Parasite Effects on Host Behavior

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Preliminary data from a baseline survey (Santiago Bass, unpub data) suggested that *Fundulus heteroclitus* (Linneus) from Mill Creek, a restored tidal marsh located in the Hackensack Meadowlands District, have severe digenean trematode cyst infections of the gills (several thousand parasites) relative to other sites examined (several hundred parasites). Little if any research has been done to examine potential behavioral or physiological effects of high endoparasite loads in the gills of fish, nor its implication to restored tidal marshes. Fish with highly infected gills may be experiencing parasite-induced behavioral modifications which in turn could increase their consumption by wading birds (final host) thus increasing their parasite loads as well. *F. heteroclitus* may also be experiencing conditions similar to hypoxia, as their respiration may become severely compromised from parasite saturated gills. These hosts may try to compensate for decreased oxygen extraction by counteracting the parasite invasion either behaviorally (surface breathing, reduced activity) or physiologically (increase gill surface area, increased number of red blood cells). Consequently, a more focused experimental study of fish from restored sites and unrestored sites within the Hackensack Meadowlands District was undertaken to investigate gill parasites and their relation to physiology and behavior, and to examine if the process of restoration is potentially influencing either parasite or snail (1^{st} intermediate host) abundance.

The aim of this study was to answer the following hypotheses: 1) Restored sites will have fish with greater numbers of larval digenean trematode gill parasites than fish from unrestored sites. 2) Fish with extremely high gill infections will have lower respiration rates than those with lower infections. 3) Fish with lower infections will have greater stamina versus those with significantly higher infections. 4) Excessive parasite loads will induce physiological (e.g., changes in blood size and/or volume) and/or morphological changes (e.g., additional gill branching) in the gills to compensate for likely oxygen loss, and 5) Restored sites will have higher numbers of first intermediate snails hosts (*Littoridinops tenuipes* Couper) than unrestored sites. *L. tenuipes* was chosen as the potential 1^{st} intermediate host as intensive surveys during the course of this study for other snail species (e.g., *Littorina littorina*, *Ilyanassa obsoleta, Melampus bidentatus*) were unfruitful.

MATERIALS & METHODS

Six sites within the Hackensack Meadowlands District; Mill Creek (MC), Skeetkill Creek (SK), Richard W. DeKorte Park (RD), Kingsland Creek (KC), Vince Lombardi (VL), and Cedar Creek (CC) were examined. *F. heteroclitus* were collected using seines from each site. Fish were returned to the lab, kept in an aerated 10 gal tank and allowed to acclimate for approximately one week until tested. Fish were fed a combination of Tetramin® fish flakes and dried shrimp (*Gammarus* sp.) twice daily.

Gill Parasite Abundance

Gills were removed and placed in a Petri dish with DI water to keep wet. Individual gill filaments were then examined under a dissection microscope, the number of metacercarial cysts recorded, and prevalence calculated. Gills collected were placed in individual vials of 10% formalin for later reference as needed. 20 fish from each site were examined.

Vertical Positioning & Conspicuous Behavior

Groups of 10 fish (5 male, 5 female) were placed in a 10 gal tank that had the upper third delineated with a line to represent the surface region and the lower two-thirds designated as the pelagic and benthic habitats. After allowing the fish to acclimate for one hour alone, they were videotaped to remove potential confounding effects of having an observer present. The first 10 minutes of footage was discarded to reduce any residual effects resulting from the initial human presence (e.g., turning camera on). The number of individuals found at the surface (in the upper third region) were recorded at each minute interval over a 15 minute period. Then, each individual fish was observed separately for a 5 min period, and any unusual conspicuous, aberrant behaviors such as flashing and jerking were recorded.

Activity

One fish at a time was placed into a 10 gal tank with a 5 cm^2 grid drawn on the bottom and allowed to acclimate for one half hour. Activity rates were measured by counting the number of lines crossed in a one minute period. P values <0.05 were considered statistically significant.

Respiration

F. heteroclitus respiration rates were measured by placing weighed fish in individual 2.8 L Erlenmeyer flasks with a magnetic stirrer on a Themolyne Nuova II stirring plate, to keep the water mixed continuously. Initial dissolved oxygen (DO) levels were measured using a YSI DO meter (model 51B), and the flasks sealed with parafilm. DO was measured again after 30 min and the difference between initial and final readings recorded. Respiration rate was calculated per gram fish (mg/g fish). 25 fish from each site were tested.

Stamina

Fish stamina was measured using individuals placed in a 2.8 L Erlenmeyer flask. A 63 mm magnetic stirrer was added to the flask/fish set-up and positioned on a Themolyne Nuova II stirring plate. Fish swam against a current generated by the fixed stirring action over a 15 min period and the time to exhaustion was recorded. Fish were deemed exhausted when they succumbed to the current's force, and were subsequently caught-up in the vortex. 25 fish from each site were tested. P values <0.05 were considered statistically significant.

Blood Collection & Analysis

Following standard fish hematological methods (Wedemeyer and Yasutake, 1977), whole blood was taken directly from the severed caudal peduncle of 15 fish (lightly anesthetized with MS-222) into a 75 mm (50 µl) heparinized microcapilliary tube (Fisherbrand™) and sealed with tube sealant compound (Chāseal™). Microcapillary tubes were placed in an IEC microcapillary centrifuge and spun for 5 minutes at approximately 10,000 rpm, after which the tubes were read immediately to avoid CO_2-induced swelling. Color of supernatant was recorded and the percent of packed red blood cells (PRBC) was calculated as follows:

$$\text{Hematocrit (\%)} = \frac{\text{Length RBC column}}{\text{Total length of blood column}} \times 100$$

Using rainbow trout (*Salmo gairdneri*), Houston and DeWilde (1968) concluded that hematocrit values may be substituted for RBC counts and hemoglobin determinations in routine evaluations of hematological status. Hemoglobin content was estimated by normalizing data as specified by Bachand and Leray (1975) and Walsh et al. (1990). They determined that 1 gm Hb is equivalent to 4 to 5 ml (or gm) of packed red cells or hematocrit.

Differential blood smears were then prepared from the 15 fish and the thin blood film on the slide was allowed to air dry. Slides were then stained using Quickstep Wright-Giemsa stain (Fischer Scientific), rinsed in distilled water, and allowed to air dry. The area of 100 RBCs were calculated using ImageJ (National Institutes of Health, 2006). Fish were subsequently placed in individual 50 mL Falcon™ tubes and fixed in 10% formalin.

Gill Morphology

Gills were removed from preserved fish and placed in a Petri dish with DI water to keep wet and examined under a dissection microscope. Individual gill filament numbers were recorded and abnormalities such as additional branches/forks and their number of occurrences were recorded. 20 fish from each site were examined. *P* values <0.05 were considered statistically significant.

Snail Abundance

Snail populations of the coastal marsh snail (*L. tenuipes*), believed to be the first intermediate host for one of the digenean trematode species found encysted in *F. heteroclitus* gills were estimated. The numbers of snails occupying the immediate area surrounding the study sites were estimated by sampling mud flats at low tide between June and September of the summer of 2006. Six surficial sediment samples were taken using watch glasses (area=23.75 cm^2) at approximately six foot intervals, parallel to the marsh surface. Samples were returned to the lab where they were rinsed through a 1 mm^2 mesh sieve. Snails were collected once a month over a three month period in the summer, and their numbers recorded.

Statistics

One-way ANOVA, Tukey HSD and Covariance Matrix, Kruskal-Wallis test and Pearson correlation analyses were performed using Statistix 8 statistical package and Canonical Correlations using SAS 9.1. *P* values <0.05 were considered statistically significant.

RESULTS

Gill Parasite Abundance

Highly significant differences ($P<0.001$, df=5, $F_{83.3}$) in gill parasite abundance were found among sites using a one-way ANOVA, with four groups of homogenous means found using Tukey HSD (Figure 1). Sites are Mill Creek (MC), Skeetkill Creek (SK), Richard W. DeKorte Park (RD), Kingsland Creek (KC), Vince Lombardi (VL), and Cedar Creek (CC).

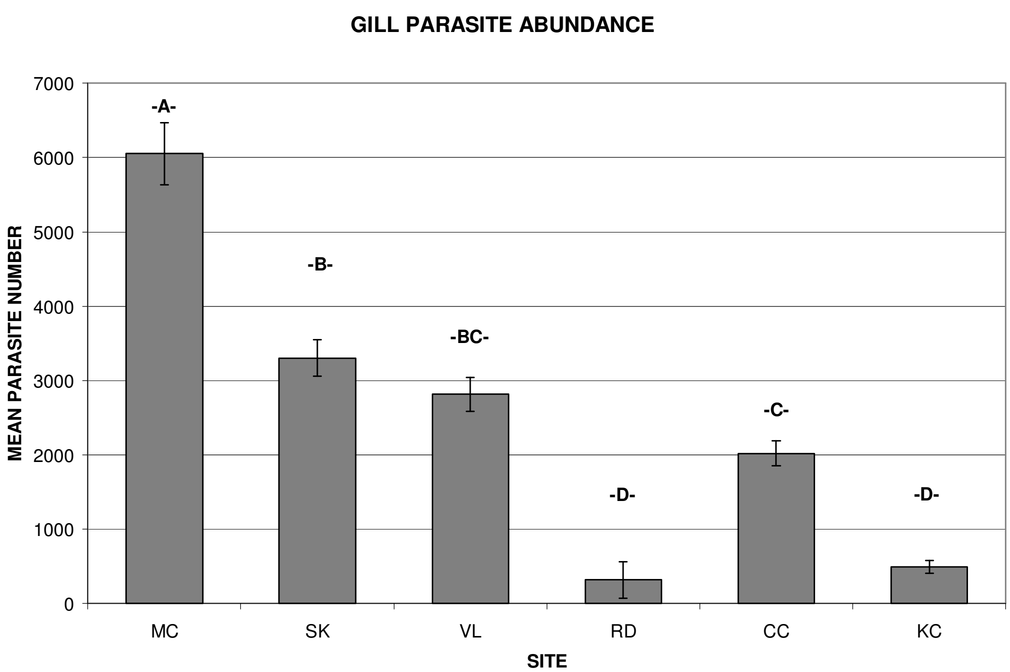

Figure 1. Mean (±SE) number of with Tukey HSD comparison of gill parasites.

Five macroparasite species were commonly found associated with the gills (Table 1); *Ascocotyle (Phagicola) diminuta*, *Echinochasmus schwartzi*, *Dactylogyrus* sp., *Fundulus prolongis*, and *Ergasilus funduli*. A sixth species; *Argulus flavens* was rarely observed (prevalence << 1%) and therefore was not included. Both *A. diminuta* (range 29-7549) and *E. schwartzi* (range 54-1887) were found in 100% of all fish from the six sites examined, and made up 99% of the total number of gill parasites collected. *Dactylogyrus* sp (range 0-56) was observed in 95% of all fish, making it the second most common parasite species. 55% of all fish examined were infected by *F. prolongis* (range 0-38), and less than half (42%) were infected by *E. funduli* (range 0-28).

Table 1. Gill parasite mean (±SE), range and prevalence across sites.

SPECIES	SITE					
	MC (n=21)	SK (n=21)	VL (n=21)	RD (n=21)	CC (n=21)	KC (n=21)
A. diminuta						
Mean ±SE	4789 ±319	2514 ±178	2452 ±187	122 ±11	1551 ±121	111 ±22
Range	1181 - 7549	1005 - 4291	987 - 3923	36 - 233	525 - 2816	29 - 408
Prevalence (%)	100	100	100	100	100	100
E. schwartzi						
Mean ±SE	1197 ±80	629 ±45	625 ±47	182 ±16	388 ±30	445 ±88
Range	295 - 1887	251 - 1073	247 - 981	54 - 350	131 - 704	115 - 1632
Prevalence (%)	100	100	100	100	100	100
Dactylogyrus sp.						
Mean ±SE	9 ±2	6 ±1	6 ±1	11 ±2	19 ±3	6 ±2
Range	1 - 41	0 - 22	0 - 22	2 - 25	1 - 56	0 - 29
Prevalence (%)	100	86	91	100	100	95
F. prolongis						
Mean ±SE	1 ±0.28	0.81 ±0.21	0.14 ±0.10	0.95 ±0.20	3 ±2	1 ±0.27
Range	0 - 4	0 - 3	0 - 2	0 - 3	0 - 38	0 - 4
Prevalence (%)	71	48	10	62	29	57
E. funduli						
Mean ±SE	0.95 ±0.64	0.86 ±0.26	0.76 ±0.23	0.67 ±0.28	3 ±0.74	4 ±2
Range	0 - 13	0 - 4	0 - 4	0 - 5	0 - 12	0 - 28
Prevalence (%)	19	52	48	33	67	33

A Canonical Correlation comparing gill parasites and the three behavioral parameters; vertical positioning, conspicuousness, and activity, was run. Despite strong correlations between *A. diminuta* (0.6792) and *Dactylogyrus* spp. (-0.6686) with both the time spent at the surface (0.7542) and conspicuousness (0.6371), no significant correlation (P=0.36, $F_{1.10}$) was found between gill parasites abundance and each of the behaviors examined (Table 2).

Table 2. Canonical Correlation structure comparing gill parasites and behavioral variables.

	Canonical Variables		
Original Variables	*CV1*	*CV2*	*CV3*
A. diminuta	**0.6792**	0.4528	*0.3152*
E. schwartzi	*0.4342*	0.3089	0.1551
Dactylogyrus sp.	**-0.6686**	0.3816	0.4127
F. prolongis	-0.2426	0.6096	-0.5542
E. funduli	-0.4663	-0.1617	0.5063
	W1	*W2*	*W3*
Verical Position	***0.7542***	0.3242	-0.5711
Conspicuousness	**0.6371**	-0.108	*0.7632*
Activity	0.3212	-0.9327	*-0.1643*

Wilks' Lambda P=0.3597, $F_{1.10}$

Vertical Positioning

Overall, fish with the highest parasite infections (VL, SK and MC) spent significantly more time at the water's surface (p=0.004; df=5; $F_{3.76}$) than fish with lower parasite infections when using a one-way ANOVA, with three groups reported using Tukey HSD. VL (mean=4.60 min ±0.702SE) and SK (mean=3.80 min ±0.545SE) spent the most time at the surface (46% and 38% respectively) and comprised the first group. MC (mean=3.40 min ±0.567SE), RD (mean=2.73 min ±0.556SE) and CC (mean=2.53 min ±0.624SE) were grouped together as intermediates, spending 35%, 27% and 25% of their time at the surface respectively. The second group consisted of KC fish, which only spent approximately 14% of their time (mean=1.40 min ±0.400SE) at the surface (Figure 2).

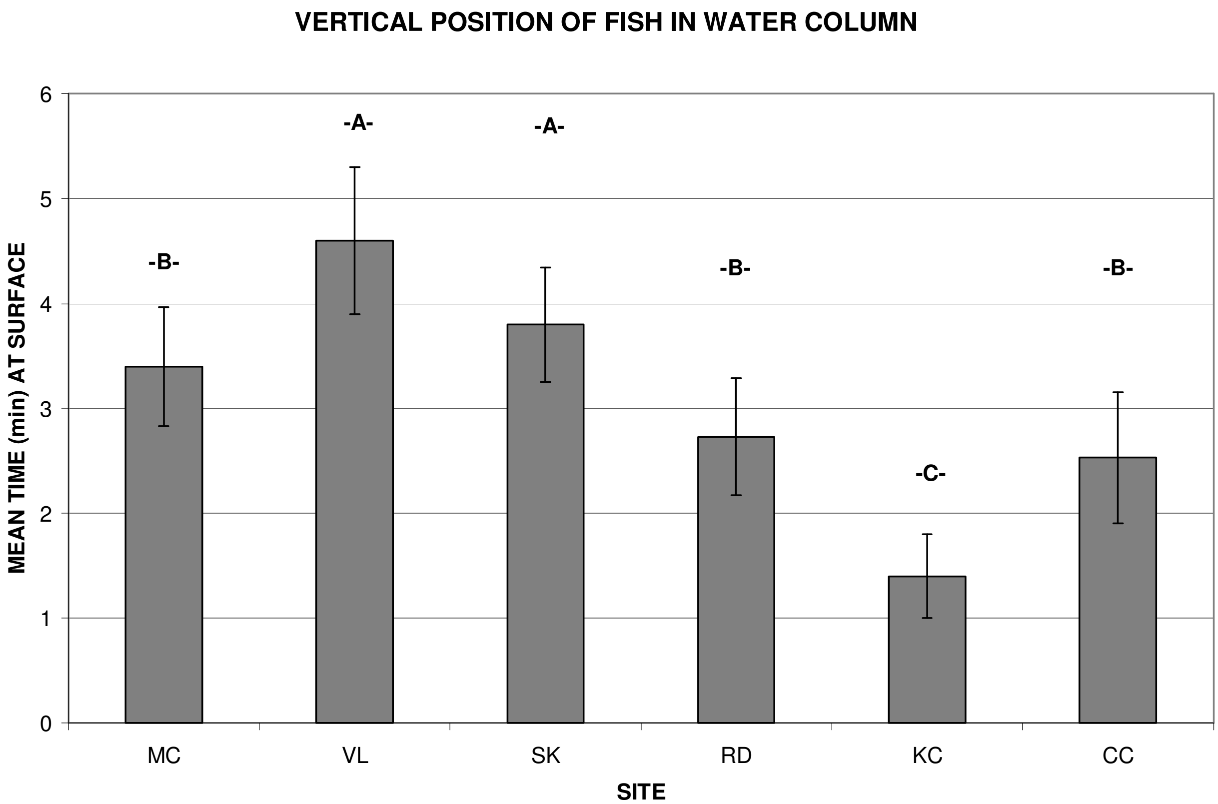

Figure 2. Mean amount of time (min) spent at surface of tank.

Conspicuous Behaviors

Highly significant differences (P=0.003, df=5, $F_{4.16}$) were found among sites with respect to the number of conspicuous behaviors observed using a one-way ANOVA. Tukey HSD determined that there were two groups of homogenous means (Figure 3). Fish from SK performed the greatest mean number (5.3 ±2.06SE) of individual conspicuous behaviors and were placed into one group. VL and MC were grouped together as intermediates with means of 4.2 (±0.74SE) and 1.8 (±0.51SE) respectively. The second group containing CC (1.2 ±0.55), KC (1.1 ±0.35), and RD (0.4 ±0.22) were the least conspicuous, performing the fewest aberrant behaviors.

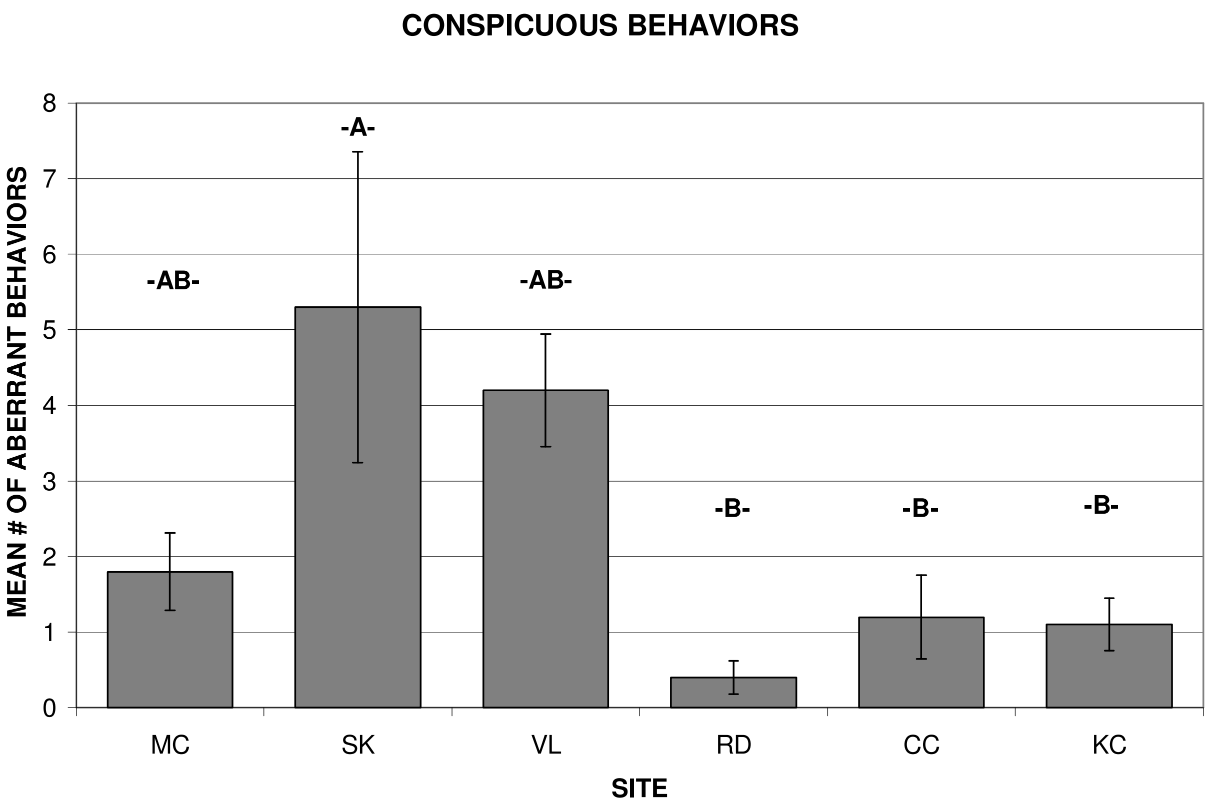

Figure 3. Mean number of individual conspicuous fish behaviors per population.

Activity

No significant differences (*P*=0.712; df=5; $F_{0.58}$) were found between sites when activity levels were analyzed using a one-way ANOVA. SK fish had the highest activity with an average of 63.24 ±6.10SE lines crossed in a minute's time and CC had the lowest activity with 50.28 (±6.10SE) lines crossed in the same period (Table 3).

Table 3. Mean number of lines crossed per minute.

SITE	MEAN	±SE
MC	55.76	6.10
SK	63.24	6.10
VL	53.57	6.65
RD	51.00	6.10
CC	50.28	6.10
KC	55.48	6.10

Respiration

Using a one-way ANOVA, highly significant differences (P<0.001, df=5, $F_{7.9}$) in respiration rates (mg/g) were found between sites, and two groups found using Tukey HSD (Figure 4). No correlation was found between respiration and parasite abundance (R^2=0.035).

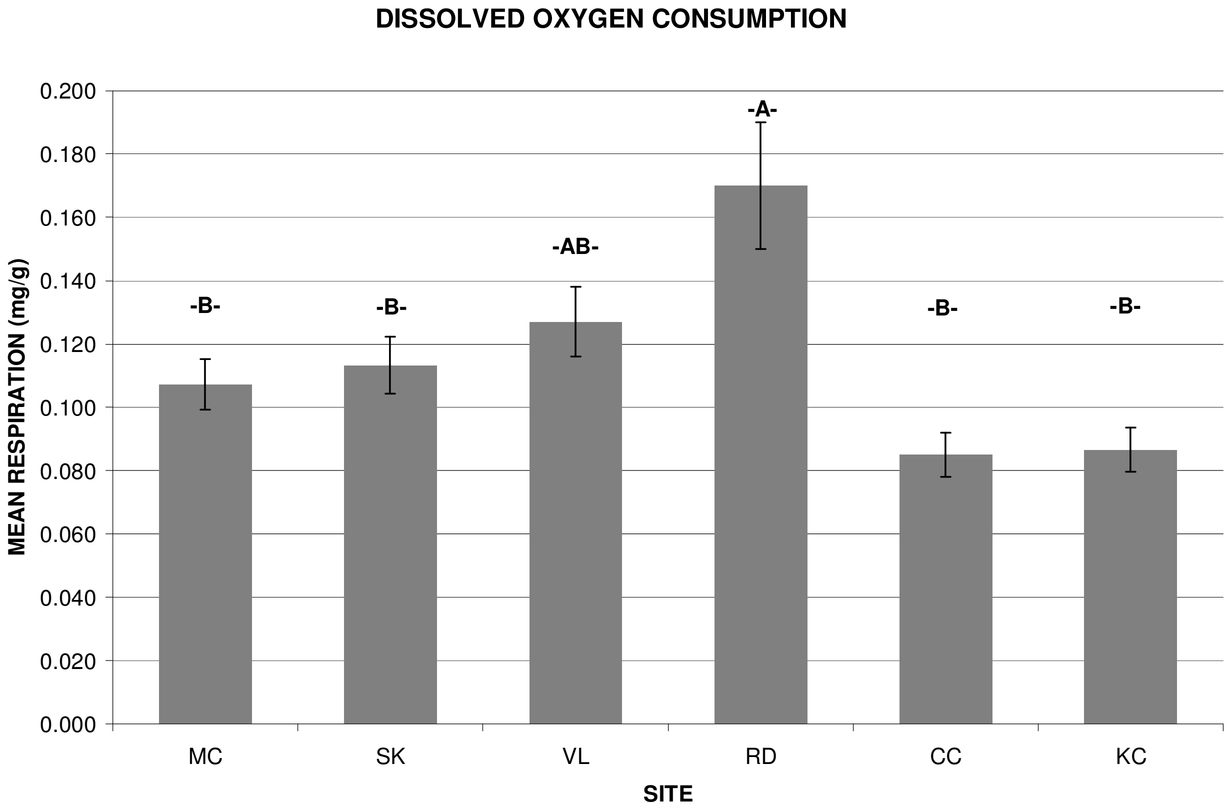

Figure 4. Mean dissolved oxygen consumption (mg/g) over 30 min.

A Principal Component Analysis (PCA) was used and it was determined that of the five gill parasites observed, the digenean trematode metacercariae of *A. diminuta* (38%) and *E. schwartzi* (22%) were accountable for approximately 60% of the total variation seen with Eigenvalues of 1.9029 and 1.0915 respectively (Table 4). These two species were also very strongly correlated with one another, with a correlation of 0.8748. A Canonical Correlation (Table 5) was then run using these two components (species) with the physiological parameters of this study. Although highly significant (P<0.001, $F_{10.98}$, df=8) differences were reported, no correlation was found between either species and respiration.

Table 4. Principal Component Analysis of gill parasite species.

	Eigenvalues of the Correlation Matrix			
	Eigenvalue	*Difference*	*Proportion*	*Cumulative*
1	1.9029	0.8115	0.3806	0.3806
2	1.0915	0.0822	0.2183	0.5989
3	1.0093	0.1361	0.2019	0.8007
4	0.8732		0.1746	0.9754
	Eigenvectors			
	Prin1	*Prin2*	*Prin3*	*Prin4*
A. diminuta	0.6982	0.0882	-0.0173	0.0453
E. schwartzi	0.6939	0.0964	-0.0402	0.1123
Dactylogyrus sp.	-0.023	0.7572	0.1123	-0.6424
F. prolongis	-0.0023	0.0862	0.9592	0.2694
E funduli	-0.1748	0.6341	-0.2559	0.7072

Table 5. Canonical Correlation structure comparing gill parasites and physiological variables.

	Canonical Variables	
Original Variables	*CV1*	*CV3*
A. diminuta	***0.9999***	0.012
E. schwartzi	-0.1041	***-0.9946***
	W1	*W3*
PRBC	-0.0392	***0.7129***
RBC Size	***0.9902***	-0.0292
Respiration	0.1727	-0.01
Stamina	0.0492	***0.5164***

Wilks' Lambda P<0.001, $F_{10.98}$

Stamina

Highly significant differences (P=0.002, df=5, $F_{4.10}$) were found among sites when analyzed using a one-way ANOVA with respect to stamina. Two groups of similar means were found with a Tukey HSD (Figure 5). The Canonical Correlation (Table 5) comparing the two species components to stamina, found a highly significant (P<0.001), strong negative correlation between *E. schwartzi* (-0.9946) and stamina (0.5164).

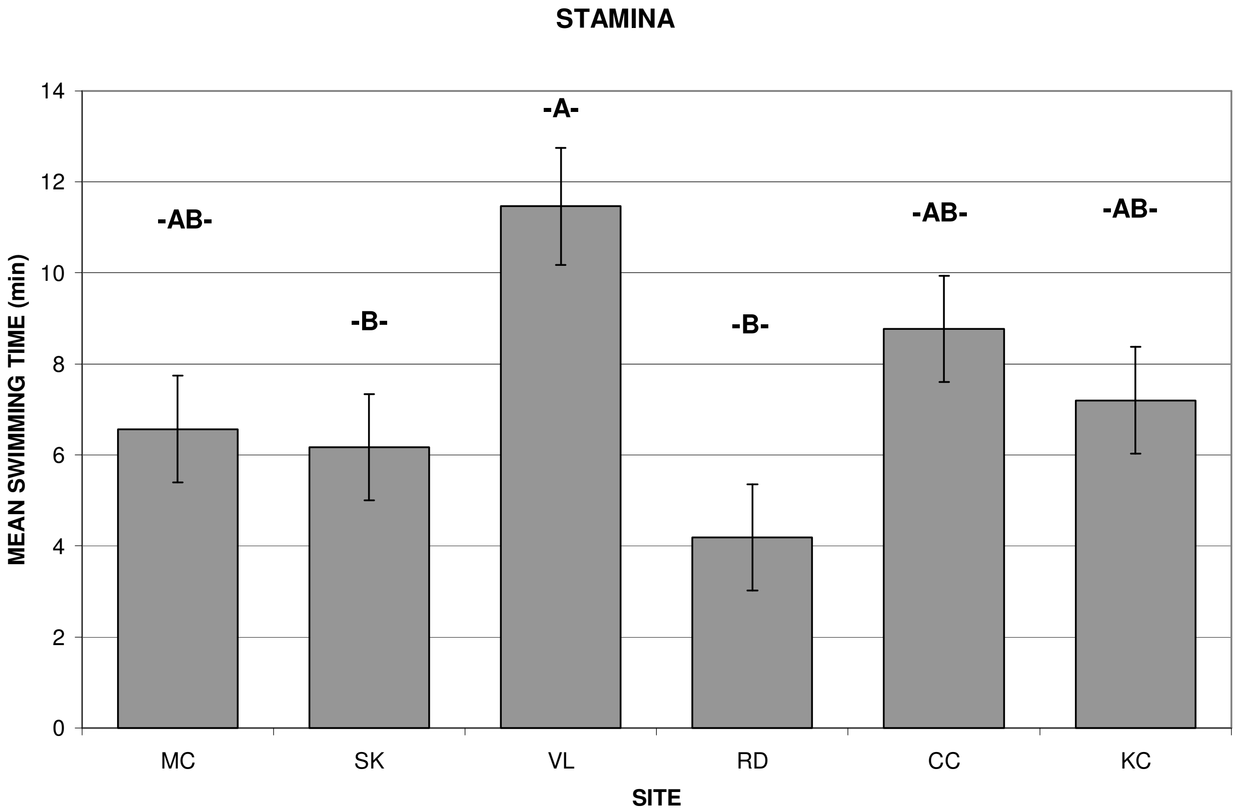

Figure 5. Average fish stamina (min) for all sites.

Blood Analyses

Using a one-way ANOVA, highly significant differences in red blood cell (RBC) area [mm] (*P*<0.001, df=5, $F_{52.6}$) and percent packed red blood cell (PRBC) volume (*P*=0.007, df=5, $F_{3.46}$) were also found among sites. Tukey HSD found three groups of means for RBC area (Figure 6), and two for PRBC (Table 7).

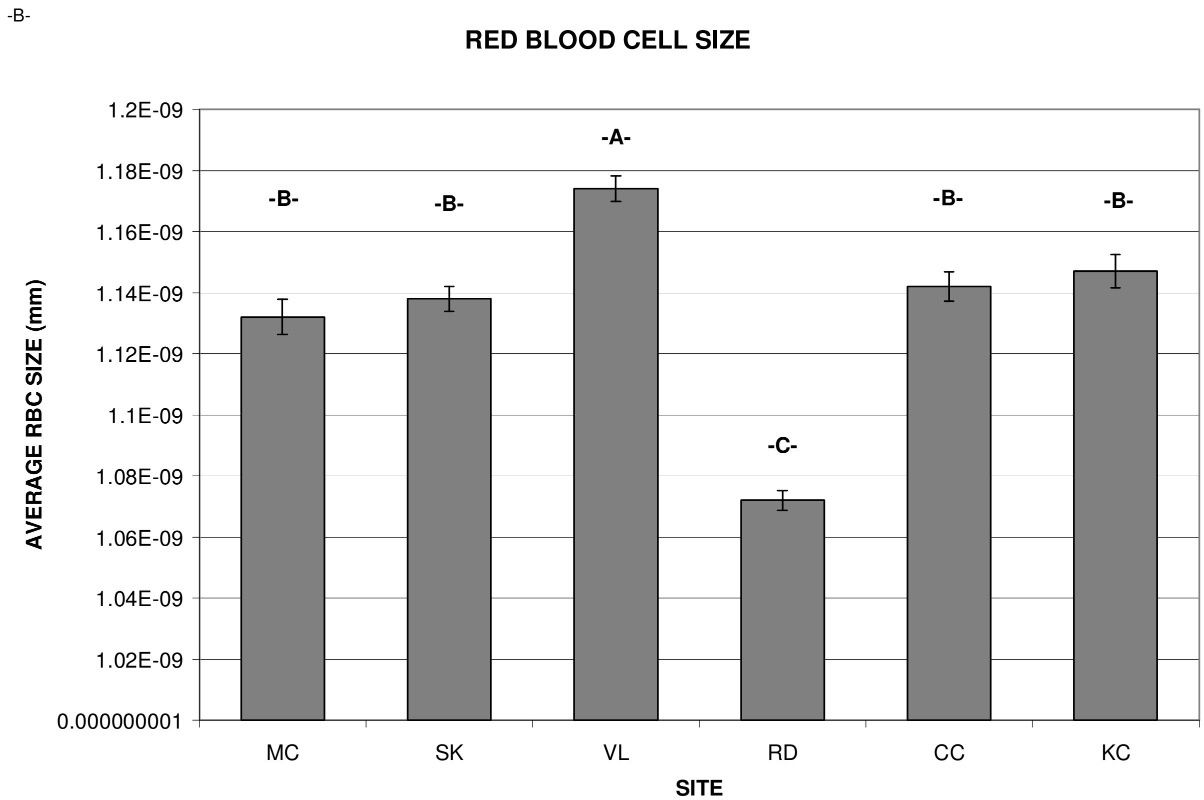

Figure 6. Average red blood cell size (mm).

The Canonical Correlation (Table 5) comparing the two species components to blood, found a highly significant ($P<0.001$), strong positive correlation between *A. diminuta* (0.9999) and RBC area (0.9902), and a strong negative correlation between *E. schwartzi* (-0.9946) and PRBC (0.7129).

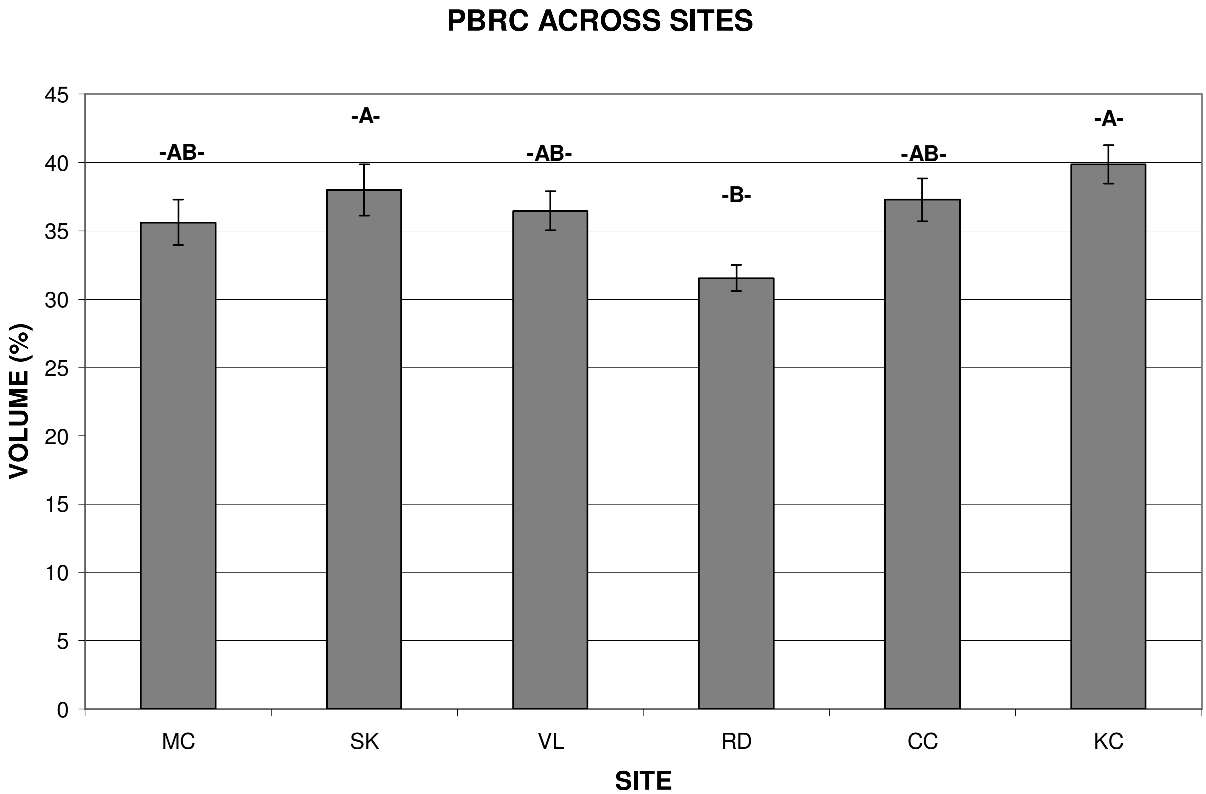

Figure 7. Mean PRBC % of total whole blood volume.

Gill Morphology

Although the total number of gill filaments per individual fish were not significantly different among sites (P=0.367, df=5, $F_{1.10}$) using a one-way ANOVA, there was a moderately positive correlation (R^2=0.4116) when compared to individual fish weight. The number of additional gill branches observed among sites, however, was found to be statistically significant (P<0.001, df=5, $F_{6.98}$). Using a Covariance Matrix, it was determined that 99% of the variability observed in gill branching was due to *A. diminuta* (λ=-0.9769), and the remaining 1% from *E. schwartzi* (λ=-0.2135). Highly significant (P=0.002) strong correlations were found for *A. diminuta* (0.8282) and *E. schwartzi* (0.7201) with additional gill branching (1.0) using a canonical correlation.

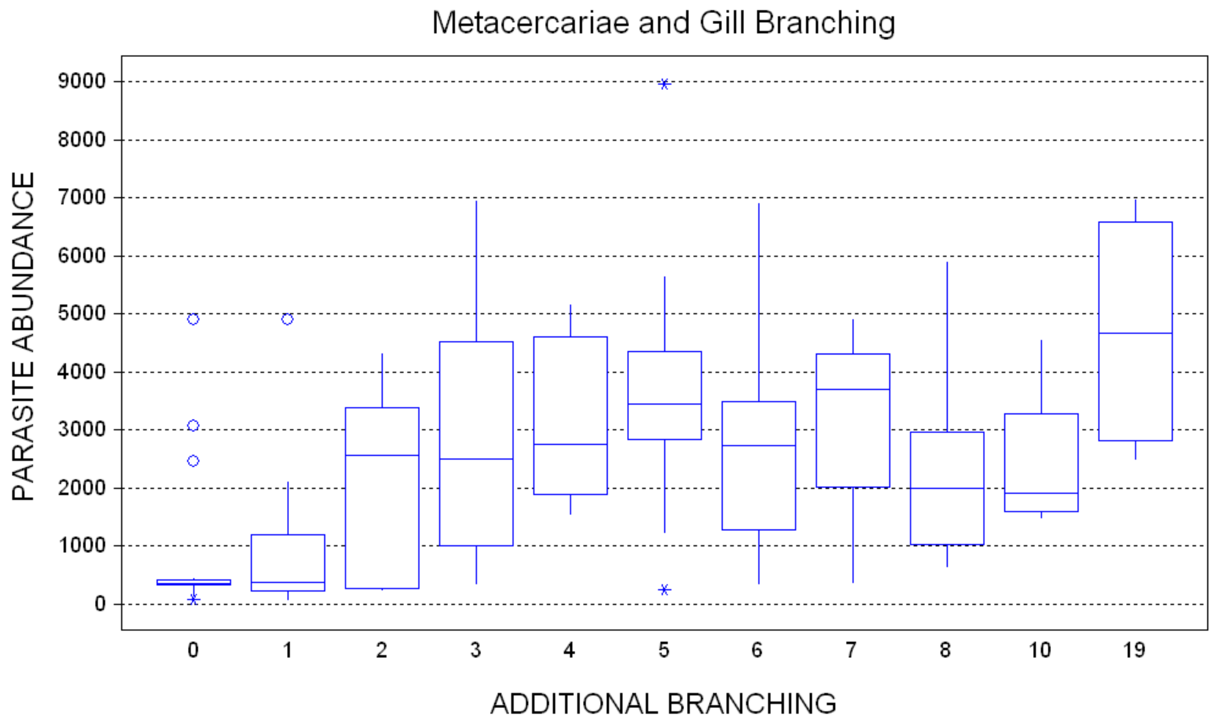

Figure 8. Digenean trematode metacercariae abundance relationship to gill branching.

A Canonical Correlation (Table 5) was run without parasites to compare gill structure and blood with respiration. A highly significant (*P*<0.001), very strong negative correlation was found between respiration (-0.9885) and filaments (0.8012), and to a lesser extent with RBC size (0.4488). A highly significant (*P*<0.001), strongly positive correlation was found between stamina (1.0) and additional branches (0.7249), and to a lesser extent with microhematocrit volume (0.4608). It was negatively correlated with filament number (-0.4312). Additionally, the size of fish RBCs (0.4488) were found to negatively correlate with respiration (-0.9885). As RBCs increased in size, respiration decreased. A less important negative correlation (0.3191) was found between blood volume and respiration (-0.9885), and a larger positive correlation (0.4608) was found with stamina (1.0).

Table 5. Canonical Correlation structure comparing physiological and morphological variables.

	Canonical Variables	
Original Variables	*CV1*	*CV2*
Branches	0.104	***0.7249***
Filaments	***0.8012***	-0.4312
Blood size	0.4488	0.1378
Blood volume	0.3191	0.4608
	W1	W2
Respiration	***-0.9885***	-0.1512
Stamina	-0.008	***1***
Wilks' Lambda $P < 0.001$, $F_{3.60}$		

Snail Abundance

Several snail specimens from the restored sites were dissected to determine whether snails maintained any viable digenean trematode infections. Large cercariae were found occupying the reproductive organs within the visceral mass, rather than the digestive gland where cercariae are typically found. These unknown cercariae are brownish with pronounced black eyes, with dark streaks on both sides of the body. Additionally, extremely large masses of metacercariae were found in the reproductive organs within the visceral mass as well (M. Mazurkiewicz pers. comm.).

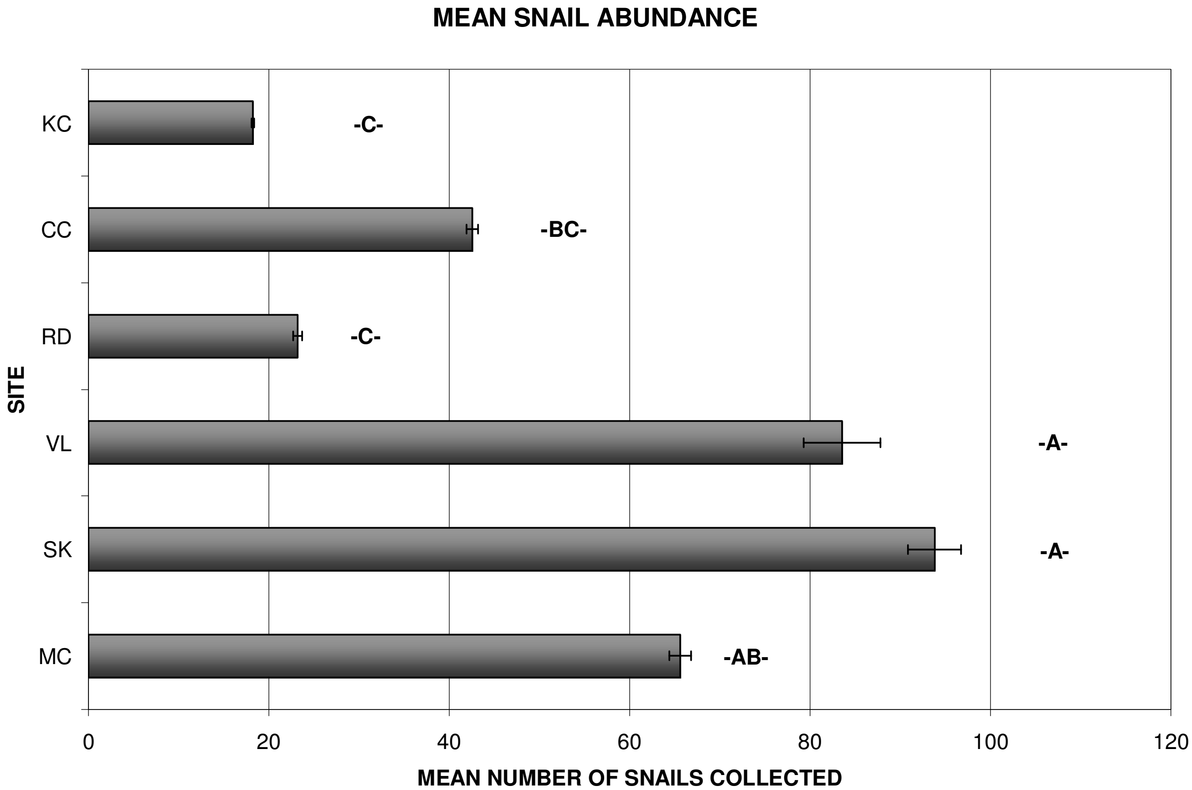

Figure 9. Mean number of snails collected from restored and unrestored sites.

Highly significant differences (P<0.001, df=5, F_{126}) were calculated among sites sampled using a Kruskal-Wallis one-way nonparametric ANOVA, with three groups of homogenous means found with an all-pairwise comparisons test. Overall, SK (580), VL (454), and MC (214); the three restored sites, had more snails collected in total than the three unrestored sites; CC (73), RD (19), and KC (6). SK (mean rank=93.83) and VL (mean rank=83.56) were placed together with similar means in group one. MC was placed as an intermediate with a mean rank of 65.61. These were the sites that had high gill infections. CC (mean rank=42.58) was a second intermediate between MC and the third group containing RD and KC with mean ranks of 23.19 and 18.22 respectively (Figure 9). Finally, although highly significant (P<0.001, R^2=0.2417), no strong correlations were found between gill parasite abundance and snail abundance (Figure 9).

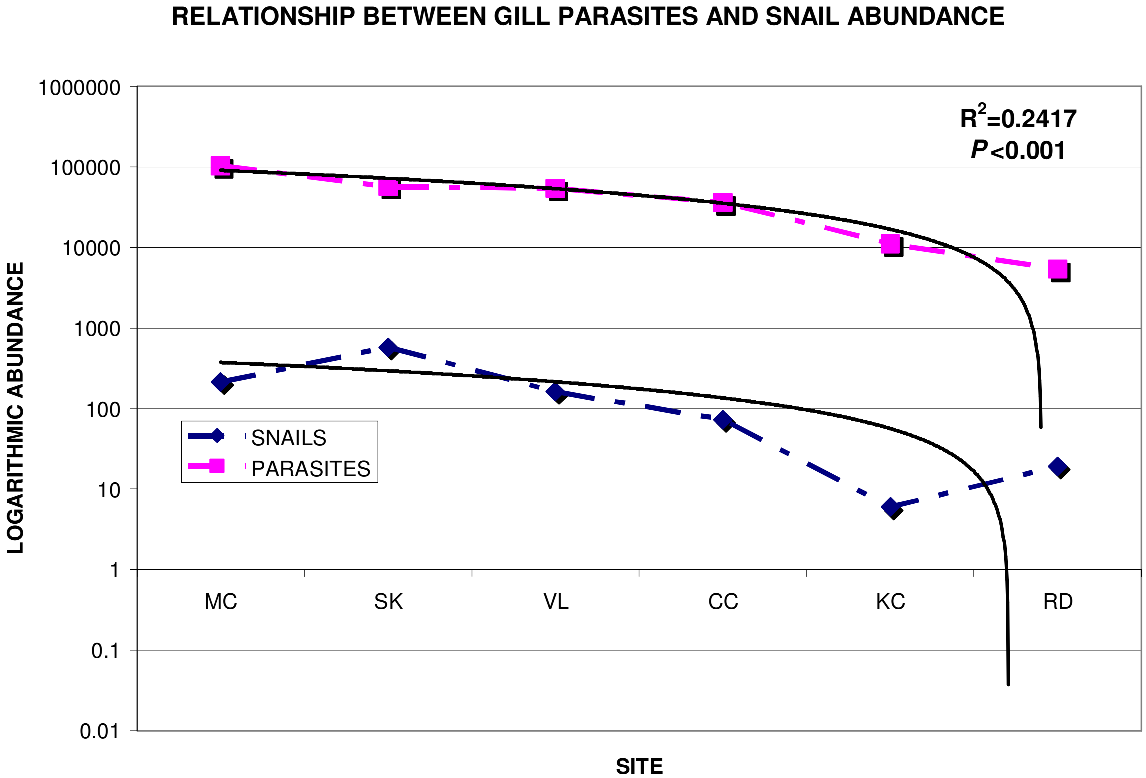

Figure 9. Correlation between gill parasites and snail abundance.

DISCUSSION

Highly significant differences were seen among sites with respect to gill parasite and snail abundance, vertical positioning, conspicuous behaviors, respiration rates, stamina, blood conditions, and gill morphology. Restored sites had the greatest number of gill parasites overall. Though five species were commonly found across all sites, there were two digenean trematode metacercariae that were dominant in both number and prevalence; *A. phagicola* and *E. schwartzi*. Additionally, restored sites had the highest number of snails collected. Although an unrestored site, CC had comparable mean parasite abundances to the restored sites (MC, SK and VL) examined, yet had significantly fewer snails. We believe one reason that this may have been seen is CC's relatively close proximity to the 206-acre wetland mitigation bank, Marsh Resources, Inc. (MRI). Doctor's Creek flows through this restored tidal marsh connecting directly to the Hackensack River, which flows downstream past Cedar Creek. If MRI is similar to the other restored sites examined in this study, one can presume that there may be a large snail population supporting parasite reproduction. Most digenean trematode cercariae live approximately 24 hrs. and are sophisticated swimmers with muscular tails (Lafferty, 2002). This infective stage has been previously observed (Jimenéz-García and Vidal-Martínez, 2005) traveling distances of approximately 50 m by means of both active (e.g., swimming) and passive (e.g., water currents) transport. However, it is unknown whether the tidal currents within the Hackensack River are 1) strong enough to carry the cercariae far enough downstream to CC, or 2) whether the cercariae will survive and/or remain infective long enough to reach CC. Another possibility is that there is a mixing of *F. heteroclitus* populations between the two sites. Although their typical home range is approximately 38 m along tidal creek banks (Lotrich, 1975), some have been known to move as much as 375 m, and up to 15 ha within [restored] salt marshes (Teo and Able, 2003). Once again, if it is presumed that MRI is similar to the other restored sites examined in this study, its fish population should also be highly infected with digenean metacercaria. Either or both of these two scenarios would help explain the large number of digenean trematodes observed at CC. Additionally, as suggested in the Box and Whisker plot (Figure 8), there appears to be a very strong relationship between high gill parasite abundances (median infections greater than 1000 metacercariae), and the number of additional gill branches observed.

Overall, fish with the highest parasite infections (VL, SK and MC) spent as much as 46% of their time at the water's surface. This is highly unusual behavior, as it has been reported by Bretsch and Allen (2006) that like other species trying to avoid predators, *F. heteroclitus* prefer deeper waters rather than shallow habitats. The highly significant differences seen in parasitized fish's overall preference to remain at the surface in addition to their increased conspicuousness, indicates that the heavily parasitized fish from restored sites are more likely to be predated upon by picivorous birds than at unrestored sites. One of several studies by Bethel and Holmes (1973) investigating behavioral modifications using an amphipod-acanthocephalan parasite-host system reported overt behavioral changes of amphipods (*Gammarus lacustris*) infected with *Polymorphus paradoxus* cystacanths. Amphipods carrying stages that were infective to the definitive host, the mallard duck (*Anas platyrhynchos*), behaved in more conspicuous ways, becoming positively phototactic, and spending more time skimming at the water's surface, and clinging to floating objects, thus increasing potential predatory risk. Consequently, another experimental study using a heron as a predator, confirmed the suggestion that the risk of predation increases for fish nearer the surface (Kramer *et al.*, 1983).

Highly significant differences were also found for respiration with RD having the highest respiration rate of all sites tested, which inherently makes sense. The parasitized fish's gills are having an increasingly difficult time extracting dissolved oxygen from the liquid medium. This is contrary to what has been published on parasite affects on host respiration. Studies using the three-spined stickleback (*Gasterosteus aculeatus*) have shown that parasitized individuals not only have higher respiration rates, but also have higher resting, routine and maximum activity levels than healthy, unparasitized fish (Lester, 1978; Giles, 1987 and others). However, in these studies, the parasites infecting *G. aculeatus* are cestodes found in the gut. One study by Anderson (1975) using shrimp (*Palaemonetes pugio*) infected with the parasitic isopod *Probopyrus pandalicola,* reported that in most instances, infected shrimp had lower rates of oxygen consumption than unparasitized conspecifics, which is comparable to what is reported herein. These findings may be more similar to the Anderson (1975) study as both are examining parasites that are associated with, and directly affecting respiratory structures.

Stamina was also found to be significantly different among sites, as were RBC size and volume (PBRC). RD stood out from the other five sites, in that these fish not only had the lowest stamina, but also the smallest RBCs when compared to other sites. This was surprising as studies have consistently shown an inverse relationship between erythrocyte size and aerobic swimming ability (Dawson, 1933; Wells and Baldwin, 1990; Wilhelm Filho *et al.*, 1992). This relationship is due to the larger surface area to volume ratios and shorter diffusion distances allowing more rapid oxygenation and deoxygenation of hemoglobin as RBC volume decreases (Holland and Forster, 1966; Jones, 1979). The fact the RD also had the lowest PRBC percentage, more than likely influenced this outcome. The other five sites were more similar with higher staminas, larger RBC sizes and greater volumes. Studies have shown that if either of these two blood factors are too high, it may be an indication of stress polycythemia (Wedemeyer and Yasutake, 1977) which is a condition characterized by an abnormal increase in the number of RBCs in the blood due to stressful conditions. Stamina was found to be strongly correlated with the number of branches observed in fish. Our results show that MC, VL and CC had high stamina and many branches. However, SK had many branches, but lower stamina. Concurrently, KC had the fewest branches, but one of the highest stamina levels. There is obviously more to this story which cannot be explained in this study.

One study by Huspeni and Lafferty (2004) used larval trematode parasites as an indicator of restoration success and found that trematode prevalence nearly quadrupled at restored sites (43%) while control sites remained unchanged (26%). The restoration grading process likely increases potential habitat for both pelagic (e.g., fish as second intermediate host) and benthic (e.g., snails as first intermediate host) organisms. These newly established communities then attract wading birds (final/definitive hosts), thus enriching the wetland's trematode community from their usage. High prevalence and abundance of parasite species may indicate a healthy environment, presumably meaning that the necessary intermediate and definitive hosts are present (Overstreet 1997). Although there seems to be a developing pattern between parasite abundance and the restoration process, there are several other plausible explanations behind this phenomenon. Whether they are acting singly or in conjunction with other factors needs to be studied further. The Hackensack Meadowlands is a heavily degraded tidal marsh system and continually receives additional anthropogenic inputs from several sources. One source comes from the Bergen County Utility Authority (BCUA). In their paper, Coyner et al. (2003) discuss two means by which anthropogenic eutrophication of wading bird foraging areas with sewage effluent may be contributing to the increased prevalence of infected intermediate hosts (fish). "First, eutrophication can result in increased fish densities that will attract larger numbers of birds. Secondly, attraction of larger numbers of birds increases the chances of an infected bird contaminating the site" (Coyner et al., 2003). This coupled with the fact that the restoration grading process in itself also likely increases the number of intermediate hosts utilizing the area (e.g., fish and snails) by improving overall habitat quality, can have significant impacts. Highly significant differences in abiotic conditions (e.g., DO and salinity) were found between restored and unrestored sites. Surface DO levels at restored sites ranged from 5.3 to 5.6 mg/g and were higher than unrestored sites (ranging from 4.5 to 4.8 mg/g). Salinity was lowest (5-6 ppt) at the restored sites (MC, SK, VL), and higher (10-11 ppt) at the unrestored sites (RD, CC, KC). It is unknown if these environmental differences among sites have contributed to the high levels of infection, or to the behavioral and physiological differences found.

The wetland restoration process, however beneficial in the long run, is still an act of disturbance with activities such as grading to increase tidal flow, removal of invasive vegetative species, etc. For those systems that are able to rebound for one reason or another to become what is thought to be healthy marsh, then we can say that the restoration was a success. However, in areas that are not as quick to return to their originally intended state, problems (e.g., decreased parasite diversity) may linger. Such problems may include competition among prey species that have now been released from their parasites or difficulties in catching parasite-free fish by piscivorous birds (Huspeni and Lafferty, 2004). On the other hand, avian predators may potentially be at risk by eating a disproportionate number of highly infected prey items as may be the case in the Hackensack Meadowlands. It has already been shown that migratory birds are more apt to suffer parasite infections than their residential cohorts (Figuerola and Green, 2000), and in response, have subsequently increased the size of their immune defense organs (the bursa of Fabricius and the spleen) over evolutionary time (Møller and Erritzøe, 1998). Although, many researchers have not been able to find differences in host [bird] fitness when comparing infected and non-infected individuals, differences in the level of infection intensity (e.g., heavy vs. light), do come to light (Hudson, 1999). In fact, it has been suggested by McNeil et al. (1994) that heavy helminth infections may be an important factor responsible for aberrant migratory behaviors demonstrated in shorebirds due to the numerous physical and/or physiological disorders they cause.

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