Seasonal Energetics and Condition of Bonefish from Different Subtropical Tidal Creeks in Eleuthera, the Bahamas

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Abstract.—Body composition and fish health indices of bonefish *Albula vulpes* were investigated to elucidate the energy dynamics in poorly studied tropical tidal flat environments. In general, bonefish were composed of 72% water, 21% protein, 4% ash, and 3% lipids, which is consistent with the wet weight values of the majority of freshwater and marine fish studied to date. Significant inverse relationships between the water and lipid contents of whole-body, gonad, and liver tissues revealed that the percentage of water is a good indicator of the relative amount of stored energy in bonefish and may be used as a surrogate for lipid content in future studies. The liver was the main storage site for lipids, containing more than twice the fat in the whole body. While both abiotic (e.g., season and location) and biotic (e.g., sex and size) factors contributed to the predictive power of the general linear models generated for all constituent analyses, there were no significant differences in whole-body or liver lipid content between seasons, which may be explained by there being a consistent food supply year-round. There was, however, a significant relationship between lipid content and body size with respect to season. Lipid content decreased in the winter and increased in the summer with increasing body size, a trend that can be explained by the timing of the reproductive season in bonefish. Seasonal changes in the condition factor and gonadosomatic indices were also linked to the winter spawning season, with decreasing body condition and increasing gonad development in the winter. Observed site-specific differences in the lipid content and liver somatic indices of bonefish may be accounted for by local trophodynamics as well as subtle differences in life history strategies. The energy reserve and fish health index data collected in this study may provide a useful baseline for future comparative work and help elucidate fish energetics in dynamic tropical tidal flat systems.

In any aquatic environment, the physicochemical properties and dynamic nature of water (e.g., its high thermal conductance and viscosity and low oxygen solubility) can place constraints on how organisms function (Domenici et al. 2007). Given the tight connection between fish and their surroundings (e.g., through water temperature; Fry 1967), the fluctuating nature of environmental variables can influence how individuals partition energy between growth, repro-

duction, and survival, ultimately affecting their fitness (Claireaux and Lefrançois 2007). The strategies adopted by fish living in fluctuating environments can stem from a combination of physiological and behavioral traits, and specific understanding as to how these strategies function is fundamental to the conservation and management of fish populations.

Understanding how fish cope with fluctuating environments while meeting the energetic requirements for their life functions can be gained by estimating the proximate body composition (e.g., water, lipids, proteins, carbohydrates, ash, and energy density) and using indicators of fish health (Love 1970; Busacker et al. 1990). Estimates of proximate body composition can be valuable for modeling the changes in seasonal
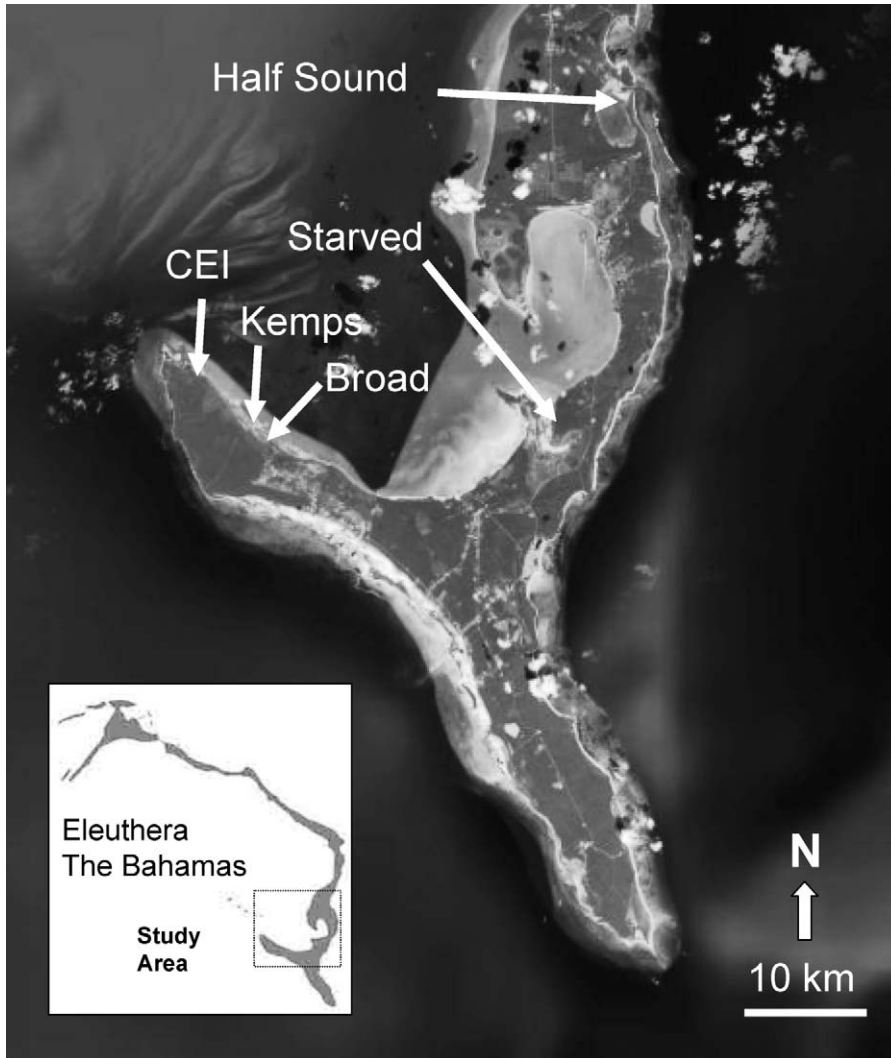
FIGURE 1.—Aerial photograph of the study area showing the locations of the tidal creeks (Kemps, Broad, and Starved) and the tidal embayment (Half Sound) as well as the Cape Eleuthera Institute (CEI).

energy dynamics and are necessary for bioenergetics-based modeling tracking the energy flow into and out of fish (Adams and Breck 1990; Madenjian et al. 2006). The different variables examined in assessing proximate body composition can lend clues to many aspects of a fish's current life history. For example, changes in protein content are considered a measure of sustainable growth, whereas the presence or absence of lipids indicates the storage of potential chemical energy (Busacker et al. 1990). Other indicators of nutritional or energy status can be used to complement proximate body composition analysis, such as Fulton's condition factor and various organosomatic indices. Unfortunately, the energetic consequences of environmental

stressors have primarily been investigated in freshwater fishes or anadromous salmonids (e.g., Cunjak and Power 1986; Fechhelm et al. 1995; Simpkins et al. 2003; Madenjian et al. 2006). Studies of subtropical and tropical fish have typically focused on aquaculture applications (e.g., Ellis et al. 1996) rather than natural environments (e.g., Montgomery and Galzin 1993; Wuenschel et al. 2006). Tropical tidal flats in particular are regions of the marine environment in which water temperature, salinity, and dissolved oxygen are in constant flux (Nybakken and Bertness 2005), thus creating physiological challenges for resident fish (Reise 1985). For this reason, indicators of energy, nutritional status, or both would provide novel insights

into the consequences of living in these dynamic aquatic systems.

Bonefishes *Albula* spp. serve as an interesting model for the study of energy dynamics in tidal marine flat systems. With a circumtropical distribution and a popular recreational fishery throughout, bonefishes play an essential role in many local economies (Pfeiler et al. 2000; Ault 2008; Danylchuk et al. 2008). In addition to their economic importance, it is hypothesized that bonefishes provide a number of fundamental services to the tidal flat ecosystems. They move into shallow flats to feed on invertebrates and small fish during high tide and then retreat to deeper water at low tide (Colton and Alevizon 1983a, 1983b; Humston et al. 2005). Through their movements and feeding habits, bonefishes effectively connect the mosaic of tidal flat habitats and have been found to limit the depth distribution of some of their prey items (see Engstrom 1984). Despite the economic value and hypothesized ecological importance of these species, there are critical gaps in the scientific knowledge of their biology. This dearth of information hampers not only the effective conservation and management of these species but also understanding of the community trophodynamics and productivity of tropical tidal flats.

The purpose of this study was to examine the body composition and condition of bonefish *A. vulpes*. The specific objectives were to (1) determine the partitioning of energy among tissues in relation to abiotic (e.g., season and location) and biotic (e.g., sex and size) factors and (2) quantify baseline levels of health and condition. Overall, we elucidate the energy dynamics of fish in tropical tidal flat systems and provide detailed information on the biology of bonefish.

Methods

This study took place in South Eleuthera, the Bahamas (24°50'05"N, 76°20'32"W), in several tidal creek systems (Kemps, Broad, and Starved) and tidal embayments (Half Sound) as well as at the Cape Eleuthera Institute (CEI) research facility (Figure 1). Preliminary genetic analysis of the bonefish species from this area indicated that all specimens were *Albula vulpes* (Danylchuk et al. 2007). Telemetry data for the bonefish in the study area show that those from Broad and Kemps creeks mix frequently, allowing the samples from these two locations to be combined (K. J. Murchie, unpublished). All research was conducted in accordance with the policies of the Canadian Council on Animal Care under an approval granted by the Carleton University Animal Care Committee (protocols B07–03, B07–05, and B07–06).

Sample collection and initial processing.—Bonefish were collected by seine net for analysis of their proximate body composition between August 27, 2006, and August 25, 2007. Sampling occurred in two distinct periods: August, to represent the summer season (May 1–October 31), when ambient water temperatures average 29°C; and February and March, to represent winter (November 1–April 30), when the mean water temperature is 23°C (Murchie, unpublished). Fish were transported to the laboratory at CEI for processing. All fish were measured to the nearest millimeter total length and wet weight was measured to the nearest gram. The viscera, gonads, and liver were removed, weighed to the nearest 0.1 g, and placed in individually labeled storage bags. The carcass of each fish was weighed to the nearest gram and then ground to a homogenous mixture with a hand-operated meat grinder. The ground sample was thoroughly mixed and run through the grinder again. After a second mixing, a subsample was extracted into a labeled storage bag and sealed. The organs and ground samples were placed in a larger labeled bag, sealed, and frozen. The samples were shipped back to the Fish Ecology and Conservation Physiology Laboratory at Carleton University, where they remained frozen until further processing for water, lipids, and ash (and trace minerals).

Proximate body composition analysis and energy density.—Water content was determined for the whole body, liver, and gonads by placing 2 ± 0.02 g of the sample in a crucible and drying it overnight (18 h) to a constant mass at 80°C. Each sample was then cooled in a desiccator and reweighed to the nearest 0.0001 g to determine water content (Crossin and Hinch 2005). Dried samples were crushed into a fine powder with a glass pestle, and a portion (0.2 g) was used in the lipid extraction procedure.

The lipid content of the whole body, liver, and gonads of all fish was determined using the Smedes and Askland (1999) modification of the chloroform–methanol extraction technique developed by Bligh and Dyer (1959). Samples were combined thoroughly with chloroform, methanol, and distilled water in a 1 : 2 : 0.8 ratio and placed in an ultrasonic bath for 15 min. An additional 1 mL of chloroform and 1 mL of distilled water were added and the samples centrifuged at $126 \times$ gravity for 10 min. The solvent layer containing the lipids was extracted with a pipette and filtered through sodium sulfate and quartz wool into preweighed aluminum boats. The extraction procedure was repeated on the supernatant, and the extracted lipids were left overnight in the fume hood to allow the chloroform to evaporate. The aluminum boats were dried for 1 h at 60°C and reweighed to the nearest 0.0001 g to calculate the percentage of lipids by dry mass. These values were then converted to percent lipids by wet mass. All

TABLE 1.—Proximate composition (% water, lipids, ash, and protein by wet mass) and energy density (MG/kg) in whole-body, gonad, and liver tissues of female (F) and male (M) bonefish sampled in summer and winter in various locations in Eleuthera, the Bahamas. Values are given as the means ± SDs.

| Season | Location | Sex | N | Whole body | | | | |
				Water	Lipids	Ash	Protein	Energy density
Summer	Broad–Kemps creeks	F	4	72.6 ± 2.2	1.7 ± 0.6	4.4 ± 0.8	21.4 ± 1.3	4.9 ± 0.3
		M	8	72.6 ± 3.2	2.2 ± 1.0	4.3 ± 1.1	20.9 ± 2.4	5.0 ± 0.8
	Half Sound	F	3	73.2 ± 0.6	3.3 ± 0.5	3.7 ± 0.7	19.8 ± 0.8	5.2 ± 0.2
		M	7	74.4 ± 2.6	3.2 ± 1.2	3.6 ± 0.8	19.0 ± 1.2	5.0 ± 0.7
	Starved Creek	F	6	75.5 ± 2.0	2.8 ± 1.1	3.0 ± 0.3	18.7 ± 1.3	4.8 ± 0.5
		M	10	74.5 ± 3.0	3.5 ± 1.4	3.3 ± 0.8	18.7 ± 1.7	5.0 ± 0.7
Winter	Broad–Kemps creeks	F	21	73.1 ± 2.8	2.6 ± 1.0	3.8 ± 1.2	20.6 ± 2.0	5.1 ± 0.6
		M	43	71.5 ± 2.3	3.3 ± 1.4	4.0 ± 1.4	21.2 ± 2.1	5.5 ± 0.7
	Half Sound	F	15	73.8 ± 2.1	1.9 ± 0.8	3.4 ± 0.5	20.8 ± 1.7	4.9 ± 0.5
		M	14	74.7 ± 1.9	1.8 ± 0.9	3.4 ± 0.5	20.2 ± 1.6	4.7 ± 0.4
	Starved Creek	F	13	69.1 ± 2.5	3.6 ± 1.6	4.7 ± 1.5	22.7 ± 1.8	5.9 ± 0.7
		M	30	67.8 ± 2.4	4.5 ± 1.3	4.7 ± 1.4	23.0 ± 2.0	6.3 ± 0.7

samples were analyzed in duplicate; the differences between replicates never exceeded 2%.

Ash and protein were determined for whole-body samples only owing to the mass of tissue required. The methods outlined by Crossin and Hinch (2005) were used to determine ash and protein content. After the replicate samples (described above) were analyzed for water content, the crucibles containing the dried samples were combusted for 2 h in a muffle furnace at temperatures between 500°C and 600°C. After the samples were cooled in a dessicator, the remaining ash was weighed to determine the percentage of trace minerals by wet mass. As before, differences between duplicate samples never exceeded 2%.

The percent of whole-body protein (C_P) was determined by the relationship

$$C_P = 100 - (C_W + C_L + C_A),$$

where C_W, C_L, and C_A are the percentages of water, lipids, and ash, respectively (Berg et al. 1998; Hendry et al. 2000). Given the fractions of whole-body lipids and protein of each fish, the whole-body energy density (d; MJ/kg) can be calculated from the equation

$$d = fD_f + pD_p,$$

where f and p are the fractions of lipids and proteins from the samples (g/kg) and D_f and D_p are the energy densities of lipids and protein (MJ/g; Breck 2008). The values for the latter two variables (0.0362 and 0.0201 MJ/g, respectively) were taken from Brett and Groves (1979).

To calculate fish health indices such as Fulton's condition factor (K; Anderson and Neumann 1996), the gonadosomatic index (GSI), and the liver somatic index (LSI) (Barton et al. 2002), the following equations were used:

$$K = [\text{whole body weight (g)/total length (mm)}^3] \times 10^5;$$

$$GSI = [\text{gonad weight (g)/whole body weight (g)}] \times 100;$$

$$LSI = [\text{liver weight (g)/whole body weight (g)}] \times 100.$$

Data analysis.—All statistical analyses on collected and derived data were performed in JMP 7.0.2 (SAS Institute, Cary, North Carolina). Maximal type 1 error rates were set at 0.05. The normality and homogeneity of variance assumptions were evaluated with the Shapiro–Wilk or Kolmogorov–Smirnov goodness-of-fit tests and Levene's test, respectively (Sokal and Rohlf 1995). Regression analyses were used to determine any relationships between the percentages of water and lipids in various tissues and the percentages of water and protein in the whole body (see Salam and Davies 1994). A general linear model (GLM) was used to assess whether the proximate compositions of water, lipids, ash, and protein and the energy density of whole-body, gonad, and liver tissues varied by season, location, sex, or size. Tukey–Kramer honestly significant difference (HSD) tests were performed to determine significant differences between means (Day and Quinn 1989). Relationships between indices of fish condition and proximate composition were evaluated by means of regression analysis (e.g., Kaufman et al. 2007). The effects of season, location, and sex on K were evaluated by means of a three-way analysis of variance (ANOVA) followed by Tukey–Kramer HSD tests. To evaluate the effects of season, location, and sex on the GSI and LSI, the proportional

TABLE 1.—Extended.

	Gonad		Liver	
Season	Water	Lipids	Water	Lipids
Summer	67.1 ± 5.1	8.6 ± 3.4	70.2 ± 0.9	5.7 ± 1.2
	53.7 ± 18.6	23.7 ± 25.3	70.5 ± 0.9	5.7 ± 0.8
	70.6 ± 2.0	7.6 ± 2.1	70.3 ± 1.0	6.6 ± 0.5
	55.4 ± 18.5	20.8 ± 19.0	70.8 ± 2.4	6.1 ± 1.8
	61.5 ± 12.8	17.8 ± 13.6	72.4 ± 1.6	6.8 ± 0.7
	41.6 ± 18.7	39.9 ± 20.0	71.1 ± 2.1	7.5 ± 1.9
Winter	60.6 ± 6.6	12.0 ± 3.5	71.2 ± 1.8	7.9 ± 1.9
	66.9 ± 12.0	11.3 ± 14.0	70.0 ± 2.0	7.9 ± 2.1
	65.1 ± 7.2	10.5 ± 5.6	70.1 ± 1.9	8.0 ± 1.9
	64.1 ± 10.8	14.1 ± 13.3	70.8 ± 2.2	7.4 ± 2.1
	58.4 ± 13.0	13.6 ± 12.0	71.7 ± 2.4	5.3 ± 1.5
	73.6 ± 9.9	5.2 ± 11.1	69.1 ± 2.8	7.8 ± 2.7

data (gonad or liver weight divided by total fish weight) was arcsine-square-root transformed and evaluated by means of a three-way ANOVA followed by Tukey–Kramer HSD tests. Total length was not included in these analyses because all health indices incorporate some measure of fish size.

Results

Proximate Body Condition and Energy Density

A total of 174 bonefish (442 ± 47 mm total length [mean ± SD]) were captured and processed for proximate body composition. An additional 21 bonefish were captured in the summer season but not included in the data set because their sex could not be determined. The wet weight of the fish generally consisted of 72% water, 21% protein, 4% ash, and 3% lipids. The mean energy density of the whole body was 5.4 ± 0.8 MJ/kg. On average, the livers had a higher lipid content (7.4 ± 2.2%) than the whole body, but the percentage of fat was highest in the gonads (13.4 ± 15.0%). There were significant negative relationships between the percentages of lipids and water for the whole-body, gonad, and liver tissues (Figure 2; % lipids = 25.2 – 0.308 · % water [$r^2 = 0.5$, $F = 169.12$, $P < 0.0001$]; % lipids = 78.7 – 1.027 · % water [$r^2 = 0.9$, $F = 1,591.47$, $P < 0.0001$]; and % lipids = 51.898 – 0.633 · % water [$r^2 = 0.427$, $F = 128.23$, $P < 0.0001$]). The protein stores in whole body also had a strong negative relationship with whole-body water content (% protein = 58.9 – 0.527 · % water [$r^2 = 0.6$, $F = 298.27$, $P < 0.0001$]).

The proximate compositions of the various tissue samples were not similar across all sample locations (Table 1). To best understand the influence of season, location, sex, and size on the various tissue constituents, the results of the GLM must be
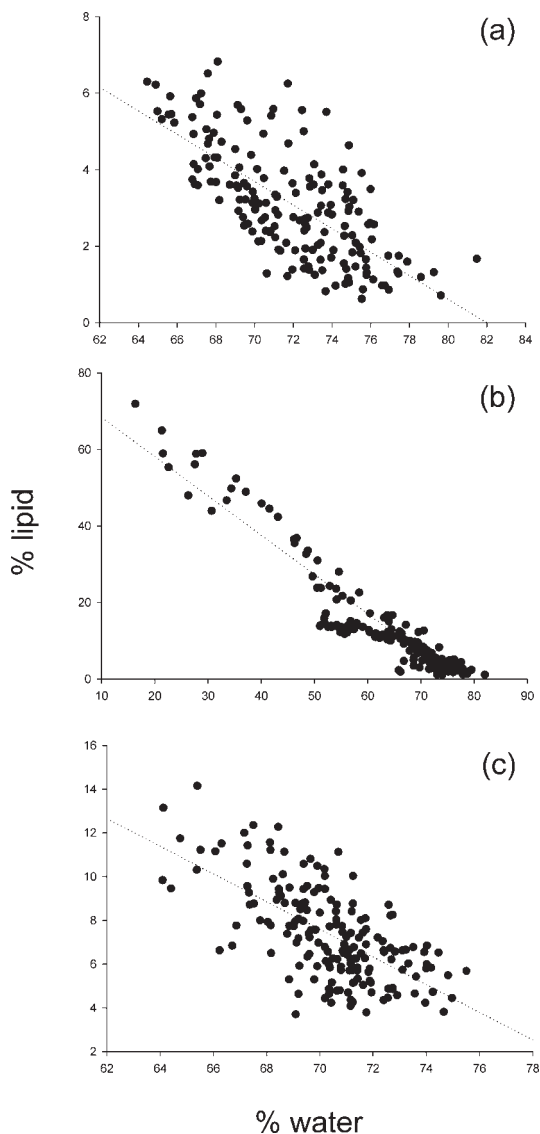

FIGURE 2.—Estimated relationships between the percentage of water and the percentage of lipids by wet mass for (a) whole body, (b) gonad, and (c) liver samples for bonefish collected at Eleuthera (see text for equations).

examined. The GLM for whole-body water content was significant ($F = 9.341$, $P < 0.0001$), explaining 58.9% of the variability in this factor. There was also a significant interaction between the season and location terms ($F = 8.825$, $P = 0.0002$) (Table 1). Bonefish from Broad and Kemps creeks and Half Sound showed no seasonal differences in water content, whereas those from Starved Creek had higher values in the summer than in the winter (Figure 3a). The GLM for whole-body lipid content
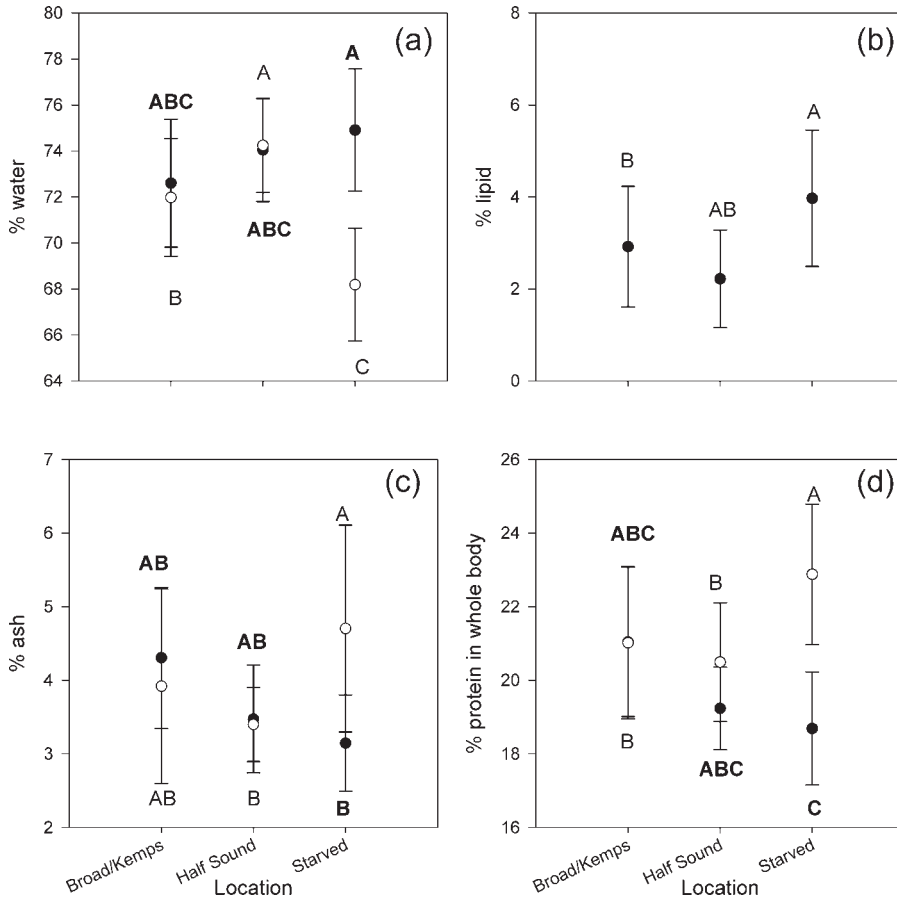
FIGURE 3.—Mean constituent analyses for **(a)** water, **(b)** lipids, **(c)** ash, and **(d)** protein in whole-body samples of bonefish collected from Broad and Kemps creeks, Half Sound, and Starved Creek in the summer (closed circles) and winter (open circles). The error bars represent standard errors. Values with different letters are significantly different ($P < 0.05$; bold = summer, ordinary type = winter).

had an r^2 value of 0.5 ($F = 7.917$, $P < 0.0001$). Location and the interaction between season and total length were significant ($F = 4.505$, $P = 0.013$ and $F = 14.745$, $P = 0.0002$, respectively; Table 2). Lipid content was higher in bonefish from Starved Creek than in those from Broad and Kemps creeks (Figure 3b). There was a significant relationship between lipid content and total length in both seasons, but in opposite directions (Figure 4a, b); whole-body fat content increased with fish size in the summer (% lipids $= -0.581 + 0.007 \cdot$ TL [$r^2 = 0.104$, $F = 4.185$, $P = 0.048$]) and decreased in the winter (% lipids $= 11.9 - 0.020 \cdot$ TL [$r^2 = 0.266$, $F = 48.533$, $P < 0.0001$]).

The variation in whole-body ash content was explained by the GLM ($r^2 = 0.2$, $F = 1.855$, $P = 0.015$), and an interaction between season and location was found ($F = 3.955$, $P = 0.021$; Table 2). There was

no difference in the percentage of ash between seasons for fish from Broad and Kemps creeks and Half Sound, whereas those from Starved Creek had higher ash content in the winter than in the summer (Figure 3c). The GLM for whole-body protein content was significant ($F = 3.884$, $P < 0.0001$), explaining 37.3% of the variability. There was a significant interaction between season and location ($F = 8.346$, $P = 0.0004$) (Table 2). Protein content only varied significantly by season at Starved Creek, the highest values being recorded in the winter (Figure 3d). The energy density of the bonefish was predicted by the GLM ($r^2 = 0.528$, $F = 7.290$, $P < 0.0001$), with a significant interaction between season and total length ($F = 6.501$, $P = 0.012$; Table 2). There was no relationship between energy density and bonefish size in the summer ($r^2 = 0.082$, $F = 3.217$, $P = 0.081$; Figure 4c). However, there appears to be a slight

TABLE 2.—Evaluation of the variables in the general linear models to determine whether the whole-body proximate composition and energy density of bonefish is dependent on season, location, sex, or size. All significant values are designated with asterisks.

| Variable(s)	Water		Lipids		Ash		Protein		Energy density	
	F	P	F	P	F	P	F	P	F	P
Season	0.885	0.349	1.206	0.274	0.177	0.685	0.636	0.426	1.535	0.217
Location	0.193	0.825	4.505	0.013*	0.879	0.417	0.742	0.478	0.912	0.404
Sex	0.154	0.695	0.510	0.475	0.340	0.561	0.268	0.605	0.017	0.895
TL	0.0002	0.988	0.191	0.663	0.473	0.493	0.041	0.840	0.175	0.677
Season × location	8.825	0.0002*	0.699	0.499	3.955	0.021*	8.346	0.0004*	1.769	0.174
Season × TL	2.278	0.133	14.745	0.0002*	0.320	0.572	0.002	0.962	6.501	0.012*
Season × sex	0.335	0.564	0.276	0.600	0.319	0.573	0.424	0.516	0.006	0.937
Location × TL	0.284	0.753	1.990	0.140	0.205	0.815	0.018	0.982	1.023	0.362
Location × sex	0.603	0.549	0.018	0.982	0.212	0.809	0.433	0.649	0.236	0.790
TL × sex	0.587	0.445	0.021	0.884	0.045	0.833	0.787	0.376	0.232	0.631
Season × location × TL	1.427	0.243	0.075	0.928	1.378	0.255	0.375	0.688	0.321	0.726
Season × location ×sex	1.310	0.273	0.010	0.990	0.786	0.456	0.797	0.453	0.297	0.744
Season × TL × sex	1.856	0.175	0.334	0.564	0.121	0.728	1.269	0.262	1.235	0.268
Location × TL × sex	0.451	0.638	0.334	0.715	0.092	0.913	0.880	0.417	0.076	0.927
Season × location × TL × sex	0.915	0.403	0.117	0.890	0.058	0.943	1.300	0.276	0.816	0.445
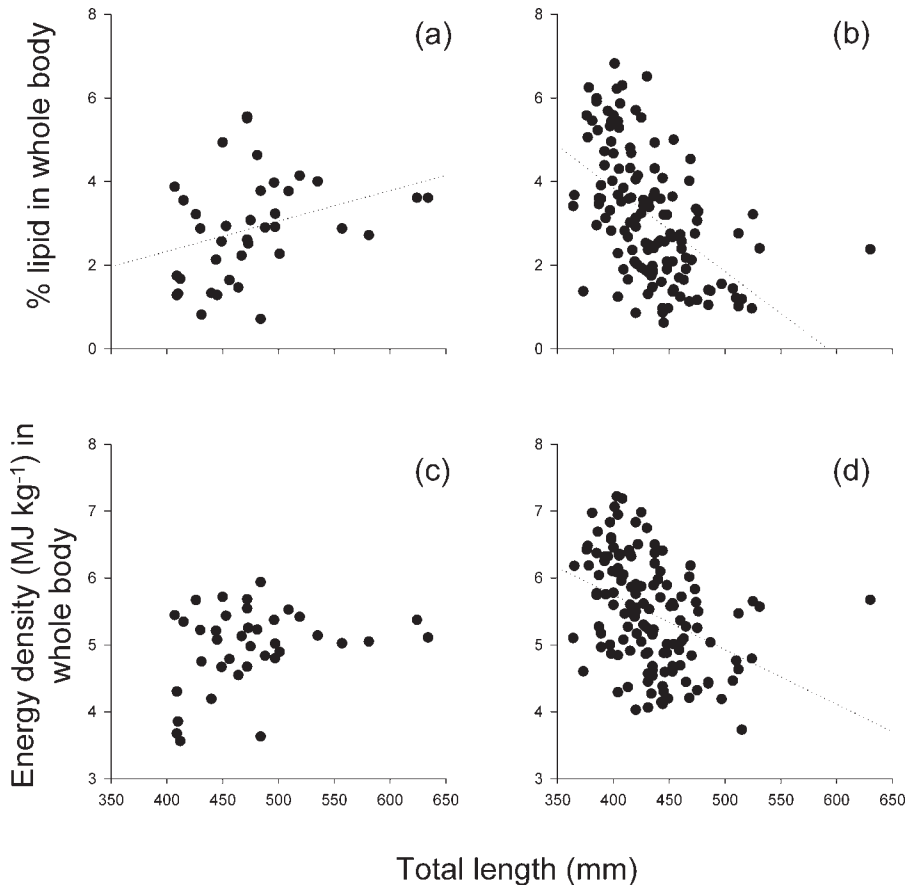
FIGURE 4.—Estimated relationships between total length and whole-body lipid content and energy density for bonefish sampled in (a) and (c) summer and (b) and (d) winter in Eleuthera. The equations of the lines in (b) and (d) are % lipids = 11.9 − 0.020 · TL and energy density = 9.01− 0.008 · TL, respectively (see text for the equations in [a] and [c] and summary statistics).

TABLE 3.—Evaluation of the variables in the general linear models to determine whether the proximate composition of bonefish gonads is dependent on season, location, sex, or size. All significant values are designated with asterisks.

Variable(s)	Water		Lipids	
	F	P	F	P
Season	0.046	0.830	0.000	0.995
Location	1.165	0.315	1.581	0.209
Sex	0.021	0.884	0.090	0.765
TL	2.107	0.149	2.001	0.159
Season × location	2.958	0.055	2.812	0.063
Season × TL	6.807	0.010*	4.703	0.032*
Season × sex	0.651	0.421	0.164	0.686
Location × TL	1.888	0.155	0.986	0.376
Location × sex	0.104	0.901	0.159	0.853
TL × sex	1.142	0.287	1.332	0.250
Season × location × TL	0.465	0.629	1.101	0.335
Season × location × sex	0.532	0.589	0.501	0.607
Season × TL × sex	0.958	0.329	1.659	0.200
Location × TL × sex	0.159	0.853	0.038	0.963
Season × location × TL × sex	2.770	0.066	2.106	0.125

tendency for energy density to decrease with increasing total length during the winter (energy density = 9.01 − 0.008 · TL [$r^2 = 0.2$, $F = 24.907$, $P < 0.0001$; Figure 4d).

The GLMs also explained a significant portion of the variability in the water and lipid contents of bonefish gonads ($r^2 = 0.4$, $F = 4.852$, $P < 0.0001$ and $r^2 = 0.4$, $F = 3.770$, $P < 0.0001$. respectively). Interactions were found between season and total length for both gonad constituents (Table 3). No relationship was found between water content and total length (Figure 5a, b) or between lipid content and total length (Figure 5c, d) in either season ($P > 0.05$). The GLMs produced for the water ($r^2 = 0.2$, $F = 1.981$, $P = 0.008$) and lipid contents ($r^2 = 0.3$, $F = 2.176$, $P = 0.003$) of bonefish livers had no significant terms (Table 4).

Fish Health Indices

The condition factor ranged from 0.928 to 1.571 and was significantly higher in the summer than in the winter for both males and females ($P < 0.0001$; Tables 5, 6). Location was also a significant factor in the three-way ANOVA ($P = 0.028$; Table 6), bonefish being in better condition at Half Sound than at Starved Creek (Figure 6a). The winter GSI levels were greater than those reported for the summer in both sexes ($P < 0.0001$; Tables 5, 6). Location and the interaction between season and location were also significant variables for GSI (Table 6). The values of the index differed between bonefish from Starved Creek (mean = 3.064) and those from Half Sound (mean = 0.791) (Figure 6b; all $P < 0.05$). The summer values for GSI did not vary across locations, but the winter values

were highest in Starved Creek, followed by Broad and Kemps creeks, and then Half Sound (Figure 6c). The LSI values ranged from 0.178 to 1.005 and differed between sexes ($P = 0.010$; Tables 5, 6). They were higher in the bonefish from Starved Creek than in those from Half Sound ($P < 0.05$; Figure 6d). Furthermore, Starved Creek bonefish had significantly higher LSI values in the summer (mean = 0.642) than the fish at the other two locations (Figure 6e). There is a positive relationship between K and whole-body fat content for bonefish ($K = 1.28 + 0.016$ · % lipids [$r^2 = 0.06$, $F = 11.150$, $P = 0.001$; Figure 7).

Discussion

This study represents the first assessment of the nutritional status of wild bonefish and one of the few energetic studies focusing on a wild tropical fish inhabiting tidal marine flats. In general, the proximate body composition values determined for bonefish (72% water, 21% protein, 4% ash, and 3% lipids) are consistent with the wet weight values for the majority of freshwater and marine fish studied to date (70–80% water, 20–30% protein, and 2–12% lipids; Love 1970). A well-documented trend is the inverse relationship between water and fat (see Craig 1977; Hartman and Brandt 1995; Kaufman et al. 2007). For bonefish, the percentage of water is a good indicator of the relative amounts of energy stored in the whole body, a lower percentage of water equating to a greater lipid and protein content. Significant inverse relationships between water and fat were also documented for gonad and liver tissues. Depending on the energetic status of the fish (Idler and Bitners 1959; Love 1970), tissue water content has often been recommended or used as a surrogate for lipid or energy content in a number of fish species (e.g., Salam and Davies 1994; Plante et al. 2005; Trudel et al. 2005) because water replaces catabolized lipids. Future studies of bonefish may benefit from the use of the predictive equations derived for these relationships, as determining the water content of various tissues is much less labor-intensive and costly than determining the lipid content.

Lipid deposition is the most efficient mode of energy storage because lipids can easily be metabolized when needed (Jobling 1994) and can be depleted without reducing the physical performance of the fish (Hendry et al. 2000). Contrasting the lipid content of various tissues is useful for determining the main location of lipid storage. The location varies among fish species, but lipids have been found in the liver, between the myomeres of the muscles, in the mesentery, along the lateral line, and at the base of fins (Arrington et al. 2006). In bonefish, the liver appears to be the main site for lipid storage, as the mean density in the liver (7.4%)
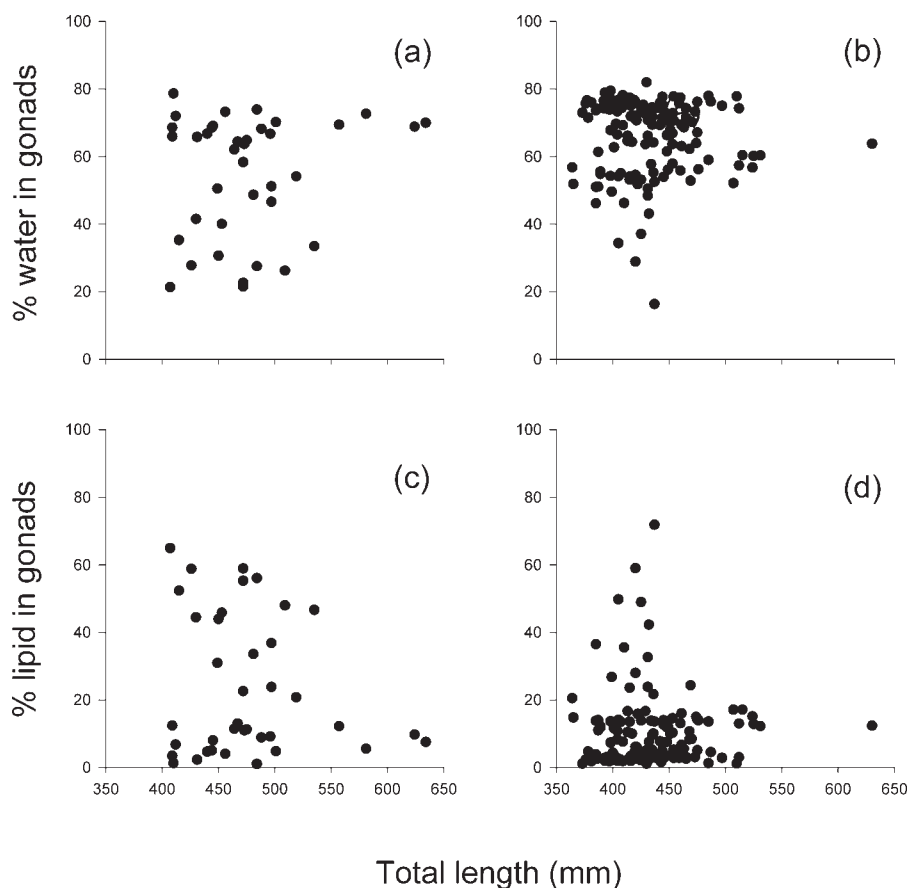
FIGURE 5.—Scatterplots of the data for the percentages of water and lipids (wet mass) versus total length for bonefish sampled in **(a)** and **(c)** summer and **(b)** and **(d)** winter in Eleuthera.

was more than double that in the whole body (3.1%). In addition to serving as a storage depot for lipids, the liver is the major site of lipid biosynthesis (Henderson and Tocher 1987), which may contribute to the higher values there. Regardless of the main location of lipid storage, the presence of lipids indicates that surplus energy is available for future maintenance, growth, and reproduction (Kaufman et al. 2007). Because it is often assumed that body condition indices are good indicators of the lipid status of fish (Kaufman et al. 2007), the relationship between K and the percentage of whole body lipids was examined for bonefish. Although a positive relationship ($F = 11.150$, $P = 0.001$) was detected, only 6% of the variability in K was explained by whole-body lipid content.

The highest amount of stored energy in bonefish was in muscle protein (21%). This is similar to the result documented by Hendry et al. (2000) for sockeye salmon *Oncorhynchus nerka*. Muscle protein is important for structural and performance-related func-

TABLE 4.—Evaluation of the variable(s) in the general linear models to determine whether the proximate composition of bonefish livers is dependent on season, location, sex, or size.

	Water		Lipids	
Variable(s)	F	P	F	P
Season	0.688	0.408	1.354	0.247
Location	0.872	0.420	0.254	0.776
Sex	0.000	0.998	0.080	0.778
TL	0.033	0.856	0.017	0.895
Season × location	0.635	0.531	2.797	0.065
Season × TL	1.310	0.254	0.492	0.484
Season × sex	0.155	0.695	0.153	0.697
Location × TL	1.799	0.169	0.885	0.415
Location × sex	0.834	0.437	0.719	0.489
TL × sex	0.164	0.686	0.052	0.820
Season × location × TL	0.766	0.467	1.803	0.168
Season × location × sex	0.018	0.982	0.016	0.984
Season × TL × sex	1.004	0.318	2.365	0.126
Location × TL × sex	0.490	0.614	1.195	0.306
Season × location × TL × sex	0.555	0.576	0.275	0.760

TABLE 5.—Health indices (Fulton's condition factor [K], gonadosomatic index [GSI], and liver somatic index [LSI]) for female and male bonefish collected in Eleuthera in the summer and winter. Values are means ± SDs.

Health index	Females		Males	
	Summer ($n = 13$)	Winter ($n = 40$)	Summer ($n = 25$)	Winter ($n = 87$)
K	1.38 ± 0.08	1.30 ± 0.09	1.40 ± 0.10	1.31 ± 0.09
GSI	0.39 ± 0.20	2.7 ± 2.4	0.25 ± 0.13	2.4 ± 2.2
LSI	0.59 ± 0.20	0.56 ± 0.18	0.45 ± 0.16	0.51 ± 0.17

tions (e.g., swimming; Hendry et al. 2000), and deposition of protein is the most effective way to grow because each gram of protein also binds 3–4 g of water (Jobling 1994). From a life history perspective, allocating more energy to protein storage and thus growth may be more important for bonefish because greater body size may decrease the susceptibility to predation by lemon sharks *Negaprion brevirostris* and barracudas *Sphyraena barracuda* that also reside in tropical marine flats (Cooke and Philipp 2004). Additionally, since bonefish appear to spend a great deal of time foraging while moving in and out of the tidal flats (Colton and Alevizon 1983b), the turnover rates for consumed energy are probably high. Future studies that investigate the daily field activity budget of bonefish would be beneficial in quantifying the amount of time that fish spend foraging as opposed to avoiding predation, as activity is variable and could significantly contribute to the total energy budget (Boisclair and Leggett 1989). A laboratory study that investigates the tissue turnover time and metabolic rate of bonefish would also provide clues to bonefish energetics.

Both abiotic (e.g., season and location) and biotic (e.g., sex and size) factors influenced the proximate body composition of bonefish in a variety of ways. While not all factors were significant predictors of the various constituents, all contributed to the predictive power of the general linear models. As a main effect, season was not a significant variable. In particular, there was no significant difference in whole-body or liver lipid content between seasons. While such differences are expected (and observed) in northern

temperate regions where fish use stored energy to fuel metabolic activity during harsh winter conditions (e.g., brook trout *Salvelinus fontinalis* [Cunjak and Power 1986]; Atlantic salmon *Salmo salar* [Shackley et al. 1994]; and broad whitefish *Coregonus nasus* [Fechhelm et al. 1995]), species inhabiting subtropical and tropical waters in which the availability of food is more stable seasonally may allocate more energy to growth than to storage (Wuenschel et al. 2006). Consistent with this, we found no seasonal differences in whole-body lipids, water, ash, protein, or GSI. However, interactions between season and location were found. For example, somatic protein and ash were lower and somatic water higher in bonefish from Starved Creek in the summer. Starved Creek fish also had higher somatic lipid levels than fish from the other locations. This may be due to site-specific differences in diet, prey availability, or subtle differences in life history strategy (Love 1970; Cunjak and Power 1986; Hoey et al. 2007). Starved Creek lies 1.2 km from the settlement of Rock Sound, so potential anthropogenic inputs of nutrients could possibly affect the trophodynamics at this locale. Investigations of the trophodynamics and nutrient sources using stable isotope analysis would provide further insight.

The interaction between season and size was a significant predictor in the GLM of whole-body lipids and energy density as well as gonad lipids and water content. The percentage of whole-body lipids and energy density increased significantly with bonefish size in the summer and decreased in the winter. The decrease in winter can be explained by the timing of

TABLE 6.—Results of three-way ANOVA evaluating the influence of season, location, and sex on various health indices for bonefish. All significant values are designated with asterisks.

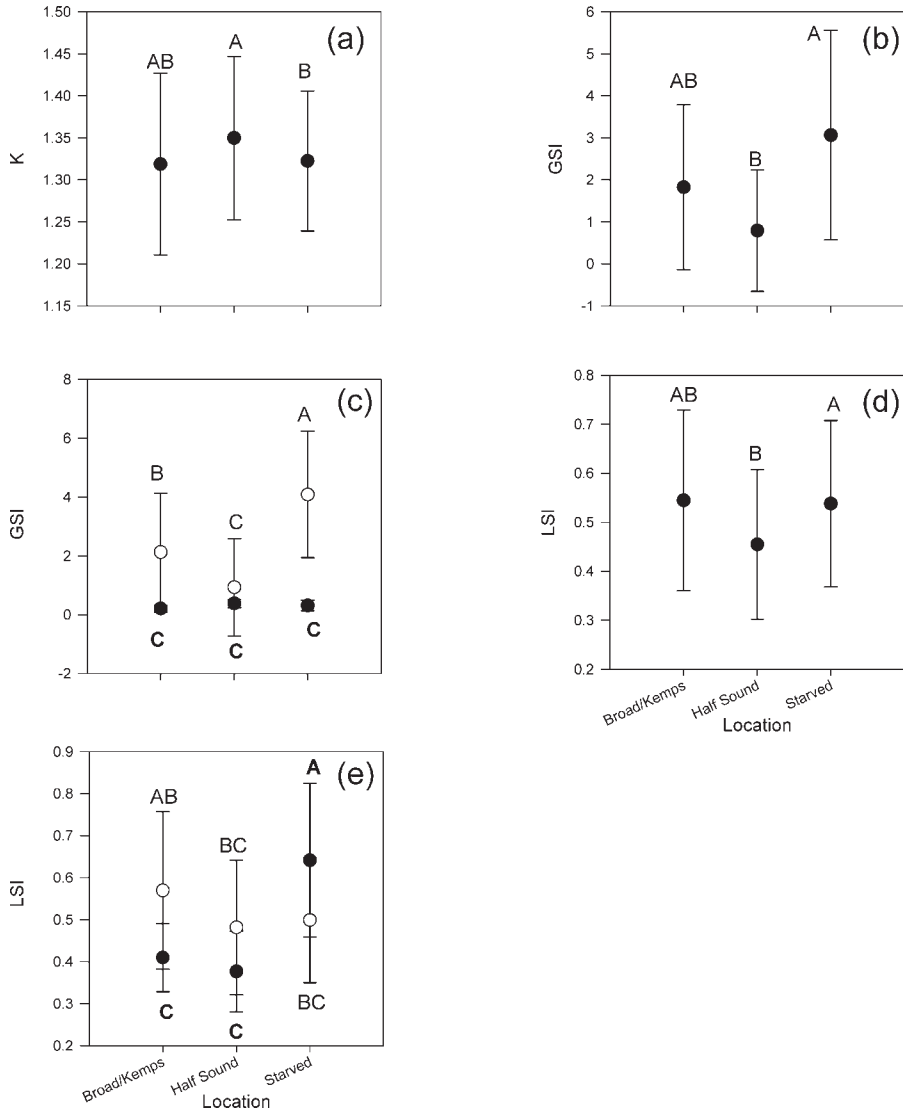| Variable(s)	K		GSI		LSI	
	F	P	F	P	F	P
Season	25.118	<0.0001*	35.569	<0.0001*	0.925	0.338
Location	3.667	0.028*	6.074	0.003*	6.605	0.002*
Sex	0.439	0.508	0.881	0.349	6.718	0.010*
Season × location	2.681	0.072	6.554	0.002*	9.650	0.0001*
Season × sex	0.006	0.938	0.271	0.603	1.146	0.286
Location × sex	0.824	0.441	0.983	0.377	0.113	0.893
Season × location × sex	0.753	0.473	1.212	0.300	0.599	0.551

FIGURE 6.—Mean health indices for bonefish sampled from Broad and Kemps creeks, Half Sound, and Starved Creek. Panel **(a)** shows the results for Fulton's condition factor (K), panel **(b)** those for the gonadosomatic index (GSI), panel **(c)** those for the GSI disaggregated by season, panel **(d)** those for the liver somatic index (LSI), and panel **(e)** those for the LSI disaggregated by season. Summer values are represented by closed circles, winter values by open circles. The error bars represent standard errors. Values with different letters are significantly different ($P < 0.05$; where both summer and winter values are shown, bold letters designate the summer ones.)

the reproductive season (Crabtree et al. 1997; Danylchuk and coworkers, CEI, unpublished data). Fish health indices also reflected the winter reproductive period, with lower fish condition and higher GSI values in the winter. Liver lipid and water concentrations were not significant variables in the GLM for LSI, but location, sex, and the interaction between season and location were. Differences in LSI between the bonefish at Half Sound and those at Starved Creek were found,

and those differences could be due to site-specific differences in prey content. The differences in LSI with respect to sex are unlikely to be caused by differences in energy storage strategy since no sex differences were detected for liver protein or water content, but it may be that female bonefish acquire different types of lipids than males. Analysis of the fatty acid composition of bonefish livers could elucidate this difference. In general, it is suggested that a study examining the
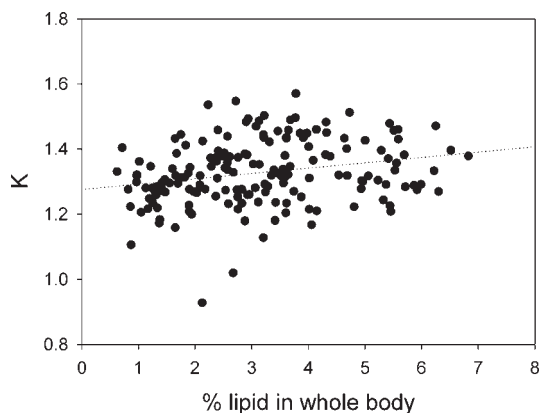
FIGURE 7.—Estimated relationship between condition factor and the whole-body percentage of lipids in bonefish (see text for equation).

proximate body composition of bonefish on a monthly basis be conducted to further parse out the potential combined effects of reproductive timing and water temperature (Montgomery and Galzin 1993; Kurita 2003).

In conclusion, an assessment of the proximate body composition and condition of bonefish improved our understanding of the strategies that fish use for making a living in tropical tidal flat environments. Ultimately, the data from this study will serve as the basis for the development and parameterization of a bioenergetics model for bonefish, providing fisheries managers with a useful tool for understanding bonefish production (Hansen et al. 1993). In addition, a bonefish bioenergetics model could be used in combination with bioenergetics models for other tropical flat organisms (e.g., lemon sharks; Sundström and Gruber 1998) to study predator–prey relations and truly understand ecosystem-level trophodynamics (Adams and Breck 1990; Hansen et al. 1993). Ideally, proximate body composition data such as we report in this study would be combined with information on trophic relationships (from stable isotope analyses), feeding ecology (from field studies), field activity levels, and growth to generate a comprehensive understanding of bonefish bioenergetics and trophic relations. Our observations also provide a baseline for future biomonitoring programs. Since bonefish are benthivores occupying shallow coastal habitats, they may be appropriate as bioindicators (Leamon et al. 2000; Plante et al. 2005). Energy reserve and fish health index data from Eleuthera may provide a useful baseline for the bonefish populations in areas such as Florida, where human development along the coastline is much more substantial. However, caution should be exercised

since there are numerous physiological and environmental factors that can influence the data, as observed in this study.

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