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Persistence of the Nematode, *Oswaldocruzia pipiens* (Molineidae), in the Pacific Treefrog, *Hyla regilla* (Hylidae), from California

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Abstract.—Multi-year samples of *Hyla regilla* from three California counties (Los Angeles, Orange, Riverside) as well as single-year samples from three additional California counties (Humboldt, Imperial, Santa Clara) were examined for helminths. Gravid individuals of one species of cestode, *Distoichometra bufonis* and two species of nematodes, *Oswaldocruzia pipiens* and *Rhabdias ranae* were found. Metacercariae of two species of trematodes, *Alaria* sp. and *Clinostomum* sp. and larvae of one nematode species, *Physaloptera* sp. were also found. Of the helminths found in the multi-year samples, only *O. pipiens* was persistent; however, it was not stable.

Although many amphibians species have been recorded as hosts for helminth parasites (Prudhoe and Bray 1982; Baker 1987), the majority of this work has concentrated on faunistic surveys typically based upon collections taken during a single season. A general conclusion drawn from these studies is that helminth communities of anurans are depauperate and isolationist in character (Barton 1997). However, two measures of helminth communities developed by Meffe and Minckley (1987) are not addressed in these single season collections: persistence, a measure of presence or absence of a species over time, and stability, a qualitative measure based upon the relative constancy of species abundances over time. These measures can only be addressed with long-term studies; unfortunately, there are few published accounts of long-term variation (biennial or greater) in helminth infracommunities of anurans. In Poland, Grossman and Sandner (1953), in Russia, Markov and Rogoza (1949, 1953), and in England, Lees (1962) published data on seasonal variation in the prevalence of helminths of *Rana temporaria.* Also from Poland, Kuc and Sulgostowska (1988) published data collected from *Rana ridibunda* over a period of four years.

The Pacific treefrog, *Hyla regilla* (Baird and Girard 1852), is a small anuran that occurs from British Columbia to the tip of Baja California Sur, México and east to western Montana and eastern Nevada (Stebbins 1985). Pacific treefrogs prefer low plant growth near water and frequent a variety of habitats from sea level to elevations of 3540 m including grassland, chaparral, woodland, forest, desert oasis and farmland (Stebbins 1985). There are seven reports of helminths from northern populations of this frog (Millzner 1924; Douglas 1958; Lehmann 1965; Macy 1960; Efford and Tsumura 1969; Brooks 1976, Johnson et al. 1999) and one report from southern California (Koller and Gaudin 1977).

Multi-year collections of *H. regilla* from three California counties (Los Angeles, Orange, Riverside) as well as single season collections from three California

Table 1. Number (N), SVL, and collection dates of *Hyla regilla* from California counties with number infected by and prevalence (%) of anuran parasites.

County	N	SVL ± SD range (mm)	Collection dates	*Distoichometra bufonis*	*Oswaldocruzia pipiens*	*Rhabdias ranae*
Humboldt	3	39 ± 1.5, 38–41	1997, Aug	—	—	—
Santa Clara	18	34 ± 4.1, 21–40	1998, Jan–May	2 (11)	3 (17)	—
Imperial	2	31 ± 1.4, 30–32	1959, Mar	—	2 (100)	—
Los Angeles	14	30 ± 4.3, 19–35	1943, Mar	—	3 (21)	—
	2	30 ± 0, 30	1944, Mar	—	—	—
	1	25	1963, Mar	—	—	—
	17	30 ± 3.7, 25–36	1971, Jan–Apr	—	7 (41)	—
	2	34 ± 5.0, 30–37	1972, Apr	—	2 (100)	—
Orange	12	35 ± 5.0, 30–48	1957, Feb–Mar	—	—	—
	10	33 ± 4.8, 27–43	1958, Feb	—	—	—
	5	33 ± 7.2, 24–41	1961, Feb	—	1 (20)	—
	4	37 ± 5.0, 30–41	1962, Mar	—	—	—
	8	33 ± 4.4, 29–40	1963, Feb	—	—	—
	2	32 ± 1.4, 31–33	1964, Feb	—	—	—
	2	34 ± 5.7, 30–38	1965, May–Jun	—	—	—
	8	36 ± 2.6, 32–39	1966, Mar	—	1 (13)	—
	2	34 ± 5.0, 31–38	1967, Mar–May	—	1 (50)	1 (50)
	8	29 ± 3.3, 24–35	1972, Feb	—	1 (13)	—
Riverside	1	35	1965, June	—	1 (100)	—
	1	24	1967, May	—	—	—
	3	26 ± 5.0, 21–31	1969, July	—	2 (67)	1 (33)
	1	18	1972, Aug	—	—	—

counties (Humboldt, Imperial, Santa Clara) were examined for helminths. The purpose of this paper is to evaluate persistence and stability of helminths harbored by *H. regilla* from California.

Materials and Methods

One hundred twenty-eight *H. regilla* (mean ± 1 SD snout-vent length [SVL] = 32 mm ± 5 SD, range 18–48 mm) from 6 counties in California were examined (collection data by year is given in Table 1). There were 47 female (SVL = 33 mm ± 6 SD, range = 18–43 mm) and 81 male (SVL = 32 mm ± 4 SD, range = 19–48 mm) frogs in the total sample. All frogs had been fixed in 10% formalin and preserved in 50% ethanol. The body cavity of each frog was opened by a longitudinal incision and the gastrointestinal tract was removed and slit longitudinally. The esophagus, stomach, small intestine, large intestine, lung, urinary bladder, coelom and liver were searched with a dissecting microscope for helminths. Helminths were placed in a drop of undiluted glycerol on a glass slide and allowed to clear for study with a compound microscope. Nematodes were identified from these slides. Cestodes and trematodes were stained in hematoxylin and mounted in balsam for identification. Where applicable, difference in prevalence between male and female frogs was examined by the chi-square test (χ^2), numbers of helminths per male and female hosts were evaluated by the Kruskal Wallis test, and correlation analysis (r) was used to relate intensity of infection to age (as estimated by SVL).

Results

Gravid individuals of one species of cestode, *Distoichometra bufonis* Dickey 1921; and two species of nematodes, Oswaldocruzia pipiens Walton 1929, and *Rhabdias ranae* Walton 1929 were found. Metacercariae of two species of trematodes, *Alaria* sp. and *Clinostomum* sp., and larvae of one species of nematode, *Physaloptera* sp., were also found. Only *Oswaldocruzia pipiens* was found to be persistent (Table 1). Number, prevalence (number of infected hosts divided by number of hosts examined), and abundance (number of individuals of a parasite species divided by the number of hosts examined) for each helminth species by locality are given in Table 2. Voucher specimens were placed in vials of 70% ethanol and deposited in the U.S. National Parasite Collection (USNPC): *Alaria* sp. (mesocercaria), 88611; *Clinostomum* sp. (metacercaria), 88612; *Distoichometra bufonis,* 88614; *Oswaldocruzia pipiens,* 88615; *Rhabdias ranae,* 88609; *Physaloptera* sp. (larvae), 88610.

Thirty four frogs (27%) were found to harbor 157 helminths ($\bar{x}$ = 5 ± 5 SD per infected frog). Of these, 29 were larvae of helminth species not capable of completing their life cycles in anurans. Mean helminth species richness for infected frogs was 1.0 ± 0.2 SD, range = 1–2 species. Eighteen of 81 male frogs (22%) harbored 59 helminths; 16 of 47 female frogs (34%) harbored 98 helminths. Prevalence of helminth infection (χ^2 = 2.13, 1 df, $P > 0.05$) as well as number of helminths harbored per host (Kruskal Wallis test = 2.6, 1 df, $P > 0.05$) was not significantly different between male and female frogs. Thirty three frogs (26%) harbored a single species of helminth; 1 frog (1%) harbored 2 species. The smallest infected frog was 26 mm SVL. There was no correlation between number of helminths and SVL (r = 0.002, $p > 0.05$). Inspection of Table 2 will show that in no two localities did *H. regilla* harbor the same combination of helminth species and that the helminths preferred specific host locations. This is the first report of metacercariae of *Clinostomum* sp. and larvae of *Physaloptera* sp. in *Hyla regilla.*

Discussion

Barton (1997) described anuran helminth communities as depauperate and isolationist. Depauperate is used in the sense that few helminth species are found in a host species from a particular locality. Table 2 supports the use of depauperate to describe helminth communities harbored by *H. regilla.* Isolationist is used in the sense that helminth density is low, helminth species are unaffected by one another and some niches are absent. Table 2 also supports the use of isolationist to describe helminth communities of *H. regilla.* In addition, none of the helminth species found in this study was unique to *H. regilla.* Of the six helminth species present, *Alaria* sp., *Clinostomum* sp., and *Physaloptera* sp. are incapable of completing their life cycles in anurans. Species of *Alaria* utilize mammals as final hosts and species of *Clinostomum* utilize birds as final hosts; metacercariae of both are commonly found in frogs (Smyth and Smyth 1980). Larvae, but no adults, of *Physaloptera* sp. have been reported from anurans (Anderson 1992). *Distoichometra bufonis* and *Rhabdias ranae* are common parasites of anurans (Brooks 1976; Baker 1987). *Oswaldocruzia pipiens* has been reported from frogs, toads, salamanders, lizards and turtles (Baker 1987). The term generalist should

Table 2. Number (N), prevalence as % (P), abundance (A) and host site of helminths from *Hyla regilla* by California county (north to south).

Helminth	Humboldt			Santa Clara			Los Angeles			Orange			Riverside			Imperial		
Host site	N	P	A	N	P	A	N	P	A	N	P	A	N	P	A	N	P	A
Trematoda																		
Alaria sp. (metacercaria)	—	—	—	3	5	0.2	—	—	—	—	—	—	—	—	—	—	—	—
cysts in skeletal muscle																		
Clinostomum sp. (metacercaria)	2	33	0.7	13	5	0.7	—	—	—	—	—	—	—	—	—	—	—	—
cysts in mesentery																		
Cestoda																		
Distoichometra bufonis	—	—	—	11	11	0.6	—	—	—	—	—	—	—	—	—	—	—	—
small intestine																		
Nematoda																		
Oswaldocruzia pipiens	—	—	—	3	17	0.2	72	33	2.0	16	6	0.3	15	50	2.5	5	100	2.5
small intestine																		
Physaloptera sp. (larvae)	—	—	—	—	—	—	—	—	—	3	3	0.1	—	—	—	—	—	—
stomach																		
Rhabdias ranae	—	—	—	—	—	—	—	—	—	10	5	0.2	1	17	0.2	—	—	—
lungs																		

Table 3. Reports of *Distoichometra bufonis, Oswaldocruzia pipiens* and *Rhabdias ranae* from California by County.

Helminth Host	County	Prevalence (%)	Reference
Distoichometra bufonis			
Bufo boreas	Los Angeles	19/255 (7)	Koller and Gaudin 1977
	Los Angeles	4/69 (6)	Goldberg et al. 1999
Hyla regilla	Los Angeles	2/232 (1)	Koller and Gaudin 1977
	Santa Clara	2/18 (11)	this study
Oswaldocruzia pipiens			
Bufo boreas	Butte	not stated	Ingles 1936
	Kern	not stated	Ingles 1936
	Los Angeles	119/255 (47)	Koller and Gaudin 1977
	Los Angeles	10/30 (33)	Goldberg et al. 1999
	Riverside	1/11 (9)	Goldberg et al. 1999
	San Diego	not stated	Ingles 1936
Hyla regilla	Imperial	2/2 (100)	this study
	Los Angeles	161/232 (69)	Koller and Gaudin 1977
	Los Angeles	12/36 (33)	this study
	Orange	4/61 (7)	this study
	Riverside	3/6 (50)	this study
	Santa Clara	3/18 (17)	this study
Rana aurora	Butte	not stated	Ingles 1936
	Kern	not stated	Ingles 1936
	San Diego	not stated	Ingles 1936
Aneides lugubris	not stated	21/31 (68)	Goldberg et al. 1998
Elgaria multicarinata	Los Angeles	2/96 (2)	Goldberg and Bursey 1990
Rhabdias ranae			
Hyla regilla	Los Angeles	28/232 (12)	Koller and Gaudin 1977
	Orange	1/61 (2)	this study
	Riverside	1/6 (17)	this study
Rana boylii	not stated	not stated	Ingles 1936
	Marin/Sonoma	2/11 (18)	Lehmann 1965

also be used as a descriptor of the helminth communities of *H. regilla* (Table 2). Thus, in each county studied, *H. regilla* harbors a depauperate, isolationist community composed of generalist helminths.

More importantly, data from Los Angeles and Orange Counties (Table 1) suggest that none of these species of helminths maintains a stable population in *H. regilla* and that only *O. pipiens* is persistent, present some years with widely differing prevalences and absent other years. Several questions arise, namely, is absence of a helminth species an artifact of sample size, is *H. regilla* an atypical host, and do stable populations of these helminths exist in these localities? The first question cannot be answered by the data examined; it could only be determined by a long-term study utilizing a large number of hosts. Others have reported these helminths from *H. regilla* (Table 3), thus *H. regilla* is a suitable host. The life cycle of *D. bufonis* is unknown but Joyeux (1927) regards the life history of nematotaeniid cestodes to be direct, that is, without an intermediate host; infection of a new host occurs through ingestion of cestode eggs. Both *O. pipiens* and *R. ranae* have direct life cycles and infection occurs by integumental penetration (Anderson 1992). These three helminths are generalists in that they are capable of infecting a number of hosts (Brooks 1976; Baker 1987). Table 3 lists California records of these three species; however, long-term studies are not available to test stability in these hosts. The advantage to generalist helminths is that a particular host is unimportant; infection in a particular host may fluctuate (Table 3), but the helminth population maintains an overall density.

The conclusion that we draw is that a generalist helminth population may vary drastically within a particular host over time, but within its population of hosts, the helminth species is persistent and most likely stable.

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