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Source: Journal of Parasitology, 96(3):482-490.
Published By: American Society of Parasitologists
DOI: http://dx.doi.org/10.1645/GE-2188.1
URL: http://www.bioone.org/doi/full/10.1645/GE-2188.1

J. Parasitol., 96(3), 2010, pp. 482–490

ECOLOGY OF THE BRAIN TREMATODE *EUHAPLORCHIS CALIFORNIENSIS* AND ITS HOST, THE CALIFORNIA KILLIFISH (*FUNDULUS PARVIPINNIS*)

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ABSTRACT: We describe the distribution and abundance of the brain-encysting trematode *Euhaplorchis californiensis* and its second intermediate host, the California killifish (*Fundulus parvipinnis*), in 3 estuaries in southern California and Baja California. We quantified the density of fish and metacercariae at 13–14 sites per estuary and dissected 375 killifish. Density (numbers and biomass) was examined at 3 spatial scales, i.e., small replicate sites, habitats, and entire estuaries. At those same scales, factors that might influence metacercaria prevalence, abundance, and aggregation in host individuals and populations were also examined. Metacercaria prevalence was 94–100% among the estuaries. Most fish were infected with 100s to 1,000s of *E. californiensis* metacercariae, with mean abundance generally increasing with host size. Although body condition of fish did not vary among sites or estuaries, the abundance of metacercariae varied significantly among sites, habitats, estuaries, and substantially with host size and gender. Metacercariae were modestly aggregated in killifish ($k > 1$), with aggregation decreasing in larger hosts. Across the 3 estuaries, the total populations of killifish ranged from 9,000–12,000 individuals/ha and from 7–43 kg/ha. The component populations of *E. californiensis* metacercariae ranged from 78–200 million individuals/ha and from 0.1–0.3 kg/ha. Biomass of *E. californiensis* metacercariae constituted 0.5–1.7% of the killifish biomass in the estuaries. Our findings, in conjunction with previously documented effects of *E. californiensis*, suggest a strong influence of this parasite on the size, distribution, biomass, and abundance of its killifish host.

One of the most common trematodes in southern California and Baja California estuaries (Martin, 1955; Hechinger et al., 2007), *Euhaplorchis californiensis* (Heterophyidae) spends a critical part of its life parasitizing the brain of the California killifish (*Fundulus parvipinnis*), one of the most common fishes in these estuaries (Allen et al., 2006; Hechinger et al., 2007). *Euhaplorchis californiensis* employs 3 hosts in its life cycle, i.e., horn snails (*Cerithidea californica*), California killifish, and several species of fish-eating birds (Martin, 1950). The cercariae swim from their first intermediate host snail, penetrate the skin of killifish, and migrate to the brain as metacercariae, presumably following blood vessels or nerve tracts (McNeff, 1978; Hendrickson, 1979; Haas et al., 2007). Once inside the braincase, metacercariae encyst in the meningeal layer and on the brain surface. Infected killifish display 4 times as many conspicuous swimming behaviors as uninfected ones, rendering them 10–30 times more likely to be eaten by birds, the parasite's final host (Lafferty and Morris, 1996). Infected fish exhibit parasite-dependent alterations in serotonin and dopamine metabolism, which could underlie some of the odd swimming behaviors (Shaw et al., 2009). Aside from the altered neurotransmitter activity and the parasite's increased trophic transmission (Lafferty, 1999), infected fish remain healthy overall, with body-condition indices comparable to those of uninfected fish (Shaw, 2007).

California killifish are key components of estuarine fish communities (Kwak and Zedler, 1997; West and Zedler, 2000; Madon et al., 2001; Allen et al., 2006), ranging from Morro Bay in central California down to Bahia Almejas in southern Baja California (Miller and Lea, 1972). Killifish encounter *E. californiensis* throughout this geographic range. Uninfected killifish populations occur only in locations where horn snails are absent, i.e., usually in small sloughs and coastal lagoons that are periodically closed to tidal influx. However, most of the mid-sized and large estuaries throughout the killifish's range contain both horn snails *C. californica* (Macdonald, 1969) and *E. californiensis* (R. F. Hechinger, pers. obs.). Therefore, we hypothesize that a large proportion of all killifish are likely infected with *E. californiensis*.

Here, we assess the ecology of California killifish and *E. californiensis* in 3 estuaries with horn snail populations in southern California and Baja California. Given the recent focus on examining parasites and parasitism at ecosystem scales (e.g., Kuris et al. 2008), we provide information on the spatial density (numbers and biomass) of both hosts and parasites. While host density is a familiar term, parasite density (in terms of parasites per unit area of space) is estimated rather infrequently (for examples, see Zander, 2005; Kuris et al., 2008). Our study addresses questions concerning host and parasite density at 2 scales. First, we ask what factors contribute to patterns observed at the small scale of our replicate sampling sites, which occur in various habitats. Then, we explore whether the site-level patterns translate to differences in host and parasite density for populations within the entire estuary.

We additionally examine factors known to influence parasitism in individual hosts, e.g., how habitat and host size influence infection prevalence and abundance. As with the analyses of host and parasite densities, we scale up our findings concerning parasitism in individuals to reflect the levels of parasitism characterizing entire killifish populations in the various habitats and estuaries. We also investigate the extent of aggregation among *E. californiensis* metacercariae in killifish. Parasites are commonly aggregated among hosts, which presumably results from the additive effects of random events during transmission (Shaw and Dobson, 1995). However, intensity-dependent mortality can reduce aggregation by removing the most heavily infected individuals that comprise the tail end of the distribution. Because risk of predation on killifish increases with intensity of *E. californiensis* (Lafferty and Morris 1996), we expect that aggregation may change as prevalence increases with age class. This work was conducted as part of a larger-scale study assessing the role of parasites in estuarine ecosystems (i.e., Kuris et al., 2008).

MATERIALS AND METHODS

Field collection and dissection

We sampled killifish from multiple sites within 3 tidal estuaries: Estero de Punta Banda (EPB) (31°46′30″N, 116°36′42″W, 706.6 ha, October

Received 3 June 2009; revised 22 December 2009; accepted 16 February 2010.

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DOI: 10.1645/GE-2188.1

2002), Carpinteria Salt Marsh (CSM) (34°24′06″N, 119°32′13″W, 60.9 ha, July 2003), and Bahia Falsa in Bahia San Quintín (BSQ) (30°30′95″N, 116°01′49″W, 144.3 ha, July 2004). We used ArcGIS software (ESRI, Redlands, California) to randomly select sites based on satellite images of each estuary, stratified by habitat type, i.e., channels, mudflats, and pans (non-vegetated, shallow depressions within vegetated marsh) (details in Kuris et al., 2008). California killifish can also use a fourth habitat, vegetated marsh, which we chose not to sample for logistical reasons. Fish gain access to marsh habitat only during high spring tides (West and Zedler, 2000). To ensure that we examined an entire killifish population, we sampled during mid-tide levels, when marsh habitat was dry.

To estimate killifish density at each site, we used 2-pole seines (7.6 m × 1.8 m, 4.8-mm mesh) to sweep a prescribed area demarcated by 2 blocking nets (10.7 m × 1.8 m, 4.8-mm mesh) (PERL, 1990; Steele et al., 2006). Sampling area shape was determined by site topography and vegetation, but generally consisted of a rectangle (channels), triangle (mudflats), or an ellipse (pans), as delineated by the blocking nets, an elevated shoreline, when present, or both. We seined 4 times within the blocking seines, alternating direction after each haul. After this, we pulled in both blocking nets to form a fifth haul. We pooled killifish from all hauls into plastic tubs and counted the total number of individuals.

We randomly sampled killifish size–frequency distributions by haphazardly sweeping hand-nets (batch netting) through the fish captured in our hauls. The first 50 individuals were measured for total length (TL), then sorted into 10-mm size classes, and the remaining fish were counted. At each site, 10 of the measured individuals were selected for parasitological examination (sample size varied at some sites). One fish from each 10-mm size class was isolated to represent the size range of the catch. Then, we targeted additional large fish for dissection; it was expected that they would harbor more parasites and more diverse parasite assemblages. To ensure greater sample sizes for these larger size classes, we weighted the number of individuals selected for necropsy by size class cubed. As described below, parasite counts were subsequently weighted according to the natural abundance of the size class of the dissected host, when appropriate.

Fish were kept on ice for dissection within 24 hr, or frozen at –20 C for later examination. All individuals were assessed for standard length (SL), total length (TL), weight, and sex. We were unable to sex immature fish (mainly those <30 mm TL) due to the absence of distinguishable gonads. Dissections for *E. californiensis* consisted of 3 cuts on the top of the head, i.e., a transverse brainstem cut and a sagittal cut over each eye. This created a 3-sided flap that could be lifted forward, exposing the dorsal brain surface. Fine forceps were used to remove the entire brain, while any metacercariae remaining in the braincase were removed with a pipette and a small amount of seawater. The brain and loose metacercariae were pressed between glass slides for parasite quantification using a stereomicroscope. All research procedures were conducted in accordance with policies of the UCSB Institutional Animal Care and Use Committee.

Killifish condition, count, and biomass density (site level)

Mean body condition (K, [(wet body mass/total length^3) × 10^5]) was calculated for the dissected fish and assessed to determine whether the average killifish condition at a sample site varied across estuaries, habitats, or with size and sex. A generalized linear model (GzLM) was used due to difficulties in meeting normality and variance homogeneity required for a general linear model (GLM). All GzLMs, including those presented below, were run in JMP (SAS Institute, Inc., Cary, North Carolina) using a Poisson error distribution, a log-link function, and an overdispersion parameter test (Myers et al., 2002). If overdispersion was not significant ($P > 0.05$), we re-ran the model without the overdispersion parameter. Main effects in the GzLM for body condition included estuary, habitat (nested within estuary), TL, sex, and all first-order interactions. For this, and for all other, models, non-significant interactions ($P > 0.05$) were sequentially removed to increase power.

We also examined whether host-count density or biomass density at sample sites varied across estuaries or habitats. Host-count density for each site (no. fish/m^2) was calculated by dividing the total number of fish caught by the area seined. We estimated host biomass (wet weight, g) by generating linear regression length–weight relationships (based on TL, mm) from dissected fish at each estuary (CSM, $y = 0.00002x^{2.918}$, n = 98, $R^2 = 0.97$; EPB, $y = 0.00002x^{2.921}$, n = 63, $R^2 = 0.80$; BSQ, $y = 0.000007x^{3.187}$, N = 116, $R^2 = 0.97$, where x = weight and y = TL). Average host biomass density at each site (g/m^2) was calculated by applying these formulas to the mid-value of each size class, multiplying by the total number of fish per size class, and dividing by the area seined. We assessed host-count and biomass densities in separate GzLMs. Each model contained estuary and habitat (nested within estuary) as factors.

Parasite count and biomass density (site level)

Parallel to the hosts, we examined whether parasite count or biomass density at sample sites varied across estuaries or habitats. Parasite-count density (no. metacercariae/m^2) was calculated for each site by taking the total projected amount of metacercariae occurring at a site and dividing by the area seined (see below for calculations to project parasite abundance in the wild fish populations). Calculating parasite biomass density (parasite weight/m^2) for each site first required an estimation of the mass of an individual *E. californiensis* metacercaria. We measured the volume of 25 metacercariae by equating them to an ellipse ([vol=$(\pi)(l)(w^{2/6})$]), where l = length, w = width) and multiplied the average value (0.003 mm^3) by a conservative tissue density of 1.1 g/ml (Peters, 1983). Parasite biomass densities per site were then calculated by multiplying individual metacercariae mass by the total number of metacercariae at a site and dividing by the area seined.

Habitat- and estuary-level analyses of host and parasite densities

We extrapolated site-level host- and parasite-count, and biomass densities, to the entire populations occurring in the different habitats and estuaries. Averaging across sites within a habitat yielded habitat-level density. Multiplying this density by the total area of the habitat gave habitat-level abundance. Summing these values across habitat types gave the total population size for an estuary. We calculated mean densities for the host and parasite populations of each estuary by dividing total population numbers by the total aquatic area, i.e., excluding vegetated marsh. Total aquatic habitat area (channels, mudflats, and pans) was 13.7 ha at CSM, 352.4 ha at EPB, and 67.1 ha at BSQ. To facilitate comparison of habitat- and estuary-level density estimates, 95% confidence limits were calculated (Thompson, 2002).

Factors influencing parasite prevalence, abundance, and aggregation in individual hosts

We first examined what factors influenced levels of parasitism in individual fishes. We analyzed how estuary, habitat, site, size, and gender influenced parasite prevalence and abundance. We used logistic regression to model infection probability (prevalence). For abundance, GzLMs were used (as described above). Model factors included estuary, habitat (nested within estuary), site (nested within habitat, estuary), TL, and an estuary*TL interaction. Differences between males and females were separately assessed on 162 of 375 individuals (the subset of data for which gender information existed) by simply adding gender to the GzLMs. Non-significant interactions of gender with size and estuary were removed, as described above for other interactions.

We quantified *E. californiensis* aggregation in individual fish using the index k, an inverse measure of aggregation. Specifically, we calculated the corrected moment estimate of k, ($k = [m^2-(s^2/N)/(s^2-m)]$, where m = sample mean, s^2 = sample variance, and n = sample size), which partially corrected for sample size (Wilson et al., 2002). Small, positive values of k (<1.0) indicate aggregation. First, aggregation was examined across all individual hosts for each estuary. The full GzLM included k as the response variable, and the predictors of estuary, habitat (nested within estuary), and all first-order interactions. Then, we evaluated aggregation differences by host size, by adding size category as a factor to the GzLM. Limited sample sizes prevented evaluation of each size class, so 3 size categories (fish 0–29 mm TL, 30–59 mm, and 60–120 mm) were examined per estuary. Estero de Punta Banda had only 5 individuals sized 0–29 mm, which we combined with the 30–59-mm group for the analysis.

Factors influencing parasite abundance in killifish populations

We asked whether parasite abundance in fish populations varied across habitats and estuaries. To characterize parasitism in the fish populations, we ensured our calculations reflected the natural variation in size–frequency distribution, count density, and habitat area. The predictive models were applied from our logistic regression and GzLM analyses of

TABLE I. Generalized linear model (GzLM) statistics used to assess differences in count and biomass densities, for killifish and *Euhaplorchis californiensis*, and host condition. GzLM used a log-link function, Poisson error distribution and overdispersion parameter. Habitat (estuary) denotes habitat nested within estuary. Site (estuary, habitat) denotes site nested within habitat and estuary. TL = total length (mm). χ^2 statistic calculated using the likelihood ratio. R^2_L, analogous to R^2, is calculated from the negative log-likelihood as ([reduced-full]/reduced) (Menard, 2000). Habitat areas were not factored into models; therefore, estuary as a factor does not reflect differential habitat areas. Sampling date for each estuary: Carpinteria Salt Marsh, July 2003, Estero de Punta Banda, October 2002, and Bahia San Quintín, July 2004.

Main effect	df	χ^2	P
Killifish count density (no./m^2)			
Full model: df = 8, χ^2 = 7.3, P = 0.5, R^2_L = 0.1, overdispersion = 3.5 ($P < 0.0001$)			
Estuary	2	1.13	0.57
Habitat (estuary)	6	5.67	0.46
Killifish biomass density (kg/m^2)			
Full model: df = 8, χ^2 = 18.9, P = 0.02, R^2_L = 0.3, overdispersion = 6.0 ($P < 0.0001$)			
Estuary	2	2.36	0.30
Habitat (estuary)	6	10.95	0.09
Killifish body condition			
Full model: df = 10, χ^2 = 1.1, P = 1.0, R^2_L = 0.003, no overdispersion ($P > 0.05$)			
Estuary	2	0.40	0.82
Habitat (estuary)	6	0.16	1.00
Sex	1	0.08	0.77
Total length	1	0.17	0.88
E. californiensis count density (no./m^2)			
Full model: df = 8, χ^2 = 10.1, P = 0.3, R^2_L = 0.2, overdispersion = 58385.9 ($P < 0.0001$)			
Estuary	2	4.07	0.13
Habitat (estuary)	6	2.06	0.35
E. californiensis biomass density (kg/m^2)			
Full model: df = 8, χ^2 = 0.8, P = 1.0, R^2_L = 0.08, no overdispersion ($P > 0.05$)			
Estuary	2	0.16	0.92
Habitat (estuary)	6	0.39	1.00

dissection data (done for each estuary as described above) to estimate *E. californiensis* abundance in fish at each site. To determine whether habitat or estuary influenced the mean abundance in encountered killifish, a GzLM was run on site-level mean metacercaria abundances in fish. To calculate the typical mean abundance in fish populations of a particular habitat, mean abundance was averaged across sites from that habitat (weighted by fish density). To determine whether mean abundance differed for estuary-wide populations, it was necessary to factor in habitat area differences. These estuary-wide assessments used habitat mean abundances weighted by total habitat area. Using the predictive models to estimate parasitism (in individual fish from each site) gave the additional advantage of including all available information on the relationship of parasitism with host size and habitat type (vs. using only the information from 10 dissected fish per site).

RESULTS

Estuary sampling

It should be noted that ecosystem-level sampling of the 3 estuaries was performed at 3 different times, i.e., EPB in October 2002, CSM in July 2003, and BSQ in July 2004. As a result, sample timing, in addition to estuary characteristics, may have influenced the documented differences and similarities among estuaries (which we examine in the discussion).

Host ecology (count and biomass densities)

Killifish were the most commonly encountered fish at EPB and BSQ, accounting for 76% and 56% (respectively) of the total fish community count. Killifish comprised 40% of the total fish community at CSM. Count densities at sites did not differ significantly among estuaries or habitats (Table I), and there was also no difference in total estuary-level killifish density (Fig. 1). However, killifish biomass densities at sites varied weakly among habitats. This difference was driven by EPB channels having about 5 times higher mass density than EPB flats and pans (χ^2 = 5.6, df = 1, P = 0.02). However, when scaling up from site-level densities to entire estuaries, BSQ, given relatively high fish densities on its extensive flat habitat, appeared to have twice the killifish biomass density of EPB, although large confidence limits precluded significance (Fig. 1). Differences among estuaries in killifish biomass density were driven by differences in relative habitat areas. For example, BSQ had extensive flat habitat where killifish were abundant, while at EPB, the channel habitat favored by killifish represented just a small portion (5%) of the overall aquatic habitat.

Figure 2 shows relative size–frequency distributions of field-measured killifish. CSM and BSQ had bimodal size frequency distributions, with peaks at 20–29 mm and 70–89 mm TL. In contrast, killifish were nearly unimodally distributed across sizes at EPB, with a peak at 30–39 mm and with almost as many individuals in the 40–69-mm range, size classes that were scarce at the other 2 estuaries (Fig. 2). Body condition did not differ significantly among estuaries (Table I).

Parasite ecology (count and biomass densities)

We sampled 375 killifish for parasites (EPB, n = 116; CSM, n = 99; BSQ, n = 160). Parasite count and biomass density varied

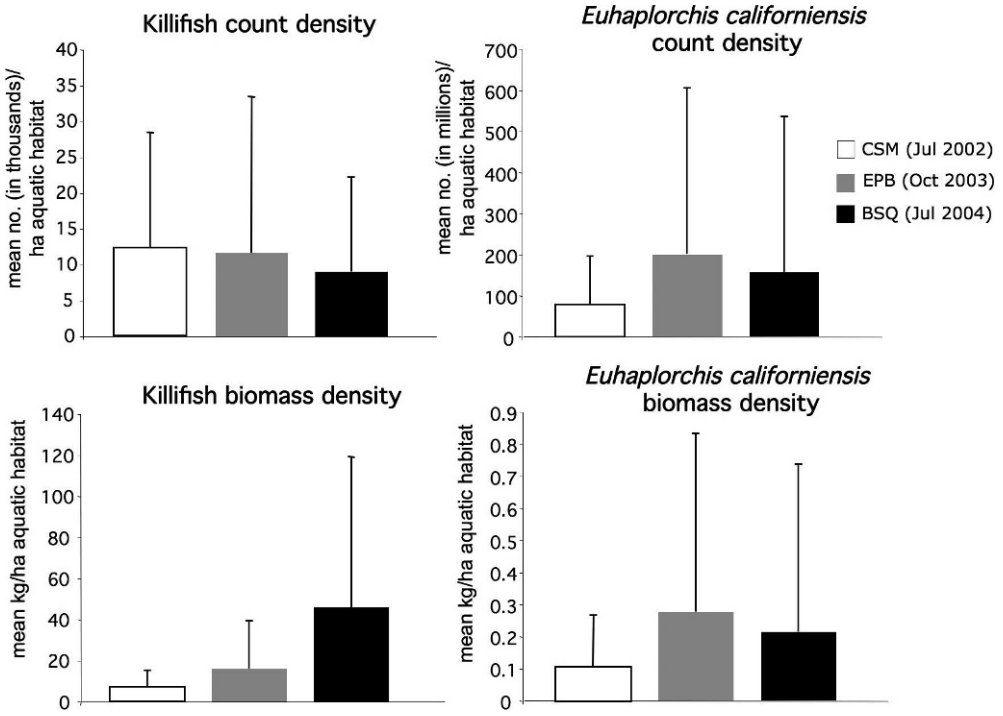

FIGURE 1. Estuary-level count and mass densities for host and parasite, per aquatic habitat. Error bars represent 95% CL for stratified means (Thompson, 2002). CSM = Carpinteria Salt Marsh, EPB = Estero de Punta Banda, BSQ = Bahia San Quintín.

widely at sites, but neither habitat nor estuary explained this variation (Table I). Estuary-level count and biomass densities of *E. californiensis* (extrapolated site-level and habitat densities) also did not vary significantly (Fig. 1). Count densities of metacercariae ranged from 78–200 million/ha and total biomass densities ranged from 0.1–0.3 kg/ha (Fig. 1). Total estuary biomass of *E. californiensis* metacercariae reached 1.7% of the total killifish biomass at EPB, 1.5% at CSM, and 0.5% at BSQ.

Parasitism in individual hosts

Euhaplorchis californiensis metacercariae in California killifish were nearly ubiquitous, with estimated estuary-level prevalences of 94% ± 0.1 SD at CSM, 98% ± 0.01 at EPB, and 100% ± 0.0 at BSQ. Logistic regression indicated that fish length was the only factor that explained the probability of being infected (prevalence), and greater than 90% of the smallest fish (<25mm) were infected. Abundance of *E. californiensis* in individual hosts strongly increased with size (Fig. 3; Table II) and varied significantly among sites and habitats, being highest in channels in each estuary, for any given fish size (Fig. 3; Table II). The size effect varied with the estuary, so that *E. californiensis* abundance increased more rapidly with host size at CSM (Fig. 3; Table II); for each 10-mm increase in size, the mean abundance of metacercariae increased 1.5 times at CSM and 1.3 times at EPB and BSQ. Parasite abundance differed significantly between sexes, with females having about 1.5 times higher abundance than males for any given size (Table II). Values for k indicated that metacercariae of *E. californiensis* in individual hosts appeared aggregated at CSM and BSQ, and slightly less aggregated at EPB (Fig. 4), although k did not differ significantly among estuaries or habitats (Table II). After accounting for host size, however, aggregation of metacercariae in smaller fish (0–29 mm) was significantly higher than in the larger fish (60–120 mm) at BSQ (Table II), with no significant differences among sizes observed at CSM and EPB. Table III lists aggregation statistics by size category.

Parasitism in fish populations

Projected mean abundance of metacercariae in killifish populations varied significantly among estuaries and habitats (Table IV). These results were also reflected when scaling up to the fish populations in the various habitats and estuaries (Fig. 5). The significant variation among habitats appeared to be largely driven by killifish in EPB channels having over 2 times greater *E.*

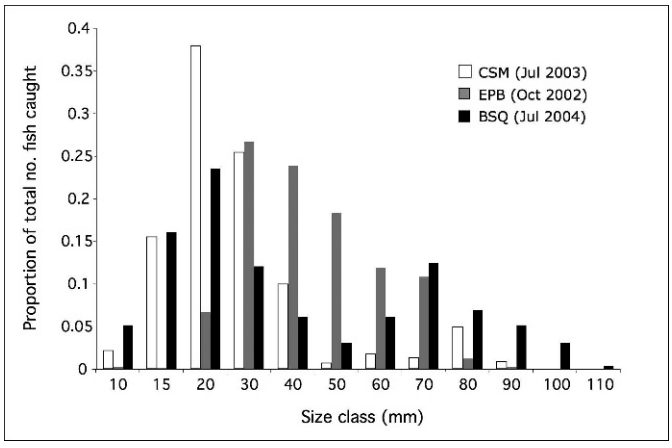

FIGURE 2. Relative size–frequency distribution of killifish at Carpinteria Salt Marsh (CSM, n = 230), Estero de Punta Banda (EPB, n = 438), and Bahia San Quintín (BSQ, n = 313).

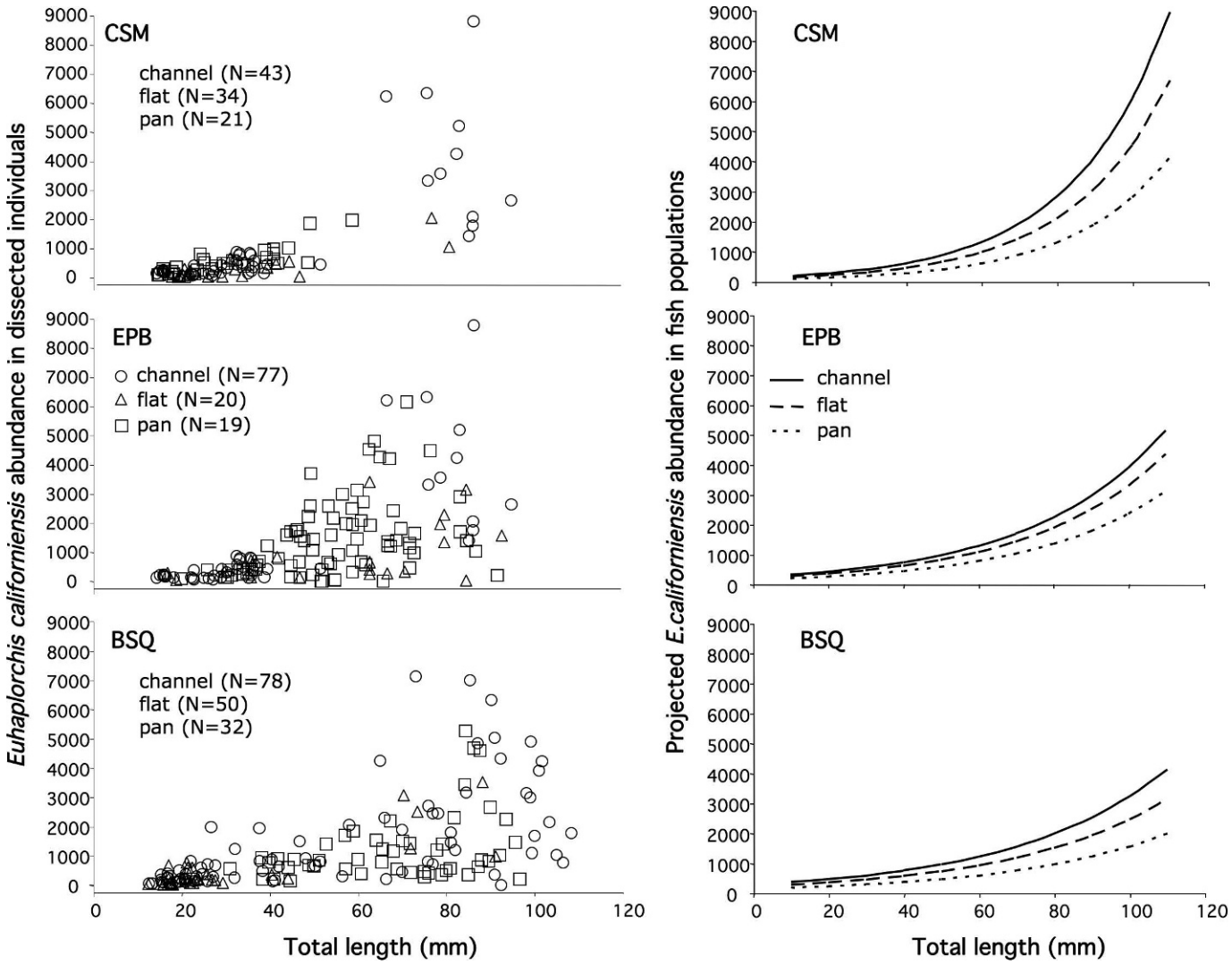

FIGURE 3. Abundance of *Euhaplorchis californiensis* in individual hosts and projected abundance in killifish populations, by habitat and estuary. CSM = Carpinteria Salt Marsh (sampled July 2003), EPB = Estero de Punta Banda (October 2002), BSQ = Bahia San Quintín (July 2004).

californiensis mean abundance than did those on EPB flats and pans ($P = 0.004$) (Fig. 5). Killifish in BSQ pans also had about 1/5 the mean parasite abundance of those in BSQ channels and flats. CSM (Jul 2003) had a lower estuary-level mean abundance of *E. californiensis* in killifish compared to EPB (Oct 2002) and BSQ (Jul 2004). The average mean infection abundance at CSM was 91 ± 105 (±95% CL), whereas EPB was 757 ± 138 and BSQ was 1,169 ± 556 (Fig. 5; Table IV).

DISCUSSION

Our results indicate an overall consistency among the 3 estuaries, despite substantial variation, in several general patterns of parasitism and in the abundance of California killifish and the trematode metacercariae of *E. californiensis*. Killifish count densities at CSM, EPB, and BSQ were consistent with previous studies, which report killifish as one of the most abundant fishes in southern California and Baja California estuaries (Perez-Espana et al., 1998; West and Zedler, 2000; Allen et al., 2006; Hechinger et al., 2007). The difference in size–frequency distributions among our estuary samples was probably due to collecting fish from EPB during the fall, as opposed to summertime collections at CSM and BSQ. California killifish breed during the spring and summer (Fritz, 1975; Perez-Espana et al., 1998), and the smaller mode of young-of-the-year fishes had likely merged with the adult size classes at EPB. This interpretation is supported by the monthly, unimodal size–frequency distributions observed by Fritz (1975) for a different southern California estuary. Older, larger fish at EPB also led to a higher killifish biomass density in channels for the same number of fish. Differences among estuaries in the relative abundance of preferred habitat helped drive variation in biomass densities of fish among estuaries.

Mean abundances of *E. californiensis* per host ranged from several hundred to over 1,000 at each estuary, consistent with the range reported in Lafferty and Morris (1996). Such high parasite numbers are uncommonly reported from host–parasite systems; among 269 systems reviewed by Shaw and Dobson (1995), only

TABLE II. Generalized linear model (GzLM) statistics used to assess differences in parasitism (abundance and aggregation) in killifish. Sampling date for each estuary: Carpinteria Salt Marsh, July 2003, Estero de Punta Banda, October 2002, and Bahia San Quintín, July 2004. See Table I for model parameters and abbreviations.

Main effect	df	χ^2	P
E. californiensis abundance in dissected killifish			
Full model: df = 46, $\chi^2 = 572.0$, $P < 0.0001$, $R^2_L = 0.6$, overdispersion = 557.7 ($P < 0.0001$)			
Estuary	2	0.45	0.80
Habitat (estuary)	6	17.46	0.008
Site (estuary, habitat)	35	113.18	<0.0001
TL	1	155.67	<0.0001
Estuary*TL	2	6.38	0.04
E. californiensis abundance in dissected male and female killifish (excluding juveniles)			
Full model: df = 34, $\chi^2 = 160.9$, $P < 0.0001$, $R^2_L = 0.6$, overdispersion = 801.5 ($P < 0.0001$)			
Estuary	2	0.70	0.70
Habitat (estuary)	6	14.35	0.03
Site (estuary, habitat)	24	58.10	0.0001
TL	1	24.11	<0.0001
Sex	1	13.06	0.0003
E. californiensis aggregation in dissected killifish, all sizes pooled			
Full model: df = 8, $\chi^2 = 6.7$, $P = 0.6$, $R^2_L = 0.2$, overdispersion = 21.3 ($P < 0.0001$)			
Estuary	2	0.78	0.97
Habitat (estuary)	6	4.50	0.60
E. californiensis aggregation in dissected killifish, 30-mm size categories			
Full model: df = 10, $\chi^2 = 26.1$, $P = 0.004$, $R^2_L = 0.4$, overdispersion = 10.1 ($P < 0.0001$)			
Estuary	2	2.07	0.36
Habitat (estuary)	6	9.04	0.17
Size category	2	12.07	0.002

5% of the studies reported mean intensities greater than 200 (we note the low number of such reports could be due to difficulty quantifying such high parasite burdens).

The increase in mean abundance of *E. californiensis* in killifish with increasing host size is a common pattern in host–parasite systems, including trematode metacercariae in fish hosts (Aho et al., 1982; Zelmer and Arai, 1998). A major reason for this is that bigger fish are older and have cumulatively been exposed to, and infected by, more metacercariae. This general and common pattern may also explain why the CSM population had the lowest mean abundance of *E. californiensis*. The relationship between mean abundance and fish size was generally the same for all estuaries, despite abundance increasing a little more rapidly with fish size at CSM (Fig. 3). Therefore, the lower mean abundance of *E. californiensis*is was likely a result of the killifish population at CSM being comprised of smaller fishes as compared to the other 2 estuaries (Figs. 1, 2, 5).

The 1.5-fold higher mean abundance of metacercariae in females compared to males of equal size could have been due to: (1) females growing more slowly than males, (2) females having higher exposure to cercariae than did males, (3) metacercariae being more likely to die after infecting males, and (4) infected males being more intensely preyed upon by birds. Fritz (1975) examined growth rates of male and female California killifish, but he presented the size–age data for individuals without distinguishing gender. Although Fritz (1975) studied growth rates of male and female California killifish, he did not distinguish gender when presenting the size–age relationship. Thus, future work should focus on distinguishing which of the above factors explain females having greater infection abundance than males.

Spatial variability in metacercariae abundance has been commonly documented (e.g., Marcogliese et al., 2001 [fish]; Smith et al., 2007 [crabs]). One factor that might explain the spatial variation observed in killifish (CSM < EPB and BSQ) is that the abundance of infection in first intermediate host horn snails varies among sites within estuaries (e.g., Lafferty et al., 1994; Hechinger et al., 2007). Subsequently, risk of infection by *E. californiensis* cercariae could also vary on a similar scale for killifish. Although we would expect fine-scale differences in infection heterogeneity to be partially integrated in a vagile host like killifish, the fact that parasite abundance differs among habitat types suggests that spatial patterns of infection in the first intermediate host do influence infection patterns in relatively mobile second intermediate hosts. An alternate (and non-mutually exclusive) explanation for the lower infection abundance at CSM is that parasite-induced trophic transmission may be more intense at CSM.

The mean abundances of *E. californiensis* metacercariae translate to a total biomass comprising close to 2% of total killifish biomass at CSM and EPB. *Euhaplorchis californiensis* biomass density was not simply a function of host biomass (Fig. 1), largely because the killifish, at any given size, at EPB were infected by more metacercariae than at the other 2 estuaries. One confounding explanation for this could be that EPB was sampled later in the season. Cercariae are generally released during warmer seasons, and metacercariae tend to accumulate in second intermediate hosts that are cumulatively exposed to more infections as the seasons progress (e.g., see Chu and Dawood, 1970; Chubb, 1979; Marcogliese et al., 2001; Sandland et al., 2001). Therefore, adult fishes at EPB may have had more time to accumulate higher metacercariae abundances. Additionally, it is

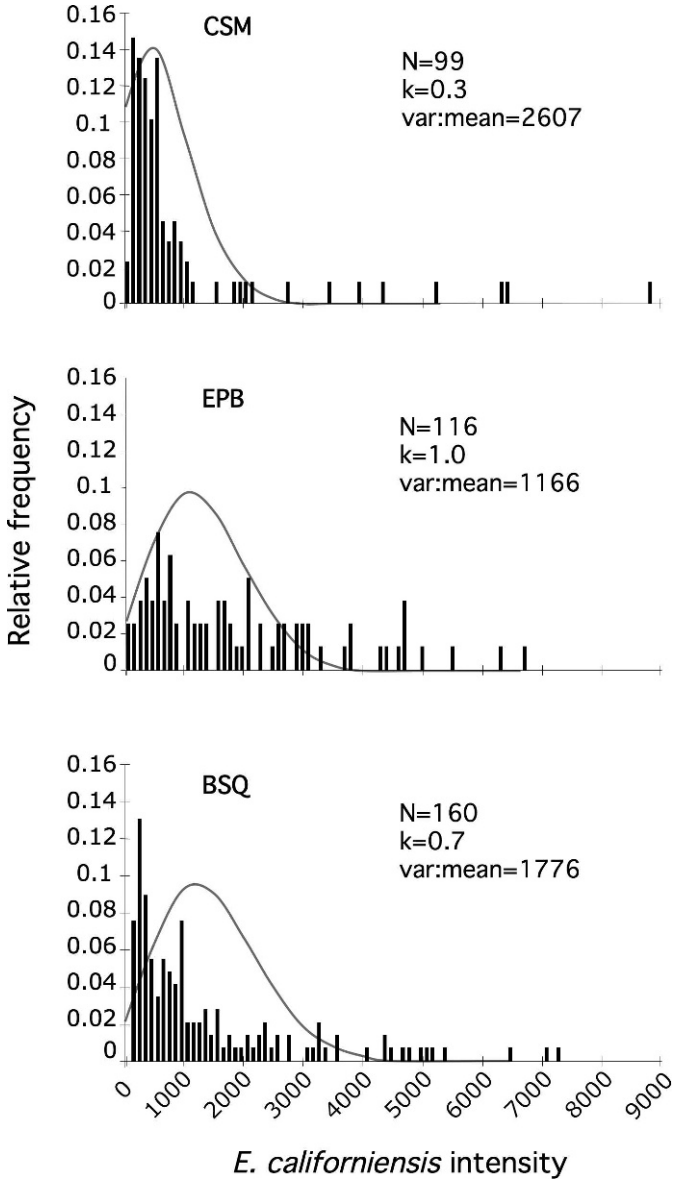

FIGURE 4. *Euhaplorchis californiensis* aggregation in dissected killifish. Fitted Poisson distribution (dashed grey lines) highlights deviation of data from projected random distribution. CSM = Carpinteria Salt Marsh (sampled July 2003), EPB = Estero de Punta Banda (October 2002), BSQ = Bahia San Quintín (July 2004).

possible that other environmental factors, such as first intermediate host density, contributed to differences in *E. californiensis* metacercariae biomass among the 3 estuaries.

Differences in intensities associated with host size accounted for most of the aggregation of metacercariae within fish populations. Further examination accounting for host size revealed that aggregation was usually modest, $k > 1$ (Shaw et al., 1998) for all but the smallest size class at BSQ, and the extent of aggregation was generally reduced at larger host sizes (as k increased) in each estuary (Table III). Our findings are unusual, when considering that aggregation generally increases with host size (Shaw et al., 1998).

Trophically transmitted parasites may be less aggregated than expected in larger, older hosts due to preferential predation on the most heavily infected hosts (Crofton, 1971; Rousset et al., 1996; Kuris, 2003). In an amphipod intermediate host, intensity and aggregation decrease in older amphipods infected with behavior-altering metacercariae (*Microphallus papillorobustus*) (Rousset et al., 1996). In contrast, such a decrease was not evident in an amphipod species in which *M. papillorobustus* does not induce behavior modification (Rousset et al., 1996). Similarly, the reduced aggregation of *E. californiensis* metacercariae in larger killifish, as indicated by k (Table III), may result from higher predation on more heavily infected individuals by piscivorous bird final hosts, as documented in the field experiments of Lafferty and Morris (1996). This may also explain the apparent, lower-overall aggegration at EPB. The later seasonal timing of our EPB sampling may have allowed this fish population to accumulate more metacercariae infections, compared with the other 2 estuaries, as well as having provided additional time for selective predation to remove the most highly infected individuals.

Parasites can greatly affect ecosystem structure and food-web dynamics by altering predator–prey links (Lafferty et al., 2008). Killifish comprise roughly half of the fish communities at CSM, EPB, and BSQ. The information presented here on the distribution and abundance of the killifish brain parasite, *E. californiensis*, integrated with the behavioral modification experiment of Lafferty and Morris (1996) and the neurobiological behavior experiment of Shaw et al. (2009), suggests that this parasite could strongly affect the size distribution, biomass, and abundance of its common host and, consequently, is an influential

TABLE III. Aggregation data for *Euhaplorchis californiensis* in killifish, by size categories. The parameter k represents the corrected moment estimate of k. CSM = Carpinteria Salt Marsh (sampled July 2003), EPB = Estero de Punta Banda (October 2002), BSQ = Bahia San Quintín (July 2004).

Estuary	Size category (mm)	n	Mean intensity ± SD	Range	k
CSM	0–29	48	176 ± 169	0–790	1.1
CSM	30–59	38	581 ± 400	0–1,950	2.1
CSM	60–120	13	3,731 ± 2,320	1,012–8,781	2.5
EPB	30–59*	73	867 ± 804	0–3,700	1.2
EPB	60–120	43	1,835 ± 1,484	0–6,150	1.5
BSQ	0–29	55	273 ± 319	1–1,995	0.7
BSQ	30–59	33	795 ± 538	140–2,046	2.2
BSQ	60–120	72	2,078 ± 1,737	6–7,130	1.4

* EPB 30–59-mm group includes 5 individuals sized 0–29 mm.

TABLE IV. Generalized linear model (GzLM) statistics used to assess differences in projected *Euhaplorchis californiensis* mean abundance in killifish populations. Habitat (estuary) denotes habitat nested within estuary. Replicates are site values, weighted by fish density. Sampling date for each estuary: Carpinteria Salt Marsh, July 2003, Estero de Punta Banda, October 2002, and Bahia San Quintín, July 2004.

Main effect	df	χ^2	P
E. californiensis projected mean abundance in killifish populations			
Full model: df = 8, $\chi^2 = 61.4$, $P < 0.0001$, $R^2_L = 0.7$, overdispersion = 795.8 ($P < 0.0001$)			
Estuary	2	22.27	<0.0001
Habitat (estuary)	6	17.47	0.008

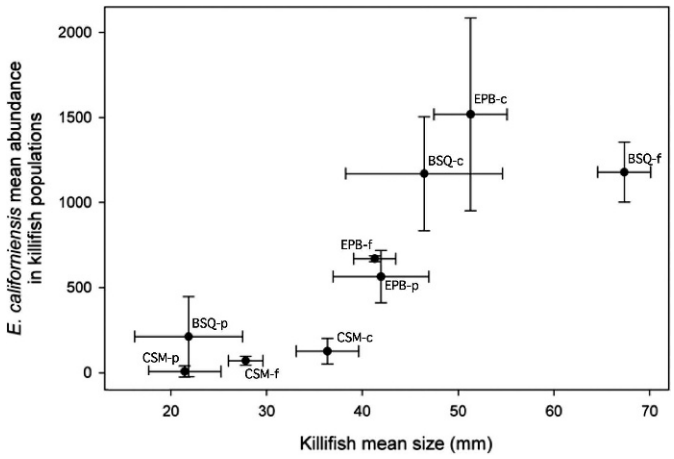

FIGURE 5. Projected mean abundance of *Euhaplorchis californiensis* by killifish mean size (total length) for each habitat and estuary. Table IV shows GLM statistics. CSM = Carpinteria Salt Marsh (sampled July 2003), EPB = Estero de Punta Banda (October 2002), BSQ = Bahia San Quintín (July 2004); c = channel, f = mudflat, p = pan.

factor in the food webs of southern California and Baja California.

ACKNOWLEDGMENTS

We thank L. Aguirre-Macedo and her CINVESTAV-IPN colleagues for assistance with EPB dissections. Our appreciation extends to the diligent UCSB undergraduate students who assisted with collection of fishes and quantification of metacercariae. The University of California Natural Reserve System granted access to CSM field sites. Funding was provided by the NSF/NIH Ecology of Infectious Diseases Program (DEB-0224565). Any use of trade, product, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. government.

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