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Influence of life history and seasonal hydrology on lipid storage in three neotropical fish species

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The seasonal dynamics of energy (lipid) storage in three neotropical fish species with differing life histories were evaluated. Lipid content was substantially greater in the liver than in dorsal musculature of all three species. Two piscivores (*Cichla temensis* and *Serrasalmus manueli*) showed large, statistically significant seasonal fluctuations in liver lipid content. Liver lipid content increased during high water, through the falling water, and into the early dry season for both piscivores. Seasonal variation in dorsal muscle lipid content was large and statistically significant for *C. temensis*, but was small and non-significant for *S. manueli*. *Cichla temensis* appeared to 'finance' costs associated with reproduction by accumulating lipids during the falling-water period when migratory prey allowed the species to subsidize their energetic dynamics. *Semaprochilodus kneri*, a migratory algivore and detritivore, showed no significant seasonal variation in dorsal muscle lipid content and minimally significant seasonal variation in liver lipid content. Statistically significant effects of lipid content on $\delta^{13}C$ was observed when tissue lipid content varied by >12%, while biological interpretation of food web statistics based on $\delta^{13}C$ values appears robust to minor variations in lipid content. Nonetheless, when lipid content varied by larger amounts (*e.g.* >35% for *C. temensis* and *S. manueli* liver tissue) lipids appeared to have a large potential effect on $\delta^{13}C$ and food web statistics calculated from such measurements may have been biased. Surprisingly, even large variation in tissue lipid content did not affect $\delta^{15}N$.

Key words: energy storage; flood pulse; lipid extraction; migration; neotropics; stable isotope ratios.

INTRODUCTION

Energy storage varies substantially among organisms with different life-history strategies. In temperate fishes, stored energy is often used to fuel metabolic activity during harsh winter conditions, long distance migrations or gonad development (Reznick & Braun, 1987; Shuter & Post, 1990; Meffe & Snelson,

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1993; Phleger *et al.*, 1995; Schultz & Conover, 1997; Schultz, 1999). Salmonids, an extreme example, may migrate long distances to their spawning grounds, develop their gonads, prepare a nest, demonstrate courtship behaviour, and spawn, all without eating (Henderson & Tocher, 1987; Phleger *et al.*, 1995). These activities are necessary to maintain reproductive output (*i.e.* fitness), and are possible only through use of stored energy. Salmonids and other organisms that 'finance' future costs of reproduction with energy stored during periods of surplus resources are termed 'capitol' breeders (Bonnet *et al.*, 1998).

Tropical environments experience minimal seasonal variation of temperature and photoperiod, variables that drive life-history patterns of many temperate organisms. Tropical floodplain rivers, however, undergo large (5–20 m) seasonal fluctuations in water level that directly impact resource availability (Sioli, 1984). During high water periods the flooded forest provides readily available food sources for herbivorous and invertivorous fishes, while during low water periods aquatic organisms are concentrated in a smaller volume of water, and prey availability for piscivores is high (Lowe-McConnell, 1964, 1987; Bayley, 1988; Goulding *et al.*, 1988; Gomes & Agostinho, 1997). In addition to influencing resource availability, flood-driven seasonality affects timing of reproduction among most neotropical fishes (Junk, 1985; Ribeiro & Petrere, 1990; Fernandes, 1997; Jepsen *et al.*, 1999). Previous research (Saldaña & Venables, 1983; Junk, 1985; Gomes & Agostinho, 1997) has documented the seasonal deposition of fat stores in migratory fishes during the period of high resource availability (*i.e.* flood), and the subsequent consumption of these fat stores during periods of either decreased resource availability (*i.e.* low water) or increased energetic costs (*e.g.* migration and reproduction). Seasonality in reproductive effort and energy storage appear to be life-history adaptations to the predictable, unimodal water level fluctuations in tropical floodplain rivers (Junk, 1985).

Temporary energy storage in fishes is achieved by production and deposition of lipids as triacylglycerols (Hadley, 1985). In some species, lipid storage primarily occurs in the liver, whereas in other species lipids may be stored between muscles, in the mesentery, along the lateral line, or at the base of fins. Variation in muscle lipid content is generally due to seasonal deposition of stored energy, in the form of triacylglycerols (Henderson & Tocher, 1987; Mommsen, 1998). These stores are consumed typically during periods of short-term (*e.g.* burst swimming) or long-term (*e.g.* long-distance migration) energy deficit. In addition to serving as a storage depot, the liver is the major site of lipid biosynthesis (Henderson & Tocher, 1987), and as such may exhibit more dynamic lipid content than muscle tissue. Two important lipids produced in the liver are triacylglycerols and vitellogenin (Jobling, 1994; Mommsen, 1998). Triacylglycerols may be stored in the liver or transported to alternate storage sites (*e.g.* muscle and mesentery), whereas vitellogenin, the precursor to egg yolk proteins, is synthesized in the liver and transported to maturing eggs *via* the bloodstream (Henderson & Tocher, 1987; Jobling, 1994).

In the present study, lipid storage was evaluated in the dorsal musculature in 14 common neotropical fish species. Furthermore, temporal variation in lipid storage was evaluated in the dorsal musculature and liver tissue in three of the most common fishes collected, which were characterized by different feeding ecologies and life histories. The pavón *Cichla temensis* Humboldt is a piscivore that provides extended brood care (Winemiller, 2001). Caribe cachamero

Serrasalmus manueli (Fernández-Yépez & Ramírez) is a piscivore that provides little or no parental care. Bocochico del Orinoco *Semaprochilodus kneri* (Pellegrin) is a migratory detritivore (*i.e.* it consumes algae and organic matter) that provides no parental care for its young. Carbon and nitrogen stable isotopic ratios (reported as $\delta^{13}C$ and $\delta^{15}N$) for each specimen were regressed against observed lipid concentrations for muscle and liver tissues in order to determine the potential effect of lipid storage on $\delta^{13}C$ and $\delta^{15}N$. If present, seasonal fat storage may substantially alter $\delta^{13}C$ and $\delta^{15}N$ (Post, 2002), which are commonly used to explore complex feeding relationships among fishes in tropical rivers (Jepsen & Winemiller, 2002; Layman *et al.*, 2006).

MATERIALS AND METHODS

STUDY SITE

Field sampling was conducted in a *c.* 35 km stretch of the Cinaruco River between 6° 31′ N; 67° 26′ W and 6° 38′ N; 67° 09′ W. The Cinaruco River is a moderate blackwater, floodplain river that originates in Colombia, crosses Venezuela's savannah (*llanos*) region, and empties into the whitewater Orinoco River. This riverine environment experiences large, predictable hydrologic variation with comparatively minor fluctuations in physical conditions (Fig. 1). Previous work has characterized fish assemblage structure

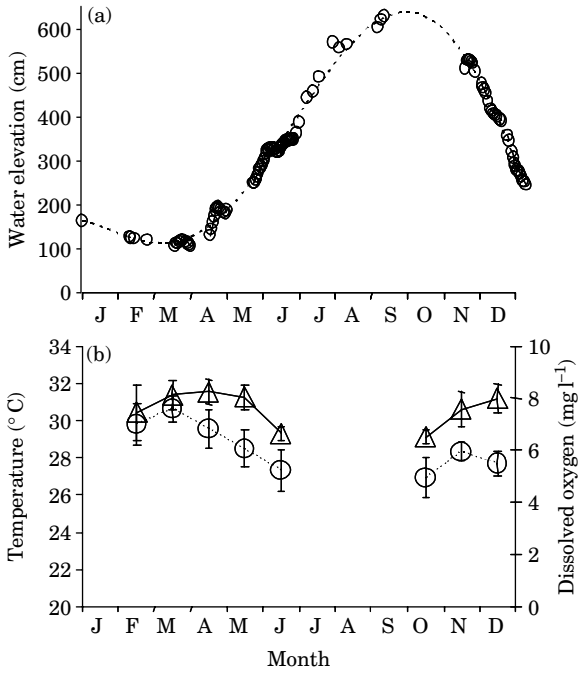

FIG. 1. Seasonal fluctuations in (a) water elevation and (b) mean ± S.D. ambient water temperature and dissolved oxygen content in the Cinaruco River during 1999. Water elevation is a relative measure used to illustrate the magnitude and timing of water level fluctuations. Mean water temperature (△) and dissolved oxygen content (○) is based on measurements taken in both the main river channel and in backwaters (*i.e.* lagoons).

and aquatic food web structure in this river (Jepsen *et al.*, 1997, 1999; Arrington & Winemiller, 2003; Layman & Winemiller, 2004; Winemiller & Jepsen, 2004, Arrington *et al.*, 2005).

FIELD SAMPLING

Fishes were collected using multiple techniques (*e.g.* angling, cast-netting and gillnetting) throughout 1999, excluding the peak flood period (July to September). In the field, specimens were identified and measured for standard length (L_S, mm) and wet mass (M_W, g). For all sampled individuals, a *c.* 2–10 g sample of dorsal muscle tissue and liver tissue was collected and preserved in the field with NaCl (Arrington & Winemiller, 2002). For each individual *C. temensis*, a species known to have substantial variation in seasonal lipid storage (Jepsen *et al.*, 1999), the following additional variables were recorded: gonad condition (scored from 0 to 5), ovary mass (M_o, g), mesenteric fat (scored from 0 to 4) and mesenteric fat mass (M_{MF} g). During field dissections, gonad condition was assessed by ranking gonad size and stage of maturity. Mesenteric fat was scored visually based on the amount of fat stored in the mesentery, and then (for a subset of the individuals) the mesenteric fat was manually removed and weighed to the nearest 0·1 g. In addition, the gonado-somatic index (I_G, where $I_G = 100M_O M_W^{-1}$) and the mesenteric fat index (I_{MF}, where $I_{MF} = 100M_{MF}M_W^{-1}$) were calculated. To examine the potential relationship between seasonal feeding patterns and lipid storage, the volume of stomach contents for individual *C. temensis* was also determined. Stomachs were examined by pressing down the posterior region of the tongue and pushing gently on the fish's stomach while holding the fish in a head-down position (Layman & Winemiller, 2004). Stomach contents were quantified volumetrically and the mean volume of stomach contents (*i.e.* prey) was calculated for each month.

LIPID EXTRACTION

In the laboratory, dorsal muscle and liver tissue lipid content was estimated using a 2 : 1 chloroform : methanol solution as described by Folch *et al.* (1957) and revised by Post & Parkinson (2001). Salt-preserved tissue samples were rinsed in distilled water, soaked in distilled water for 4 h, dried at 60° C for 48 h, and then ground to a fine powder using a mortar and pestle. A 0·5000 + 0·0001 g of dried, powered fish tissue was loaded into a 30 ml test tube, to which 8 ml chloroform and 8 ml of methanol were added (resulting in a 50 : 50 methanol–chloroform solution). The mixture was heated in a 61° C water-bath until it boiled, cooled to room temperature, and increased in volume to 25 ml by addition of chloroform. The entire volume was filtered through a No. 1 Whatman filter paper into a 125 ml separatory funnel, to which 10 ml of 0·9% saline solution was added. After shaking the separatory funnel and contents vigorously, the mixture was allowed to settle, and the bottom methanol-chloroform layer was drained into a pre-weighed aluminum dish. The contents were evaporated on a hot plate at 70° C. The weighing dish was cooled to room temperature and weighed to the nearest 0·0001 g. The mass of lipid remaining in the aluminum dish represented the mass of lipid per 0·5 g of dry fish tissue. While there are potential problems with making comparisons using per cent lipid content relative to dry mass (Shearer, 1994; Sutton *et al.*, 2000), the sampling protocol, which was designed for a stable isotope study, did not permit an allometric analysis (Jobling, 2001). Nonetheless, the analysis of per cent lipid content relative to dry mass is comparable to previous work evaluating lipid dynamics in fishes (Junk, 1985; Henderson & Tocher, 1987).

LIPID VERIFICATION

Additionally, lipids were extracted from replicate sub-samples of a single fish to evaluate the lipid extraction procedure. Large-bodied North American fishes were used

because limited amounts of tissue were available from South American fishes. To determine how much lipid remained in samples after extraction, lipids were extracted and re-extracted from six *C. temensis* samples and three *S. manueli* muscle tissue samples.

STABLE ISOTOPE ANALYSIS

Carbon and nitrogen stable isotopic ratios were determined for individual fish following Arrington & Winemiller (2002). In the laboratory, salt-preserved samples were rinsed in distilled water, soaked in distilled water for 4 h, and dried at 60° C for 48 h. Once dry, samples were ground to a fine powder using a mortar and pestle. Samples were loaded into tin capsules, and sent to the Stable Isotope Laboratory at the University of Georgia's Institute of Ecology Atlanta, Georgia, U.S.A. for analysis of carbon and nitrogen stable isotope ratios. Stable isotope values are reported using δ (delta) notation: $\delta^{15}C$ or $\delta^{15}N = 100[(R_{sample}\ R_{standard}{}^{-1}) - 1]$, where R is $^{13}C : {}^{12}C$ or $^{15}N : {}^{14}N$. Working standards were bovine ($n = 49$, $\delta^{13}C = -22{\cdot}11‰$, S.D. = 0·06‰, 48·8% C, $\delta^{15}N = 7{\cdot}47$, S.D. = 0·07‰, 10·0% N) and poplar ($n = 81$, $\delta^{13}C = -27{\cdot}34‰$, S.D. = 0·10‰, 48·1% C, $\delta^{15}N = -2{\cdot}47$, S.D. = 0·16‰, 2·7% N).

STATISTICAL ANALYSES

Evaluation of seasonal lipid dynamics was constrained to adult fishes only. Analyses, therefore, were limited to the minimum size classes observed to have mature gonads in the samples: *C. temensis* >289 mm L_S ($n = 187$), *S. manueli* >129 mm L_S ($n = 139$) and *S. kneri* >179 mm L_S ($n = 66$). The dependent variable in the analyses was per cent lipid (dry mass), the independent variable was month (January to December) and L_S was included as a potential covariate. Analyses of covariance (ANCOVA) were executed with the general linear model procedure (GLM) using SPSS after verification that none of the analyses (*e.g. S. manueli* muscle lipid content) resulted in a significant ($P < 0{\cdot}05$) interaction effect between L_S and month (*i.e.* heterogeneous slopes). Parametric assumptions were assessed using the Kolmogorov–Smirnov test of normality and Levene's test of equality of error variances. Based on these results, *S. kneri* muscle and liver lipid data were not transformed prior to analysis, but transformation was required for each of the following species-tissue combinations: *C. temensis* muscle lipid data (square root^{-1}), *S. manueli* muscle lipid data (ln), *S. manueli* liver lipid data $\left(\sqrt{\log_{10}}\right)$. Transformations of *C. temensis* liver lipid data did not meet assumptions of normality and homogeneity of variances so the non-parametric Kruskal–Wallis test was employed for the global test, while the Mann-Whitney *U*-test was used to evaluate *post hoc* pair-wise comparisons. All *post hoc* comparisons were Bonferroni corrected.

Linear regression was used to determine the following relationships for *C. temensis*: (1) I_G and gonad condition score, (2) I_{MF} and fat score, (3) dorsal muscle lipid content and I_{MF} and (4) liver lipid content and I_{MF}. Furthermore, linear regression was used to examine the potential relationship between tissue lipid content (muscle or liver) and $\delta^{13}C$ and $\delta^{15}N$ for all specimens (juveniles and adults) for of each of the three species. Linear regression was performed using SPSS.

RESULTS

LIPID VERIFICATION

Analysis of replicates resulted in small estimates of coefficient of variation (CV) [bowfin *Amia calva* L. dorsal muscle: $n = 16$, mean ± S.D. = 4·31 ± 0·19, CV = 4·40%; gizzard shad *Dorosoma cepedianum* (Lesueur) dorsal muscle: $n = 14$, mean ± S.D. = 3·53 ± 0·14, CV = 4·02%; *A. calva* liver: $n = 5$, mean ± S.D. = 11·70 ± 0·12, CV = 1·02%; flathead catfish *Pylodictis olivaris*

(Rafinesque) liver: $n = 5$, mean ± S.D. = 36·51 ± 0·96, CV = 2·62%]. The first lipid extraction yielded 88·3 ± 8·8% (mean ± S.D.) of total lipids extracted after completion of two complete extraction procedures.

LIPID CONTENT

Lipid content of the dorsal musculature for 11 common, large-bodied fishes in the Cinaruco River was characterized (Table I). These data indicate that frugivores, *Metynnis hypsauchen* (Müller & Troschel), *Mylossoma aureum* (Agassiz) and *Myleus schomburgkii* (Jardine) had the highest mean dorsal muscle lipid content (8·3, 7·1 and 6·3%, respectively). The three focal species of the present study had relatively low mean lipid content when averaged across all sampling months (*C. temensis* 4·8%; *S. manueli* 3·0%; *S. kneri* 2·2%).

Temporal analyses revealed significant seasonal patterns of lipid storage in all three species. *Cichla temensis* showed strong seasonal variation in both dorsal muscle and liver lipid content [Figs. 2(a) and 3(a)]. *Cichla temensis* dorsal muscle lipid content varied significantly among months (ANCOVA, d.f. = 8 and 17, $P < 0{\cdot}001$); effect of L_S was non-significant (ANCOVA, d.f. = 1, 177, $P > 0{\cdot}6$). There was also a highly significant effect of month on *C. temensis* liver lipid content (d.f. = 8, $P < 0{\cdot}001$). In both dorsal muscle and liver tissues, lipids were stored during the high- and falling-water periods (June to December) and early dry season (January to February) and then expended during the spawning period (February to May). Foraging activity, as measured by stomach content analyses, corresponded to the observed seasonal pattern. Mean prey volume per *C. temensis* individual peaked during the falling-water period and was substantially lower in January to June, with a notable decline from February to April (Fig. 4).

Gonad scores based on visual examination were strongly related to measured female I_G ($n = 36$, $r^2 = 0{\cdot}83$, $P < 0{\cdot}001$). Both gonad scores and I_{MF} indicated *C. temensis* gonad maturation began in late December and spawning occurred from February to May. Visually estimated fat scores corresponded well to measured I_{MF} values ($n = 101$, $r^2 = 0{\cdot}77$, $P < 0{\cdot}001$). Furthermore, the relationship between *C. temensis* dorsal muscle lipid content and I_{MF} was strongly correlated and highly significant ($n = 101$, $r^2 = 0{\cdot}70$, $P < 0{\cdot}001$). The relationship between *C. temensis* liver lipid content and I_{MF} was highly significant, although it explained little variance ($n = 98$, $r^2 = 0{\cdot}16$, $P < 0{\cdot}001$).

For *S. manueli*, dorsal muscle lipid content did not vary significantly among months [ANCOVA, d.f. = 7 and 130, $P > 0{\cdot}3$, Fig. 2(b)]. There was a significant effect of L_S (ANCOVA, d.f. = 1 and 130 $P < 0{\cdot}05$) with larger fish having higher intra-musculature lipid content. *Serrasalmus manueli* liver lipid content was significantly related to both month (ANCOVA, $P < 0{\cdot}001$) and L_S (ANCOVA, $P < 0{\cdot}001$); liver lipid stores were higher in larger individuals and were most depleted in May [Fig. 3(b)]. Lipid content of *S. kneri* dorsal muscle did not vary significantly among months [ANCOVA, d.f. = 8 and 58, $P > 0{\cdot}1$, Fig. 2(c)] or with fish size (ANCOVA, d.f. = 1 and 58, L_S, $P > 0{\cdot}2$). *Semaprochilodus kneri* liver lipid content differed significantly among months (ANCOVA, d.f. = 8 and 56, $P = 0{\cdot}011$) but the effect of L_S was non-significant (ANCOVA, d.f. = 1 and 56, $P > 0{\cdot}2$). Livers from specimens collected in April

TABLE I. Per cent lipid values (as a function of dry mass) for dorsal muscle tissue for 14 abundant fish species in the Cinaruco River, Venezuela, as sampled in 1999. Fishes are sorted by mean per cent lipid content of dorsal muscle tissue

Family	Species	n	L_S (mm) range	Per cent lipid (mean ± S.D.)	Per cent lipid (range)
Characidae	*Metynnis hypsauchen* (Müller & Troschel)	26	109–147	8·31 ± 3·49	2·41–15·87
Characidae	*Mylossoma aureum* (Agassiz)	8	152–236	7·08 ± 5·66	0·88–17·82
Characidae	*Myleus schomburgkii* (Jardine)	5	130–255	6·27 ± 3·45	3·22–11·83
Cichlidae	*Cichla temensis* Humboldt	210	180–655	4·77 ± 2·23	1·37–13·00
Characidae	*Myleus rubripinnis* (Müller & Troschel)	13	130–215	5·51 ± 4·52	1·25–14·13
Characidae	*Piaractus brachypomus* (Cuvier)	18	120–325	3·91 ± 2·41	0·96–10·86
Hemiodontidae	*Hemiodus immaculatus* (Kner)	7	140–182	3·79 ± 1·81	0·97–5·82
Characidae	*Brycon pesu* Müller & Troschel	2	75–85	3·60 ± 3·59	1·06–6·14
Characidae	*Brycon falcatus* Müller & Troschel	16	100–117	3·34 ± 2·58	0·99–9·43
Characidae	*Chalceus macrolepidotus* Cuvier	7	115–183	3·16 ± 0·85	1·85–4·56
Characidae	*Serrasalmus manueli* (Fernández-Yépez & Ramírez)	146	145–395	2·98 ± 0·94	1·57–5·58
Hemiodontidae	*Argonectes longiceps* (Kner)	2	87–91	2·78 ± 0·63	2·34–3·23
Prochilodontidae	*Semaprochilodus kneri* (Pellegrin)	105	110–300	2·22 ± 0·71	0·83–3·87
Hemiodontidae	*Hemiodus unimaculatus* (Bloch)	2	85–101	2·29 ± 0·48	1·95–2·63

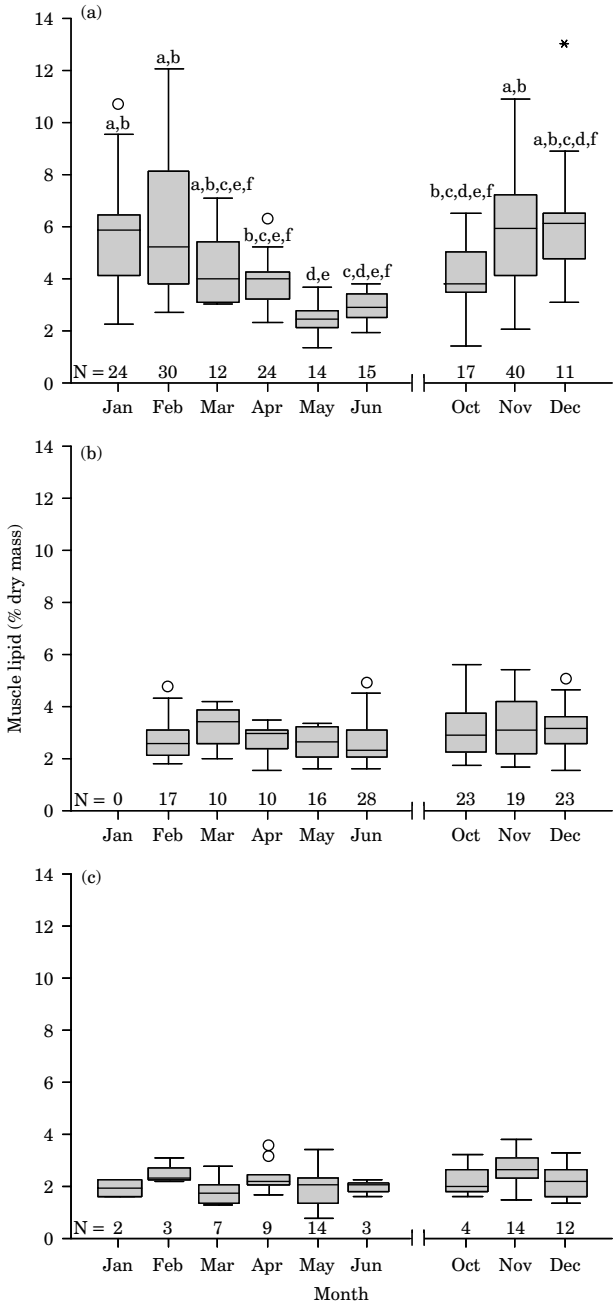

FIG. 2. Box plots of seasonal variation of muscle lipid content for (a) *Cichla temensis*, (b) *Serrasalmus manueli* and (c) *Semaprochilodus kneri*. For each month, the median (bold line in centre of box), 25th percentile (lower edge of box), 75th percentile (upper edge of box), the extent of non-outlying data (whiskers), mild outliers (circles) and extreme outliers (asterisks) are illustrated. Only *C. temensis* showed statistically significant seasonal variation in intra-musculature lipid storage ($P < 0{\cdot}001$). For *C. temensis*, months not sharing the same lowercase letter (above error bar) are significantly different ($P < 0{\cdot}05$, after Bonferroni correction for multiple comparisons).

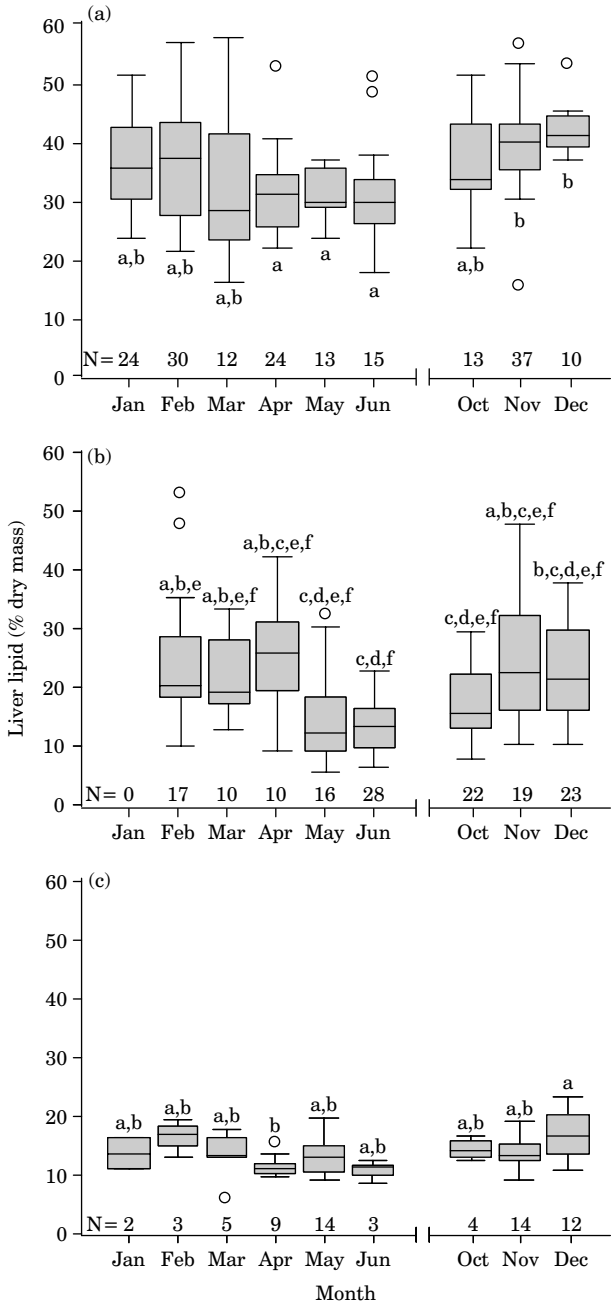

FIG. 3. Box plots showing seasonal variation of liver lipid content for (a) *Cichla temensis*, (b) *Serrasalmus manueli* and (c) *Semaprochilodus kneri*. For each month, the median (bold line in centre of box), 25th percentile (lower edge of box) 75th percentile (upper edge of box), the extent of non-outlying data (whiskers), mild outliers (circles), and extreme outliers (asterisks) are illustrated. All three species showed statistically significant seasonal variation in liver lipid content (*C. temensis*, $P < 0{\cdot}001$; *S. manueli*, $P < 0{\cdot}001$; *S. kneri*, $P = 0{\cdot}011$). For each species, months not sharing the same lowercase letter (above or below error bar) are significantly different ($P < 0{\cdot}05$, after Bonferroni correction for multiple comparisons).

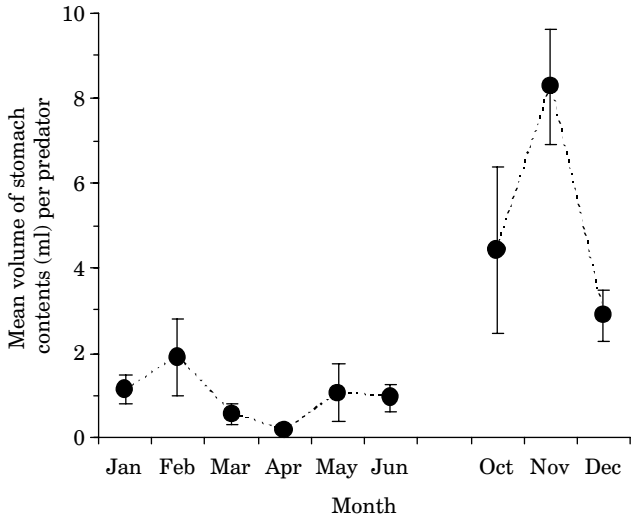

FIG. 4. Mean ± S.E. volume of stomach contents per individual *Cichla temensis*.

had significantly lower lipid content than fish collected during December [Fig. 3(c)].

STABLE ISOTOPES

Linear regression indicated variation in tissue lipid content may alter $\delta^{13}C$, though no effect was observed for $\delta^{15}N$ (Table II). In no instance was there a statistically significant relationship between either dorsal muscle or liver lipid content (as a percentage of dry mass) and the nitrogen stable isotopic ratio (Table II). Carbon stable isotopic ratios, however, were statistically related to per cent lipid content of *C. temensis* dorsal muscle tissue and *C. temensis* and *S. manueli* liver tissue. Using the statistically significant regression equations given in Table II, absolute differences in $\delta^{13}C$ due to the effect of variable lipid content was estimated at 1·17‰ (*C. temensis* muscle; lipid range 1·37–13·00%), 3·44‰ (*C. temensis* liver; lipid range 14·34–49·99%) and 2·85‰ (*S. manueli* liver; lipid range 10·36–52·93). Although statistically significant, the estimated regression (b) and correlation (r^2) coefficient for the *C. temensis* dorsal muscle relationship was sufficiently small to suggest limited biological significance (Table II). Nonetheless, when tissue lipid content showed large variation, statistically and biologically significant effects were observed relative to variation in $\delta^{13}C$.

DISCUSSION

TISSUE SPECIFIC LIPID STORAGE

Cichla temensis, *S. manueli* and *S. kneri* stored lipids differentially among tissue types throughout the year. All three species stored greater concentrations of lipids in their liver than in their dorsal muscle, with *C. temensis* possessing the

TABLE II. Linear relationships between tissue-specific lipid content and $\delta^{13}C$ and $\delta^{15}N$. Statistically significant relationships ($P < 0{\cdot}05$) are in bold

		$\delta^{13}C$				$\delta^{15}N$			
Species	n	Constant ± S.E.	b ± S.E.	r^2	P	n	Constant ± S.E.	b ± S.E.	r^2	P
				Dorsal muscle						
Cichla temensis	**196**	**−28·92 ± 0·19**	**−0·10 ± 0·04**	**0·039**	**0·005**	196	9·59 ± 0·08	−0·03 ± 0·02	0·016	0·075
Serrasalmus manueli	144	−27·65 ± 0·31	0·00 ± 0·10	0·000	0·984	144	10·44 ± 0·12	0·04 ± 0·04	0·009	0·253
Semaprochilodus kneri	99	−31·41 ± 0·42	−0·24 ± 0·18	0·018	0·181	99	7·47 ± 0·27	−0·13 ± 0·12	0·014	0·244
				Liver						
Cichla temensis	**29**	**−30·67 ± 0·89**	**−0·10 ± 0·03**	**0·282**	**0·003**	29	10·31 ± 0·33	−0·02 ± 0·01	0·080	0·130
Serrasalmus manueli	**25**	**−30·02 ± 0·50**	**−0·07 ± 0·02**	**0·309**	**0·003**	25	10·62 ± 0·24	0·01 ± 0·01	0·019	0·507
Semaprochilodus kneri	18	−33·47 ± 1·54	−0·11 ± 0·10	0·071	0·271	18	7·01 ± 0·42	0·02 ± 0·03	0·027	0·504

most, and *S. kneri* the least, lipid-rich liver. Although the liver is known to function as a dynamic lipid reservoir, it has been hypothesized that freshwater fishes do not possess lipid-rich livers under normal nutritional and environmental conditions (Henderson & Tocher, 1987). *Cichla temensis*, and to a lesser degree *S. manueli*, however, possessed lipid-rich livers [Fig. 3(a)] relative to other tissues (*e.g.* muscle) and other species (Henderson & Tocher, 1987). *Cichla temensis* use their liver, muscle and mesentery as energy storage depots, while *S. manueli* and *S. kneri* primarily store lipids in their livers and mesentery.

SEASONAL VARIATION IN LIPID STORAGE

A previous report indicated that all neotropical fish species that showed strong seasonal differences in lipid content were migratory (Junk, 1985), while results from the present study suggest that migratory neotropical fish species may store lipids (*e.g. M. aureum*; Table I). Results from the present study also suggest non-migratory species may display seasonal patterns of lipid storage due to other life-history characteristics. Seasonal deposition and consumption of lipids, *i.e.* stored energy, in *C. temensis* and *S. manueli* appears to function as a life-history adaptation in seasonally flooded tropical floodplain rivers. Energetic costs and behavioural adaptations associated with reproduction (*e.g.* gonad maturation, nest building, spawning and brood care) probably account for declining lipid content in dorsal muscle of *C. temensis* from January to May and in liver tissue of *S. manueli* between April and May. Both *C. temensis* and *S. manueli* are capital breeders (Bonnet *et al.*, 1998), that is they 'finance' costs associated with reproduction with energy assimilated during an earlier period.

The magnitude of energy required to 'finance' reproduction appears to be greater for *C. temensis* than *S. manueli*, probably because *C. temensis* completely cease feeding during reproduction and an extended period of brood care (Jepsen *et al.*, 1999; Arrington *et al.*, 2002). This period of self-imposed resource limitation has been termed a 'physiological winter' (Lowe-McConnell, 1964) and has been demonstrated previously in the Cinaruco River (Jepsen *et al.*, 1999). *Cichla temensis* and *S. manueli* began replenishing their lipid stores during the rising- and high-water periods when prey availability was lowest for piscivores. Largest increases in lipid stores occurred from October to December, a period of increased prey availability due to falling water levels and upstream migrations of *S. kneri* (Jepsen *et al.*, 1997; Winemiller *et al.*, 1997; Winemiller & Jepsen, 1998; Layman *et al.*, 2006). Seasonal variation in lipid dynamics, therefore, appears partially due to seasonal patterns of prey availability and consumption as evidenced by *C. temensis* stomach contents. These lipid stores are then utilized to 'finance' reproduction during the period thought to maximize juvenile survivorship and growth (Winemiller, 1993).

Semaprochilodus kneri, a migratory species, had one of the lowest mean muscle lipid content among all species analysed in this study (Table I), and the observed low mean muscle lipid content and low variability in *S. kneri* appears different from the congeneric *Semaprochilodus insignis* (Jardine & Schomburgk) (Junk, 1985). This may be due to differences in migratory behaviour between these species. In the Orinoco Basin, *S. kneri* migrate downstream from blackwater tributaries (*i.e.* Cinaruco River) to the whitewater Orinoco to spawn and forage in the nutrient

rich floodplain during the high water period (Winemiller & Jepsen, 1998, 2004), During the falling-water period, *S. kneri* migrate upstream into blackwater rivers (*i.e.* the Cinaruco) where they apparently remain throughout the low-water period. In the Amazon basin, *S. insignis* migrate downstream from blackwater tributaries to the Amazon River to spawn (beginning of rising water), they then migrate upstream to forage in flooded forest (early high water), and then they undertake complex dispersal migrations (late high water to low water), with continued residence in tributaries during low water (Ribeiro & Petrere, 1990). The downstream dispersal migration of *S. insignis* is referred to as the 'fat fish migration' apparently due to the accumulation of lipid stores while foraging in the flooded forest. A similar increase in lipid stores in *S. kneri* collected from the Cinaruco River was not observed. It is possible *S. kneri* store lipids while foraging on the Orinoco floodplain, and then completely deplete lipid stores during the upstream migration (*i.e.* prior to reaching the study site). Alternatively, the lack of variability in lipid content could be expected if only non-migratory individuals were collected in this study, because it has been shown that non-migrating prochilodontids (*Prochilodus mariae* Eigenmann) in the Orinoco River have substantially lower lipid content than migrating individuals (Saldaña & Venables, 1983). Given the numerical abundance of migrating *S. kneri* in the Cinaruco River, it seems unlikely that only non-migrating specimens would have been captured during migratory periods. Additionally, these explanations seem unlikely because detritivores such as *S. kneri* appear to forage continuously to meet energetic demands (Arrington *et al.*, 2002). Differences in migratory behaviour and life history appear to be a more parsimonious explanation of the differences in lipid dynamics between *S. kneri* and *S. insignis*. Migration dynamics and life history of *S. kneri* deserve further study, particularly because this species represents a critical energetic and nutritional subsidy in nutrient-poor blackwater rivers (Jepsen *et al.*, 1997; Winemiller & Jepsen, 1998, 2004).

INFLUENCE OF LIPID STORAGE ON $\delta^{13}C$ AND $\delta^{15}N$

Variation in tissue lipid content did not influence observed $\delta^{15}N$, and thus it appears in these species that $\delta^{15}N$ is relatively immune to changes in lipid content. Lipid content and $\delta^{13}C$ were significantly correlated ($P < 0{\cdot}05$) in interspecific comparisons for *C. temensis* dorsal muscle (lipid content range 1·3–13·0%), *C. temensis* liver (lipid content range 14·3–56·1%) and *S. manueli* liver (lipid content range 6·1–52·9%). Given the relatively small effect size (1·17‰) and the small coefficient of determination ($r^2 = 0{\cdot}039$), it is concluded that the biological interpretation of food web statistics based on $\delta^{13}C$ values is robust to variability in lipid content for *C. temensis* muscle samples (where range of lipid content did not exceed 12%). Nonetheless, when lipid content varies by larger amounts (*e.g.* >35% for *C. temensis* and *S. manueli* liver tissue) lipids appear to have a large potential effect on $\delta^{13}C$ and food web statistics calculated from such measurements may be biased (*i.e.* unduly influenced by lipids which are depleted in ^{13}C). Because carbon isotopic ratios are sensitive to large fluctuations in tissue lipid content, researchers should determine the magnitude of variability in lipid content of tissues prior to conducting stable isotopic studies. Standardization of lipid content (*i.e.* lipid extraction; Post, 2002) appears necessary only in tissues that show a large range of lipid content.

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