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Physiological Condition of Southwestern Willow Flycatchers in Native and Saltcedar Habitats

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EXECUTIVE SUMMARY

The Southwestern Willow Flycatcher *(Empidonax traillii extimus)* is a federally-listed endangered species that breeds in dense native- and exotic-dominated riparian habitats, including monotypic introduced saltcedar (*Tamarix ramosissima*). Some theorize that saltcedar is unsuitable habitat for the flycatcher, primarily because it generally supports a smaller and less diverse invertebrate community (and hence flycatcher food base) than native habitats (e.g., *Salix* spp.). However, differences in insect communities between native and saltcedar habitats are not proof that saltcedar habitats are inferior. The only way to evaluate whether the habitats differ in terms of dietary or energetic quality is to document actual food limitation and/or its manifestations. Measurements of an individual's body condition and metabolic state can serve as indicators of environmental stresses such as food limitation and environmental extremes. We captured 130 Southwestern Willow Flycatchers breeding in native and saltcedar habitats in Arizona and New Mexico, and measured 12 parameters of physiological condition. These parameters included body mass, fat level, body condition index, hematocrit, plasma triglycerides, plasma free fatty acids and glycerol, plasma glucose and beta-hydroxybutyrate, plasma uric acid, total leukocyte count, and heterophil to lymphocyte ratio. We found substantial sex-based differences in the condition of male and female Willow Flycatchers; 10 of the 12 physiological condition parameters varied significantly between the sexes. In all cases where male and female condition varied (except mass - males are generally larger than females), the differences suggest that males are experiencing higher stress (i.e., poorer condition) than females. We found few habitat-based differences in flycatcher condition. Only three of the 12 physiological condition parameters varied significantly between habitats. Differences in triglyceride and glycerol levels suggest that saltcedar may provide better energetic/dietary conditions than native habitats. Higher uric acid levels among flycatchers nesting in saltcedar could indicate a higher protein diet, or that the flycatchers are under food stress and metabolizing body protein; the former interpretation is more consistent with our other results. Our data show that, at least in some parts of the flycatcher's range, there is no evidence that flycatchers breeding in saltcedar habitats exhibit poorer nutritional condition or are suffering negative physiological affects.

INTRODUCTION

The Southwestern Willow Flycatcher *(Empidonax traillii extimus)* is a federally-listed endangered species (USFWS 1995) that breeds in dense riparian habitats along rivers, streams, lakes, and other wetlands. Flycatchers breed in a diverse array of riparian habitats (Sogge and Marshall 2000), including those dominated by native species such as willow (*Salix* spp.), cottonwood (*Populus* spp.), and boxelder (*Acer negundo*). However, Southwestern Willow Flycatchers also breed in habitats dominated by introduced species such as saltcedar (*Tamarix ramosissima*) and Russian olive (*Elaeagnus angustifolia*). Use of saltcedar is extensive; rangewide, 25% of Southwestern Willow Flycatcher territories are found in habitats dominated by saltcedar (Sogge et al. 2002). In Arizona, over 75% of flycatcher nests found between 1995 and 2000 were in saltcedar habitats (Paradzick et al. 2001).

Saltcedar has been implicated as a causative factor in the decline of some southwestern bird species and communities (e.g., Hunter et al. 1997), and DeLoach and Tracy (1997) and DeLoach et al. (2000) propose that saltcedar habitats are unsuitable for the flycatcher. If saltcedar habitats are suboptimal or detrimental to the flycatcher, then flycatcher conservation and recovery efforts should emphasize saltcedar control/removal and replacement with native vegetation. On the other hand, if saltcedar-dominated habitats provide adequate resources and a suitable environment for breeding flycatchers, saltcedar control/removal activities may not be needed and could even be detrimental in some cases.

DeLoach and Tracy (1997) and DeLoach et al. (2000) argue that saltcedar is detrimental to Southwestern Willow Flycatchers because (among other things) saltcedar habitats do not provide adequate food resources. Although saltcedar plants generally host fewer insects than do native willows

(see review in DeLoach et al. 2000), and recent studies (Drost et al. 1998, Drost et al. 2001) have shown that flycatcher diet varies between saltcedar and native habitats, differences in insect communities and flycatcher diet between native and saltcedar habitats are not in themselves proof that saltcedar habitats are inferior or detrimental. The only way to evaluate whether the habitats differ in terms of dietary or energetic quality is to document actual food limitation and/or its manifestations.

One way to investigate manifestations of food limitation is by measuring parameters that reflect the nutritional status of an individual. Simple clinical screening methods are available that can accurately quantify a wide variety of parameters which are useful in assessing the nutritional and immunological health of wild animals. Plasma metabolite levels depend strongly on food intake, so differences in daily pattern of food intake and changes in body mass (e.g., from differences in diet) may be reflected in concentrations of different plasma metabolites (Jenni-Eiermann and Jenni 1994). Caloric and nutritional deficits can also be manifested in the immunological condition of the individual (Glick et al. 1983, Lochmiller et al. 1993).

We used the following physiological parameters to characterize individual physiological condition of Willow Flycatchers breeding in salt cedar and native habitats.

Body Condition and Hematocrit

Fat level and Condition Index: Fat is the primary form of energy storage in birds, and energy stores fluctuate in accordance with dietary intake and metabolic demands (Blem 1990). Birds with higher levels of fat have had higher caloric intake relative to energy output than those with lower levels. Fat storage is often estimated by quantifying the amount of visible subcutaneous fat deposited in the

abdominal and furcular regions (Helms and Drury 1960). A condition index can also be used to determine the relative fat level of an individual. When standardized for body size, mass is a reasonable predicator of an individual's fat content (Odum 1960); this standardization is accomplished by dividing body mass by wing length cubed (g/mm^3) multiplied by 10,000 (per Winker et al. 1992).

Hematocrit: Hematocrit is the ratio of red blood cell volume to total blood volume (plasma + red blood cells), and is a widely used serological test to assess a bird's nutritional state (Amand 1986). A low hematocrit can indicate anemia or other mineral deficiencies or bacterial and parasite infections (Campbell and Dein 1984). A high hematocrit may be caused by dehydration (Quesenberry and Hillyer 1994, Work et al. 1999) or elevated oxygen consumption accompanying an intense workload (Carpenter 1975). Hematocrit may also reflect the reproductive activity of birds, being higher in breeding males due to increased testosterone levels (Sturkie 1986).

Plasma Metabolites

Triglycerides: In birds, energy is stored primarily as fat in the form of triglycerides - three fatty acid molecules attached to a glycerin backbone (Blem 1990). Triglycerides come directly (or via synthesis in the liver) from the diet, and presence of plasma triglycerides indicates that fats are being produced (lipogenesis) and transported to peripheral tissues (especially adipose tissue). Birds gaining mass will have higher levels of triglycerides (Jenni-Eiermann and Jenni 1994); those that are fasting or under food stress will have lower levels of plasma triglycerides than will normally feeding birds (Jenni-Eiermann and Jenni 1997).

Free Fatty Acids and Glycerol: When the body begins to metabolize stored fats (from adipose tissue), triglycerides are hydrolyzed (broken down) into free fatty acids (NEFAs; non-esterified fatty

acids) and glycerol before entering the bloodstream for transport to muscles and other organs. Levels of plasma NEFA and glycerol increase when birds are food stressed and use stored fats for their energy needs (Jenni-Eiermann and Jenni 1994, 1997).

Glucose: Glucose is the primary carbohydrate converted via the body's metabolic pathways into chemical energy to fuel metabolism. Plasma glucose levels are generally kept within narrow limits, but Swain (1987) reported hypoglycemia in birds fasted overnight, and Jenni-Eiermann and Jenni (1997) found a transient hypoglycemia that did not persist in fasting individuals.

Beta-hydroxybutyrate: When blood glucose levels fall (e.g., due to food stress), the liver synthesizes beta-hydroxybutyrate (BOHB), which replaces part of the decreased glucose, especially for use by the brain (Robinson and Williamson 1980). High levels of BOHB indicate lipid catabolism, glucose shortage, and fasting (Jenni-Eiermann and Jenni 1997).

Uric Acid: Uric acid is the end product of protein metabolism and the primary nitrogenous waste product of birds. High levels of uric acid may indicate a high protein diet or dehydration (Hochleithner 1994) and/or protein degradation during fasting (Anthony et al. 1990); low levels can indicate short-term food stress (Jenni-Eiermann and Jenni 1994).

Hematological Indices

Total leukocyte count : Leukocytes are white blood cells that fight infection and disease, and a sufficient number of circulating leukocytes is essential for an organism to produce an immune response against a bacterial or parasite infection. Total white blood cell (WBC) count is a widely used method of

assessing an organism's health (Gustafsson et al. 1994; Saino et al. 1997). Changes in WBCs, whether leukocytosis (elevated WBC count) or leukopenia (depressed WBC count), reflect ongoing disease processes of bacterial, parasitic or viral origin. Leukopenia and fluctuations in the types of leukocytes can also indicate stress on the system by a non-etiological process such as severe malnutrition (Gershwin et al. 1985), strenuous exercise (Mackinnon 1992; Nieman et al. 1989), or significant weight loss (Stinnett 1983).

Heterophil to Lymphocyte Ratio: Heterophils are phagocytic leukocytes that do not require prior exposure to an antigen in order to produce an immune response; they are part of the first line of defense against antigenic challenge. Lymphocytes are leukocytes that comprise the acquired immune response; to mount an immune response lymphocytes must be specific for each antigen. Studies show that the heterophil to lymphocyte ratio (H/L ratio) increases in response to a variety of stressors including food or water deprivation, malnutrition, injury, and extreme temperatures (Gross and Siegel 1986; Tripathi and Bhati 1997, Work et al. 1999, Vleck et al. 2000); multiple stressors usually have an additive effect (McKee and Harrison 1995). Animals respond to periods of food deprivation and malnutrition by increasing heterophil number and correspondingly decreasing lymphocyte numbers (Gross and Siegel 1986; Tripathi and Bhati 1997).

Most studies of physiological condition in passerines have focused on the post-breeding and migration period; little research has been conducted to characterize passerine condition during the breeding period (but see Horak et al. 1998a, Horak et al 1998b, Ots et al. 1998, Kern et al. 2001). To date, no studies have addressed the physiological and/or immunological condition of the Willow Flycatcher, even though those parameters of condition are influenced by the environment, may affect fitness, and can

serve to compare the relative condition of individuals in different sites or habitats. Therefore, we investigated whether measures of flycatcher physiological condition differed among individuals breeding in native and saltcedar habitat types. If saltcedar habitats produce greater physiological challenges (e.g., food and/or water limitations, disease) than native habitats, birds breeding in exotic habitats will have lower values for mass, fat level, body condition index, hematocrit, and triglycerides. In contrast, WBC count, H/L ratio, FFA, glycerol, uric acid, and BOHB should be higher in poor quality patches.

METHODS

The study was conducted during the summers of 1999 and 2000 in southcentral Arizona and southwestern New Mexico (Fig. 1). We captured, measured, and took blood samples from Southwestern Willow Flycatchers (SWWF) breeding in both native and exotic habitats. The dominant plant species within the native breeding sites were willow (*Salix* spp.), cottonwood (*Populus* spp.), and boxelder (*Acer negundo*) with less than a 15% saltcedar component. The exotic sites consisted of monotypic stands of saltcedar (*Tamarix ramosissima*) with less than a 10% native component. Native sites were located at the Salt River inflow to Roosevelt Lake, the Lower San Pedro River, and the middle Gila River in Arizona, and the Cliff/Gila valley in New Mexico. Exotic-dominated patches were found at the Tonto Creek and Salt River inflows to Roosevelt Lake, the Lower San Pedro River, the Gila River near the San Pedro River confluence, and the middle Gila River in Arizona. All sites were between 560 and 1370 m elevation.

The blood chemistry of a bird can vary with season and time of the day. In females, there is a 10 fold increase in plasma triglycerides associated with yolk production (Bacon et al. 1974). Therefore, we concentrated our capture efforts during the month after females had finished egg laying and before the young fledged the nest. Dates of capture were during June 5 – July 7 in 1999 and June 1 – June 30 in 2000. In addition, triglyceride levels can rise sharply during the early morning feeding and then level off after midmorning (Jenni-Eiermann and Jenni, 1997). In the same way, levels of BOHB are significantly lower in the early morning as compared with other times of the day. For this reason, we began sampling birds 3 hours after sunrise with daily capture times being between 0800 – 1100 hrs.

Birds were live-captured using a targeted mist netting technique (Sogge et al. 2001) whereby flycatcher songs and calls were broadcast to lure territorial flycatchers into nets. Flycatchers were removed from the nets immediately upon capture, and fitted with an aluminum numbered federal band. We captured 130 adult SWWFs at 11 different breeding sites (Table 1); 55 were captured in exotic (saltcedar) habitats, and 75 in native habitats. Birds were sexed by presence of a brood patch (females) or cloacal protuberance (males). For birds that could not be reliably sexed in the field, we determined sex by genetic analysis per Griffiths et al. (1998).For each bird we collected the following information: age, sex, fat score (Helms and Drury 1960), wing chord length, bill width, culmen length, and mass to the nearest 0.1g.

Table 1. Location and number of Willow Flycatchers captured and sampled.

	1999	2000	Total
Exotic (saltcedar) Sites			
Gila/Lower San Pedro River Confluence, AZ	5	8	13
Roosevelt Lake - Salt River Inflow, AZ	14	5	19
Roosevelt Lake - Tonto Creek Inflow, AZ	6	0	6
Middle Gila River, AZ	0	17	17
Total	25	30	55
Native Sites			
Lower San Pedro River, AZ	11	15	26
Roosevelt Lake - Salt River Inflow, AZ	0	14	14
Middle Gila River, AZ	0	11	11
Gila/Cliff, NM	0	24	24
Total	11	64	75

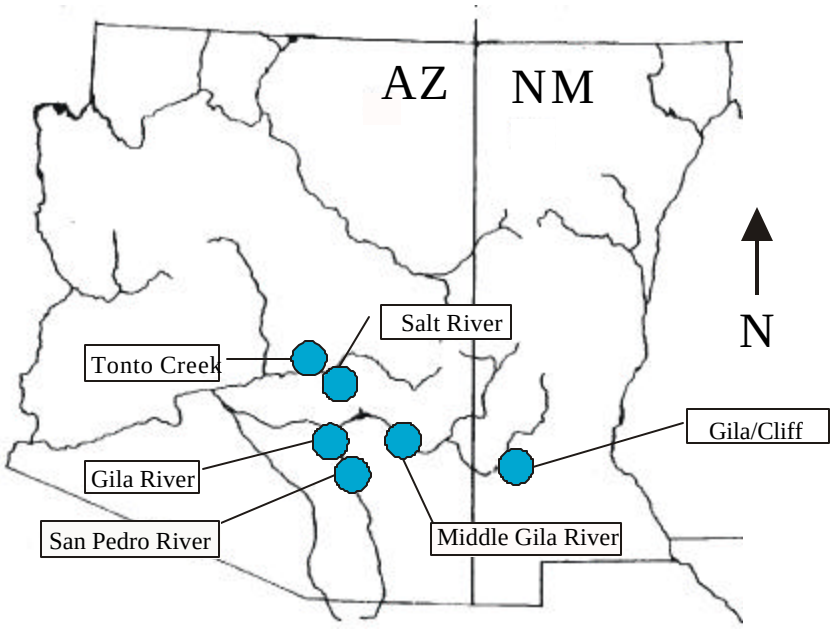

Figure 1. Map of study sites at which Willow Flycatcher physiological condition measurements were taken.

Immediately following capture, blood was taken by lancing the brachial vein with a 26 ½ guage syringe needle and drawing blood into 75-microliter heparinzed capillary tubes. Approximately 50 – 125 microliters were collected from each bird and immediately placed on ice. Next, a drop of blood was placed on a glass slide and a thin smear was made using a beveled slide. Blood smears were air-dried, fixed with 100% methanol, and air-dried again. For identification of white blood cells, slides were stained with combination Wright and Giemsa stains. Absolute WBC counts were determined by counting the total number of leukocytes per 10,000 red blood cells (RBCs) (Campbell and Dein 1984). Differential counts were done by counting individual WBC types, i.e. lymphocytes, monocytes, heterophils, eosinophils, and basophils in 100 fields of view (FOV) using oil immersion (1000x).

Within 6 hours of blood collection, we spun the capillary tubes in a clinical centrifuge for 9 minutes at 14,000 RPMs. Using digital calipers, we determined hematocrit by measuring height of red blood cell (RBC) layer and total blood sample. Blood plasma was separated from RBCs, stored frozen, then shipped to Ohio State University Department of Animal Sciences for plasma constituent analysis under the direction of Dr. Wayne Bacon.

Plasma metabolites were assayed using standard reagent kits that require small amounts of blood, ranging from 3 to 12 microliters. Glucose, uric acid, BOHB, glycerol, and triglyceride levels were determined by quantitative enzymatic tests (Sigma Chem. Co.) and NEFA (Wako Chemicals USA, Inc.). Intra – assay coefficients of variation ranged from 0.2% (triglycerides) to 3.7 % (glycerol) for the various assays. Inter – assay coefficients of variation ranged from 1.1% (glycerol) to 7.0% (uric acid).

We conducted preliminary analyses to determine if the condition indices varied with year; only NEFA varied between years. For all subsequent analyses, NEFA was separated by year. Because the process of egg production causes exceptionally high levels of triglycerides, females with triglyceride levels of 800 mg/dL or greater were excluded from the triglyceride analysis.

We tested for the effect of habitat type and sex on variation in nutritional and immunological condition with ANOVA models, using type III sums of squares; statistical significance was set at $P \leq 0.05$ All significance levels refer to two-tailed tests. White blood cell count, heterophil/lymphocyte ratio, and condition were log-transformed to normalize their distribution; all other parameters met the assumptions of normality. All analyses were conducted using SPSS statistical software.

RESULTS

Physiological condition indices can vary between the sexes, especially during the breeding season; therefore, we present results by sex. Because not all parameters were measured for all individuals (primarily due to differences in the amount of blood taken per individual), sample sizes varied among parameters (Table 2).

Body Condition and Hematocrit

We found no significant differences between habitats for mass, fat, body condition index, and hematocrit; significant main effect differences for sex were noted in three of the four parameters, excluding mass (Table 3). Females had higher fat and condition index levels, and lower hematocrit than males (Table 2, Figure 2).

Plasma Metabolites

There were significant differences among habitats for triglycerides, glycerol, and uric acid, but not for BOHB, FFA, or glucose (Table 3). We found significant differences by sex for all plasma metabolites except NEFA (Table 3). Females had higher levels of glucose, triglyceride, and uric acid, and lower levels of BOHB. Glucose showed a significant interaction effect between sex and habitat (Table 3), such that males in saltcedar had lower glucose levels than those in native habitats, while females showed the opposite trend (Figure 4).

Table 2. Condition indices for Willow Flycatchers in native and exotic habitats. Sample sizes vary among parameters because not all parameters were measured for all individuals. Column "Sig Diff" indicates whether there were statistical differences (2-way ANOVA at $P < 0.05$) among year, sex, or habitat (see also Table 3).

	NATIVE			EXOTIC			
Condition Parameter	n	Mean	SE	n	Mean	SE	Sig Diff
Body Condition and Hematocrit							
Mass (g)							
Female	26	11.6	0.49	14	10.42	1.19	sex
Male	39	12.2	0.12	31	11.98	0.42	
Fat Level							
Female	26	1.83	0.15	15	1.50	0.24	sex
Male	37	0.68	0.09	32	0.63	0.11	
Condition Index							
Female	27	0.37	0.02	14	0.31	0.04	sex
Male	37	0.34	0.04	29	0.35	0.01	
Hematocrit							
Female	25	0.48	0.01	13	0.48	0.01	sex
Male	37	0.51	0.004	26	0.50	0.005	
Plasma Metabolites							
BOHB (mg/dL)							
Female	18	11.9	1.38	5	14.57	3.12	sex
Male	35	20.41	1.21	16	19.70	1.84	
Uric Acid (mg/dL)							
Female	27	19.67	1.46	14	26.00	2.10	sex,
Male	40	16.69	1.00	32	20.16	1.18	habitat
Glucose (mg/dL)							
Female	24	332	13.8	13	373	14.8	sex
Male	40	329	7.55	32	314	8.81	
NEFA (micromol/mL)							
Female	24	3.06	0.06	15	3.32	0.07	year
Male	40	3.05	0.04	32	3.20	0.05	
Triglycerides (mg/dL)							
Female	27	144	12.1	15	191	38.1	sex,
Male	40	109	3.13	32	119	4.11	habitat
Glycerol (mg/dL)							
Female	27	39.44	3.21	15	31.74	2.77	sex,
Male	40	43.58	1.69	32	41.00	1.93	habitat
Hematological Indices							
WBC Count							
Female	24	26.69	4.94	11	28.23	4.65	sex
Male	36	12.42	2.29	26	10.07	1.18	
Heterophil/Lymphocyte Ratio							
Female	23	0.36	0.06	10	0.32	0.05	--
Male	33	0.46	0.10	19	0.93	0.46	

Table 3. Two-way ANOVA results for physiological condition parameters of Willow Flycatchers breeding in saltcedar and native habitats. Statistical significance level was set a-priori at $P < 0.05$.

(A) Fat, body condition, and hematocrit

	Mass		Fat		Body Condition		Hematocrit	
Effect	F	P	F	P	F	P	F	P
Sex	5.02	0.027	51.636	<0.001	74.34	<0.001	18.171	<0.001
Habitat	2.02	0.158	1.793	0.183	3.068	0.083	0.600	0.441
Sex x habitat	0.899	0.345	0.96	0.329	1.089	0.299	0.176	0.676

(B) Plasma metabolite levels

	Beta-Hydroxybutyrate		Triglycerides		Glycerol		Uric acid	
Effect	F	P	F	P	F	P	F	P
Sex	11.223	0.001	17.85	<0.001	7.38	0.008	9.828	0.002
Habitat	0.227	0.635	5.17	0.025	4.193	0.043	12.123	0.001
Sex x habitat	0.683	0.411	2	0.16	1.097	0.297	1.039	0.31

	NEFA -1999		NEFA - 2000		Glucose	
Effect	F	P	F	P	F	P
Sex	0.343	0.563	1.227	0.272	7.552	0.007
Habitat	0.011	0.917	3.922	0.051	1.22	0.272
Sex x habitat	1.0115	0.322	3.816	0.054	6.313	0.014

(C) Hematological Indices

	WBC Count		Heterophil/Lymphocyte Ratio	
Effect	F	P	F	P
Sex	21.083	<0.001	2.066	0.154
Habitat	0.13	0.909	0.740	0.392
Sex x habitat	0.304	0.583	1.089	0.300

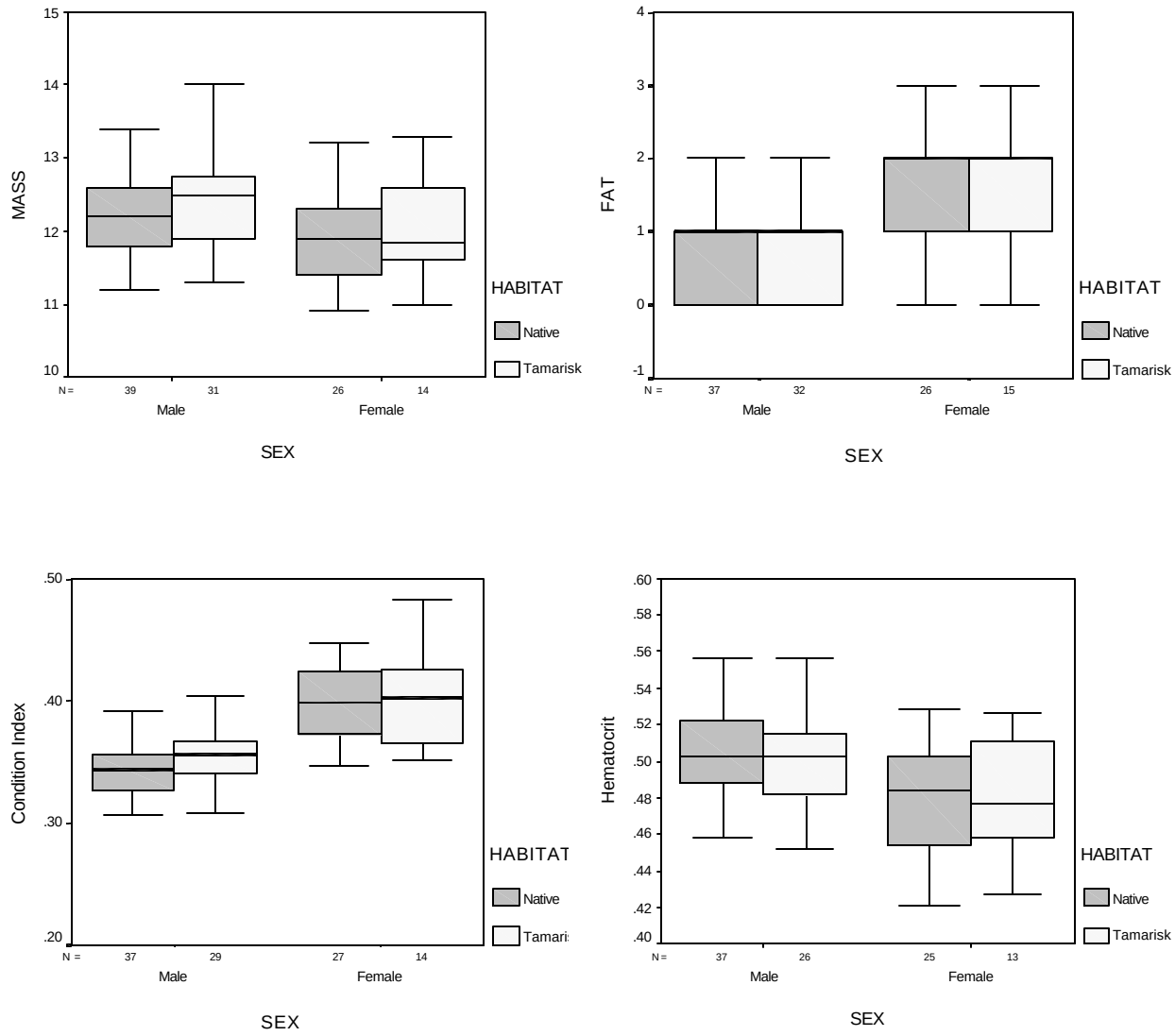

Figure 2. Comparison of Willow Flycatcher mass, fat level, condition index, and hematocrit, by sex and breeding habitat type. Differences between sexes are significant at $P < 0.05$ for all parameters; differences between habitats are not (see Table 3). Boxes represent the 25th to 75th percentile, the whiskers extend to highest and lowest values excluding outliers, and the line within the box represents the median value.

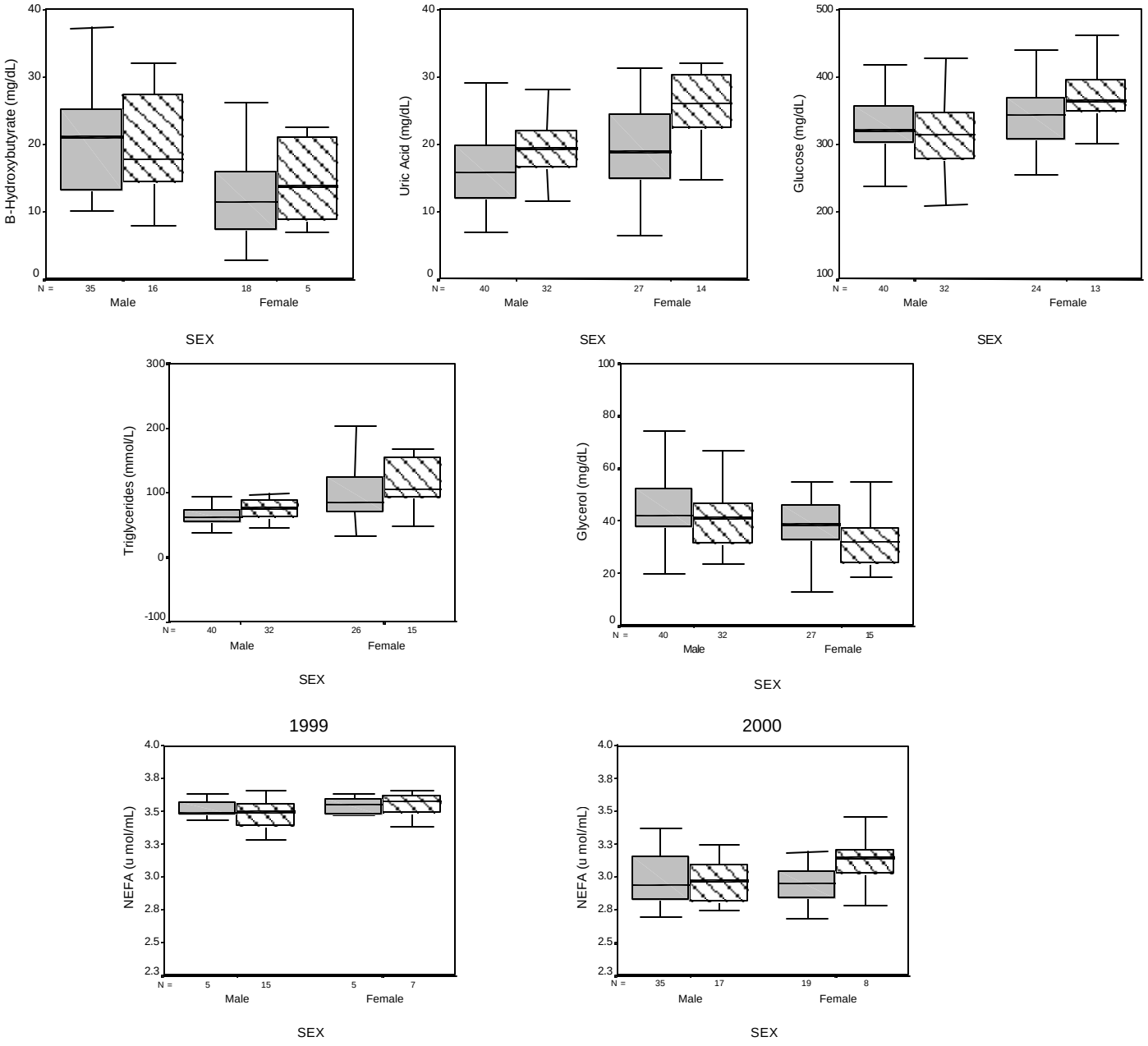

Figure 3. Comparison of Willow Flycatcher plasma metabolites by sex and breeding habitat type. Habitat differences for triglycerides, glycerol and uric acid are significant at $P< 0.05$; sex differences for all parameters except NEFA are significant (see Table 3). Boxes represent the 25^{th} to 75^{th} percentile, the whiskers extend to highest and lowest values excluding outliers, and the line within the box represents the median value.

Hematological Indices

There were no habitat differences in WBC or H/L ratio (Table 3; Figure 4). Females had a significantly higher WBC count than males, but H/L ratios did not differ significantly (Tables 2 and 3, Figure 4).

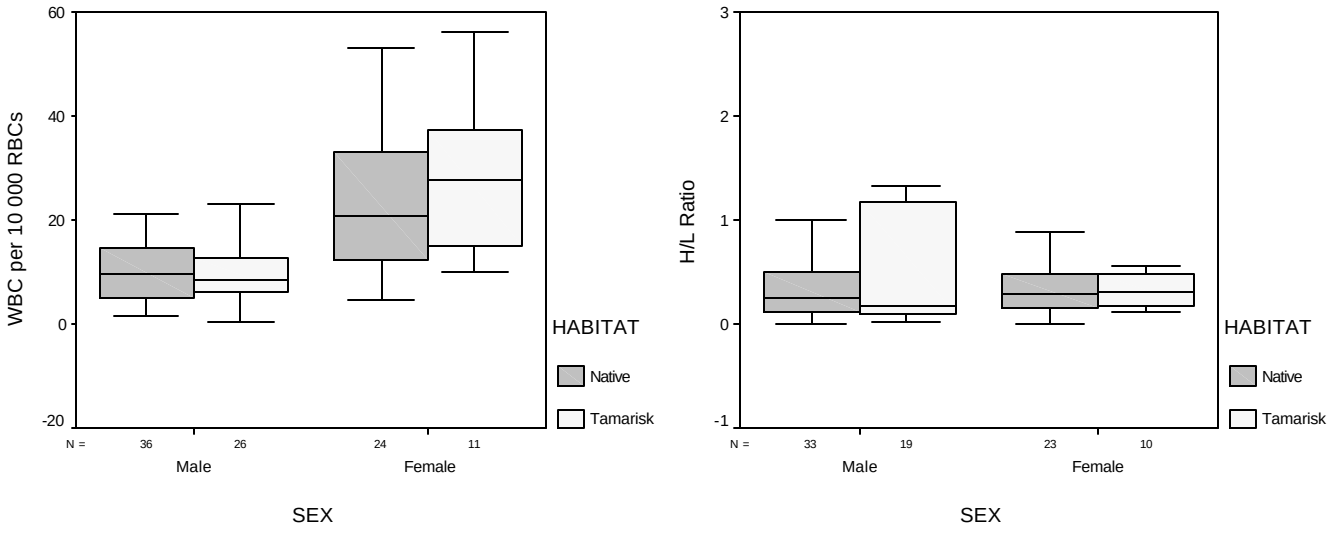

Figure 4. Comparison of Willow Flycatcher WBC count and H/L Ratio, by sex and breeding habitat type. Neither parameter differed significantly between habitats (see Table 3). Differences between sexes are significant for WBC (at $P < 0.05$) but not H/L ratio. Boxes represent the 25^{th} to 75^{th} percentile, the whiskers extend to highest and lowest values excluding outliers, and the line within the box represents the median value.

DISCUSSION

Body mass, fat levels, and plasma metabolites depend strongly on food intake; therefore, differences in daily patterns of food intake (caused by weather, differences in diet, etc.) may cause differences in daily patterns of these parameters (Jenni-Eierman and Jenni 1994). Similarly, hematological parameters (e.g., WBC, H/L Ratio) reflect longer-term stresses such as food or water limitations, injuries, or disease processes; multiple stressors usually have an additive affect (Vleck et al. 2000). Therefore, the physiological condition parameters that we measured should provide insights into both short-term and longer-term environmental stresses that might be associated with breeding in saltcedar habitats as compared to native habitats.

Sex Differences

We found substantial sex-based differences in the condition of male and female Willow Flycatchers; 10 of the 12 physiological condition parameters varied significantly between the sexes (Table 3). In all cases where male and female condition varied (except mass - males are generally larger than females; Sedgwick 2000) , the differences suggest that males are experiencing higher stress or lower food intake (i.e., are in poorer condition) than females. In both native and exotic habitats, higher levels of fat, body condition, glucose, and triglyceride indicate that females are gaining more mass than males, and lower levels of BOHB and glycerol demonstrate that females are catabolyzing their fat reserves to a lesser degree than are males (Jenni-Eiermann and Jenni 1994, 1997). Based on these patterns, the higher uric acid levels in females probably reflect a higher protein diet. The lower hematocrit could indicate that

female flycatchers are experiencing anemia or disease, or that males are subject to dehydration and/or higher energy demands (Carpenter 1975, Campbell and Dein 1984, Work et al. 1999); the latter interpretation is more consistent with our observed patterns in plasma metabolites. However, increased hematocrit may also be a function of high levels of testosterone in breeding males (Sturkie 1986). Lower WBC count found in male flycatchers may reflect depressed immune function, due to heightened energetic demands, which may subsequently increase susceptibility to disease and parasites (Folstad and Karter 1992, Vleck et al. 2000).

Only a few other studies have examined physiological condition of breeding passerines, and some of these have noted sex differences in some parameters. Gavett and Wakeley (1986) found that female House Sparrows (*Passer domesticus*) had lower hematocrits than males, but similar uric acid levels. Based on an array of parameters, Horak et al. (1998) found that male Great tits (*Parus major*) seemed to be more stressed than females prior to the egg-laying stage, but that females experienced poorer health during the breeding stage. Some sex-based differences in condition indices can be attributed to hormones and differing physiology and activity levels during the breeding season. For example, females producing eggs experience extremely high levels of plasma triglycerides (Bacon et al. 1974, Chapman et al. 1994), and territorial male birds can have lower WBC counts due to the immuno-suppressive effects of testosterone and corticosterone (Silverin 1990, Folstad and Karter 1992).

Why might male Southwestern Willow Flycatchers experience more stress and/or be in poorer condition than females? Differing behavioral and hormonal traits during the breeding season may be part of the answer. Male flycatchers are strongly territorial (Sogge 2000) and active, spending much of their time singing and defending against intrusions from conspecifics. In contrast, female flycatchers (during the

early nesting period that we sampled) are relatively inactive, periodically foraging and singing but spending most of the day incubating (Sogge unpub. data). The higher activity level of males entails higher energy demands with subsequent heightened physiological stress. Actively territorial males also have higher levels of immuno-suppressive testosterone than do breeding females. However, we did not find any difference in H/L Ratios – an indicator of chronic stress. Further research should be conducted to determine whether the higher level of breeding season stress in males is manifested in differences in annual survivorship or longevity.

Habitat Differences

Based on the parameters that we measured, there is no evidence that the physiological condition of Southwestern Willow Flycatchers is lower in saltcedar habitats. Only three of the 12 parameters varied between habitats (Table 3); and the patterns in two of these three parameters suggest that saltcedar may provide better energetic/dietary conditions than native habitats. Higher triglyceride and lower glycerol levels indicate that the flycatchers in saltcedar have more recently deposited fat (e.g., producing and storing more fat) than those in native habitat (Jenni-Eiermann and Jenni 1994, Schaub and Jenni 2001). The significantly higher uric acid levels (Table 3) for flycatchers nesting in saltcedar could indicate a higher protein diet (Hochleithner 1994), or that the flycatchers are under food stress and metabolizing body protein (Anthony et al. 1990); the former interpretation is more consistent with our observed patterns in fat levels, mass, condition indices, and other plasma metabolites.

If numerous studies have documented lower arthropod diversity and abundance in saltcedar habitats (see DeLoach et al. 2000), why did flycatchers breeding at our saltcedar sites not exhibit poorer

physiological condition than those in our native sites? One major reason may be that earlier studies did not specifically investigate the relationship between saltcedar, associated arthropods, and flycatcher diet. The flycatcher consumes a relatively diverse array of invertebrates (Drost et al 2001), and the simple fact that flycatcher diet differs in different habitats does not mean that one habitat is worse than another from a food availability perspective. Although the greater variety of prey in native habitat may offer some buffer against a temporary shortage of any particular prey species, the large number of pollinator species attracted to flowering saltcedar appears to provide a very good source of prey in this habitat (Drost et al. 2001). Given that tamarisk flowers during much of the flycatcher breeding season at our study sites, abundance of large prey items (e.g., pollinators) may more than compensate for reduced diversity of available prey types.

A recent study (SWCA 2001) of the relationship between flycatcher foraging behavior, vegetation, and arthropod populations supports the idea that food (e.g., arthropods) may not be limited in saltcedar vegetation. In a mixed stand of saltcedar and native vegetation, saltcedar exhibited more arthropods at all times of the nesting season than did native willow, cottonwood, or box elder. Homoptera and Hymenoptera, both major flycatcher prey items, comprised the majority of arthropods on both native and saltcedar vegetation. In fact, SWCA (2001) suggests that saltcedar, as a component of the habitat, may enhance flycatcher foraging opportunities.

Our findings that flycatchers breeding in saltcedar habitats do not suffer negative physiological consequences do not mean that all saltcedar-dominated riparian areas would provide suitable breeding habitat. Our study (and that of SWCA 2001) was conducted at mid-elevation (560 - 1370 m) sites, most of which were within 200 m of surface water or moist soil. Furthermore, the riparian habitats at our

study sites were usually embedded within a local matrix of desert shrub uplands (often dominated by mesquite; *Prosopis* spp.) and/or irrigated croplands. These adjacent habitats may have provided a source for many strong-flying invertebrates (e.g., bees, wasps) that were attracted to flowering saltcedar (Drost et al. 2001). Many of the earlier studies of saltcedar, its invertebrate communities, and its reduced value to wildlife were conducted at sites that were lower in elevation, hotter, and drier than those included in SWCA (2001) and this study. Indeed, in many of the extensive monotypic saltcedar stands along the lower Colorado River and elsewhere in the deserts of the southwest, high temperatures, dry conditions, and/or lack of invertebrate prey may preclude breeding by Southwestern Willow Flycatchers.

Our comparison of the effects of breeding in saltcedar versus native habitats was conducted within a specific geographic region, and is based on patterns of physiological condition of individual flycatchers. The patterns that we found could differ in other parts of the flycatcher's range. Also, there are other ways in which differences in habitat suitability could be manifested, including differences in clutch size, productivity (the number of young fledged per female per season), adult or juvenile mortality and survival, site fidelity, and breeding population age and density. Additional studies and data analyses are needed to determine whether such differences exist at our study sites and or elsewhere in the southwest. However, our results show that, at least in some settings, there is no evidence that flycatchers breeding in saltcedar habitats are suffering negative physiological effects, and reinforce the fact that negative effects must be proven, rather than assumed *a priori*.

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