism/m²), temperature (°C), salinity (psu), survival (harvest density as compared with stocking density of a pond and expressed as percentage), and turbidity, using a Secchi disk (cm). The dependent variables were intensity of infestation of *A. tuberosa*, *Epistylis* sp., and *Zoothamnium* sp. Symbols and abbreviations are as follows: β = regression coefficients, TL = total shrimp length, R² = coefficient of determination, SE = standard error of estimate.

3.5. Data on wild shrimp from the coast of Yucatan

A total of 408 wild shrimp (*P. brasiliensis*, 288, and *P. duorarum*, 120) were examined, with CL ranging from 1.44 to 2.00 cm. Table 2 shows the prevalence and MII for the three ciliates in these two shrimp species from five coastal lagoons along the Peninsula of Yucatan. In the case of *P. brasiliensis*, for *A. tuberosa*, maximum prevalence was noted in Celestun, 43% during dry season; MII was 1 ± 2 during rainy season. For *Epistylis* sp. the corresponding values were 53% in Yalahau and 21 ± 61 in Ria Lagartos, both during the north-wind season. For *Zoothamnium* sp., also during the north wind season, the highest prevalence and MII values were found in Ria Lagartos, with 66% and 66 ± 92, respectively. For *P. duorarum*, prevalence and MII for both *Epistylis* sp. and *Zoothamnium* sp. reached their highest values during the north-wind season (Table 2).

Table 2. Prevalence (%), and mean intensity of infestation (MII) \pm standard deviation (SD) of ectosymbiont ciliates of wild penaeid shrimps *Penaeus brasiliensis* and *P. duorarum* in five localities along the coast of the Peninsula of Yucatan, sampled between December 2001 and November 2002

Locality	<i>n</i>	Season	<i>Acineta tuberosa</i>		<i>Epistylis</i> sp.		<i>Zoothamnium</i> sp.	
			%	MII \pm SD	%	MII \pm SD	%	MII \pm SD
<i>P. brasiliensis</i>								
Celestún, Yuc.	30	N	—	—	50	5 \pm 10	36	10 \pm 18
	30	D	43	0.1 \pm 0.6	30	2 \pm 4	56	2 \pm 3
	30	R	6	1 \pm 2	40	3 \pm 11	23	3 \pm 12
Chabihau, Yuc.	18	N	—	—	—	—	10	2 \pm 3
	Ria Lagartos, Yuc.	N	—	—	26	21 \pm 61	66	66 \pm 92
D		10	0.3 \pm 1	—	—	13	0.03 \pm 0.2	
R		—	—	36	21 \pm 40	16	11 \pm 20	
Yalahau, Q. Roo	N	—	—	53	4 \pm 8	53	4 \pm 5	
	D	—	—	6	0.05 \pm 0.2	16	1 \pm 2	
	R	—	—	—	—	—	—	
<i>P. duorarum</i>								
Celestún, Yuc.	N	—	—	46	8 \pm 16	40	6 \pm 14	
	D	—	—	10	0.3 \pm 0.6	20	1 \pm 1	
	R	—	—	—	—	—	—	
Sisal, Yuc.	30	N	—	—	—	—	10	0.3 \pm 1

Acronyms are as follows: *n* = number of shrimp sampled, N = north-wind season, D = dry season, R = rainy season, Q. Roo = Quintana Roo state, Yuc. = Yucatan state.

4. Discussion

Results show that the three ciliates on wild shrimp from the coast of Yucatan were able to colonize and establish in the shrimp farm in Sisal. However, our assumption that ciliate infestations would reach their highest levels for all shrimp groups in the rainy hot season held significance for farmed shrimp only. The high levels seemed to be associated with artificial fertilization cycles in the farm ponds, with a corresponding absence of natural fertilization in the wild. Thus, two differences occurred between infestations on cultured and wild shrimps (Fig. 2 and Table 2). First, ciliate infestations for wild shrimp were far less than those of cultured shrimp. Second, the highest values of the two infestation parameters in cultured shrimp occurred during rainy season in contrast to the north-wind season for wild shrimp.

Epistylis sp. and *Zoothamnium* sp. reported in the present study (Fig. 2) corresponded with the organisms recorded previously on *P. vannamei* by Vidal-Martínez et al. (2002). *A. tuberosa* on *P. vannamei* was a new geographical record for this host in Yucatan. Our results contradict the findings of other studies, stating that *Zoothamnium* sp. was the most common species on shrimp from Florida, Louisiana, and Texas in the USA. Villela et al. (1970) reported that 87.5% of the pink shrimp *P. duorarum* cultured in Florida was infested with *Zoothamnium* sp. Overstreet (1973) stated that *Zoothamnium* sp. was the most common cili-

ate on white, brown, and pink shrimp cultured in Louisiana and Texas (USA). Additionally, Fontaine (1985) reported *Lagenophrys* sp., *Epistylis* sp., and *Zoothamnium* sp. in the gills of *P. setiferus* and *P. aztecus* collected in Texas (USA). Recently, Ma and Overstreet (2006) described two new species of *Epistylis*, *E. callinectes*, and *E. clampi*, from the blue crab *Callinectes sapidus* in the Mississippi area, in the Gulf of Mexico, showing that multiple species of the same genus can occur on an individual. Peritrichs are often host-specific (Görtz, 1996), and Ma and Overstreet are investigating the taxonomy of all the peritrichs on the white and brown shrimp in Mississippi, and those species may not correspond with those in Yucatan.

We did not report data on shrimp molting stage because only six individuals exhibited a soft exoskeleton during the entire year of sampling. The possibility of overlooking soft exoskeletons at the time of examination was very low since the senior author (NALT) had several years of experience working with shrimp culture. Additionally, Chan et al. (1988) have demonstrated that the anecdyosis and proecdyosis periods for *P. vannamei*, in which the exoskeleton is hard, last at least 97% of the molting cycle. Thus, the small number of molting shrimp detected apparently did not affect the assessment of the infestation parameters (Fig. 2). In any event, the results suggest that recolonization on shrimp occurred rapidly. Thus, our results concur with those found by Bauer (2002) and Itani et al. (2002)—that molting and other shrimp self-cleaning procedures are not always effective in removing ectosymbionts. Further, evidence suggests that recolonization and building up of new infrapopulations of *Epistylis* sp. in *P. vannamei* can be rapid: *Epistylis pigmaeum* population can be initiated with only one zooid, and this can develop into several hundred attached zooids after 3 days at 20°C (Gilbert and Schroder, 2003).

4.1. Differences in prevalence and intensity of infestation within and between species

Epistylis sp. had the highest prevalence relative to the two other species and remained that way during the whole year, followed by *Zoothamnium* sp. (Fig. 2a). *A. tuberosa* consistently had the lowest prevalence values. However, MII in *Epistylis* sp., and *Zoothamnium* sp. was significantly higher during the rainy season in farmed shrimp, especially with respect to the north-wind season. Although there were differences in MII among seasons, there was always a constant hierarchical relationship among the three species: *Epistylis* sp. presented the highest values of MII, followed by *Zoothamnium* sp., and then *A. tuberosa*. Furthermore, the high MII values of *Epistylis* sp. from March to August were followed by a sudden increase of the MII of *Zoothamnium* sp. in September (Fig. 2b). Possible explanations for these patterns are (a) the association of MII with environmental variables and (b) that these ciliates occur simultaneously in their ecological microhabitats and compete for substrate or food sources. In this study, the former option seems more likely, since published evidence on the presence of interspecific interactions among these ciliates is contradictory.

4.2. The microhabitat (sites) of the ciliates of the cultured shrimp through time

Each ciliate species apparently had a preference for a specific microhabitat. Thus, *A. tuberosa* preferred the exoskeleton, while *Epistylis* sp. and *Zoothamnium* sp. were observed attached preferentially to the pleopods and periopods throughout the entire year. Potential

explanations for such microhabitat specificity are related to phylogenetic host and microhabitat specificity, enhanced for chemical cues or adequate allocation for food resources. However, a combination of all these possibilities seems to be most likely explanation for the microhabitat specificity of these ciliates. Clearly, experimental designs such as those by Utz and Coats (2005) are necessary to test the above suggestions based on field work. Similar patterns of microhabitat specificity for other ciliate species have been reported by Utz and Coats (2005) and Ma and Overstreet (2006).

4.3. Association between infestation parameters of ciliates and environmental variables

Three variables detected by the multiple regression model (Table 1) helped assess *Epistylis* sp., with temperature as the one explaining the largest amount of variance. Thus, the increase in the MII of *Epistylis* sp. appears to be directly associated with the natural increase of this variable during the rainy season. The other two variables were survival and turbidity, the last one being related to pond fertilization, a typical procedure in shrimp aquaculture prior to the introduction of PLs (Gualteros-Rodríguez, 2003). This procedure creates an artificial environment rich in nutrients, enhancing the transmission of the ciliate in each pond. Thus, the longer the PLs remain in the pond, the greater the MII. This pattern occurred mainly during the rainy season, when salinity and other pond variables may have been optimal for transmission of this ciliate.

The effect of survival on the intensity of infestation of *Epistylis* sp. appears to be related to the density of shrimp present in the ponds (Table 3). Thus, the intensive culture conditions in the farm (> 100 organisms/m) in most cases promote an increase in the probability of transmission of the ciliates. In fact, even when survival would be low in some cases (Table 3), the death of infested shrimp can enhance dispersion of the zooids in the form of telotrochs to other shrimp in the vicinity. This dispersion process has been also reported by other authors such as Threlkeld and Willey (1993) and Cook et al. (1998). Similar patterns including temperature, stocking density, and survival as significant independent variables were found for *A. tuberosa* and *Zoothamnium* sp. (Table 1). However, even when the models were statistically significant for the three ciliate species, they only explained between 11 and 26% of the variance. Thus, probably several important variables were not taken into account for this study. Some of these are how long before sampling had fertilization occurred, what live foods were available in the pond, and location and amount of nocturnal light, all factors that affect the amount of available organic matter and bacteria in the water.

Table 3. Survival data of cultured Pacific white shrimp *Penaeus vannamei* from PECIS shrimp farm, Sisal, Yucatan (data provided by the farm)

Month	Season	Pond number	Age (days)	Stocking density (Sd) (No./m ²)	Harvest density (Hd) (No./m ²)	Survival (Hd/Sd) * 100
December 01	N	3	P154	98	76	77
January 02	N	15	P148–149	118	56	47
February	D	11	P154–155	105	54	51
March	D	14	P157–158	112	74	66
April	D	40	P157–158	69	43	62
May	D	5	P168–169	121	99	82
June	R	3	P172–173	136	71	52
July	R	29	P180–181	115	74	64
August	R	1	P183	114	98	86
September	R	19	P188	119	74	62
October	N	8	ND	ND	ND	ND
November	N	21	P191	118	79	67

Acronyms are as follows: n = number of shrimp sampled, N = north wind season, D = dry season, R = rainy season, P154 = postlarvae of 154 days old, No./m² = number of shrimp per square meter.

Thus, our results agree with those of Hudson and Lester (1992) and Utz (2003), which stated that shrimp are more easily infested in the rainy season (summer), although infestation persists year-round. Also, Brown et al. (1993) reported that cultured crab *Orconectes rusticus* is much more easily infested with *Epistylis* sp. in the summer, although this ciliate occurred all year-round. In fact, the increase in MII during the rainy season for all three ciliate species was directly associated to temperature, one of the relevant factors affecting abundance of ciliates, as shown by other authors (Couch, 1983; Sleigh and Lynn, 1985; Hudson and Lester, 1992; Jayasree et al., 2001; Utz, 2003) (Fig. 3). Others suggest that the most relevant factors for protozoa growth are water quality, oxygen, light, pH, and salinity. Moreover, Hudson and Lester (1992) reported in cultured *Marsupenaeus japonicus* a positive correlation among biochemical oxygen demand (BOD), water turbidity, and the mean intensity (MI) of *Zoothamnium* sp. As water quality declined, MI of this species increased. Utz (2003) did not measure pH, bacterial concentration, or dissolved oxygen concentration, but she agreed that these environmental conditions were also involved in the entire process of telotroch formation in ciliate species, infecting copepods.

4.4. Comparison between wild and cultured shrimp in terms of their ciliates

The values of the infestation parameters for *A. tuberosa* were very low and erratic on wild shrimp, and this fact does not allow any further interpretation. In contrast, the results showed that *Zoothamnium* sp. had the highest MII values on wild shrimp, followed by *Epistylis* sp., during the north-wind season (Fig. 2). This pattern might be explained by the higher shallow coastal water mix-up in the Yucatan Peninsula due to the increase in wind speed during this season, which in turn increases the transport of the swarming stages to a new host. Our results are similar in terms of ciliate composition to those of other studies,

stating that *Zoothamnium* sp. was the most common on wild shrimp species in Florida, Louisiana, and Texas (Villela et al., 1970; Overstreet, 1973; Fontaine, 1985).

Unlike on the wild shrimp dominated by *Zoothamnium* sp., cultivated shrimp had heavier infestations of *Epistylis* sp., in combination with *Zoothamnium* sp. during the rainy season. The heavier infestations on cultured shrimp compared with wild ones resulted in part from the relatively high stocking densities, making shrimp-to-shrimp transmission easier; this may also be attributed to pond fertilization procedures.

5. Conclusions

The factors affecting the increase of ectosymbiont protozoans in cultured shrimp under tropical conditions in Yucatan Peninsula appear to be related to (1) the direct effect of temperature increase during the rainy season, (2) the eutrophic condition of the ponds, and (3) the infestation parameters of the ciliates in native shrimps in contrast to farmed shrimp being more abundant during the north-wind season. An important environmental implication of this last result is whether fertilization procedures of the farm would affect the temporal dynamics of wild shrimp in the surroundings of the shrimp farm. Finally, it is not clear whether infestation levels of ciliates reached epizootic levels during the time of study because only significant associations between shrimp survival (52–86% for all year) and MII values of ciliates were for *Epistylis* sp. ($r_s = 0.30$, $n = 300$; $P < 0.01$). However, it is important to stress that the MII of the cultured shrimp reported in the present study (Fig. 2) was far higher than those reported by Overstreet (1973) in Louisiana for *Zoothamnium* sp. (1.5–4.0 for *P. aztecus* and 2.0–2.9 for *P. setiferus*) or those of Hudson and Lester (1992) for *Zoothamnium* sp. on *M. japonicus* (18.8–20.6) in Australia. Thus, the questions posed by Overstreet (1973) about the stress on shrimp produced by ciliates still need to be answered.

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