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Lipid class dynamics and storage depots in juvenile weakfish *Cynoscion regalis* and their application to condition assessment

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Lipid class dynamics, the pattern of change in the primary form and location of lipid stores and their relationship with standard length (L_S), were investigated in collections of young-of-the-year weakfish *Cynoscion regalis* for the purpose of determining the utility of this analysis as an indication of condition. The separation of total lipids into individual classes and the analysis of potential storage depots revealed the general patterns of lipid class dynamics and energy storage in *C. regalis* during their period of juvenile estuarine residency. Phospholipid and cholesterol exhibited moderate but variable ($8.1\text{--}40.0$ and $1.3\text{--}21.5\text{ mg g}^{-1}$, respectively) concentrations across the entire juvenile period and were the predominant lipid classes in juveniles $<100\text{ mm } L_S$, while wax ester concentrations were low ($c. 1\text{ mg g}^{-1}$) and exhibited the least amount of variability among lipid classes. Triacylglycerols (TAG) and free fatty acids (FFA) exhibited similar dynamics, with relatively low concentrations ($<15\text{ mg g}^{-1}$) in individuals $\leq 100\text{ mm } L_S$. In larger juveniles both TAG and FFA concentrations generally increased rapidly, though there was considerable variability in both measures ($0.0\text{--}199.7$ and $0.0\text{--}49.7\text{ mg g}^{-1}$, respectively). Increasing levels of lipids, primarily in the form of TAG, with size indicated an accumulation of energy reserves with growth, thus providing an indication of individual condition for larger juveniles. Separate analysis of liver, viscera and the remaining carcass indicated that liver and viscera did not represent a significant depot of TAG reserves. Analysis of samples derived from whole juvenile *C. regalis* thus provided an accurate estimate of energy reserves.

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Key words: biochemical condition; free fatty acids; lipid allocation, lipid storage; triacylglycerol.

INTRODUCTION

Variability in the strength of recruitment among year classes is a major focus in fisheries research and is thought to be determined largely during the early life stages of development (Houde, 1987; Ralston, 1995; Sogard, 1997). Marine transient

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species (Deegan *et al.*, 2000) spawn along the Atlantic and Gulf coasts of the U.S.A. and produce offspring that recruit to shallow estuarine habitats. For these species, post-settlement survival, growth, energy allocation and young-of-the-year (YOY) recruitment success may be governed by a number of factors (Shuter & Post, 1990; Walters & Juanes, 1993; Kneib, 2003). Initially, size-selective predation represents their primary source of mortality and therefore energy tends to be allocated to somatic growth. Later in the juvenile period, allocation of energy shifts towards long-term storage in preparation for the impending demands associated with offshore migration and overwintering (Post *et al.*, 1997; Sogard, 1997; Hurst, 2007). While overwintering, starvation due to energy deficits is considered a major form of mortality, with environmental conditions and the magnitude of energy reserves governing the likelihood of survival (Post & Lee, 1996; Schultz & Conover, 1997; Biro *et al.*, 2004; Hurst, 2007).

To understand the effects of environmental variability and competing energy demands on the survival potential of YOY fishes, there has been a focus on developing measures of biochemical condition, including those based on the energy stored as lipids. Lipids can be readily utilized during deficit periods due to food shortages or metabolically stressful conditions (Shulman & Love, 1999). While total lipid content is often used as an indicator of biochemical condition (Shuter & Post, 1990; Schultz & Conover, 1999), the ability to endure energy deficits may be dependent on the magnitude of endogenous stores of specific lipid classes (Norton *et al.*, 2001; Finstad *et al.*, 2004; Naesje *et al.*, 2006). Among these classes, triacylglycerols (TAG) are principal energy reserves for most finfishes beyond the yolk-sac stage and have been used as a principal indicator of biochemical condition (Doucett *et al.*, 1999; Norton *et al.*, 2001; Lochmann & Ludwig, 2003; Weinstein *et al.*, 2009, 2010), though other neutral lipids, such as mono- and di-acylglycerols, free fatty acids (FFA), cholesteryl esters and wax esters (WE), may be important forms of long-term energy stores (Lochmann *et al.*, 1995).

Lipid storage depots may occur in numerous locations in finfishes: subcutaneous connective tissue, skeletal muscle, the abdominal cavity (particularly in the mesenteries and pyloric caeca), the liver, interosseous tissue and at the base of the fins (Jobling *et al.*, 1998; Shulman & Love, 1999; Morgan *et al.*, 2002). As a rule, a given species has several lipid depots, with the sequence of their utilization depending on the functional state and nature of the processes requiring energy reserves. Generally, utilization of reserves is in the order: liver lipid to mesenteric lipid to muscle lipid to muscle proteins, *e.g.* in Atlantic cod *Gadus morhua* Linnaeus 1758 (Black & Love, 1986; Shulman & Love, 1999). Liver lipid concentrations, however, may not vary with physiological state. In their study of Atlantic salmon *Salmo salar* Linnaeus 1758 during migration, Doucett *et al.* (1999) noted that lipid levels in the liver were slightly reduced. They attributed this observation to the central role the liver plays in lipid metabolism and concluded that maintenance of levels in the liver was accomplished *via* lipid mobilization from areas of less importance to the liver during migration.

The objective of this study was to assess the feasibility of utilizing lipid class analysis as a metric of biochemical condition for future studies of juvenile weakfish *Cynoscion regalis* (Bloch & Schneider 1801), a marine transient species. If feasible, an additional objective was to identify the most appropriate and efficient sampling

protocol (whole individuals or specific tissues) to determine their condition. Specifically, variability in lipid classes and the relationship between mass (M) and standard length (L_S), both of whole individuals and in specific tissues, in a temperate population of YOY *C. regalis* were compared to determine: (1) the dynamics of individual lipid class concentrations over the YOY period, (2) the distribution of lipid reserves within tissues (liver, viscera and remaining carcass) and (3) the differences, if any, in estimates of lipid reserves based on analysis of whole individuals *v.* specific tissues.

MATERIALS AND METHODS

FIELD COLLECTIONS

Juvenile *C. regalis* were collected in August through to October of 1999 and 2000 with otter trawls towed near low tide for 10 min during daytime and night-time hours in tidal salt marsh creeks across the length of the New Jersey shore of Delaware Bay. Marsh creeks sampled were Alloway Creek, a 1253 ha meso-oligohaline *Phragmites australis*-dominated marsh, Mad Horse Creek, a relatively undisturbed 1558 ha mesohaline marsh, and two *Spartina alterniflora*-dominated meso-polyhaline systems: Dennis Creek, an undisturbed 1100 ha marsh, and West Creek, a nearby 234 ha formerly diked salt hay farm. In addition, juvenile *C. regalis* were collected at the mouth of Delaware Bay prior to their emigration from the system in early November of 1999.

Fish were measured for L_S to the nearest mm and quickly frozen and preserved on dry ice until transported to the laboratory and storage at -80°C (Ohman, 1996). An effort was made to collect individuals from three size classes: <60 mm (I), 60–100 mm (II) and >100 mm L_S (III). These size classes reflect ontogenetic shifts in the trophic niche of juvenile *C. regalis* from specialization on crustaceans when <60 mm, to an increasing proportion of fishes while in the second size class to roughly equal proportions of crustaceans and fishes when >100 mm (Chao & Musick, 1977; Grecey & Targett, 1996a; Nemerson, 2001).

LABORATORY ANALYSIS

Two hundred and seventy-two *C. regalis* 24–200 mm L_S (39, 145 and 88 in size classes I, II and III, respectively) were selected to discern lipid class dynamics over the juvenile period. Partially thawed fish were cleaned of gut contents (Lochmann *et al.*, 1995, 1996), then subsequently freeze-dried to a constant M before grinding to fine powder (*i.e.* amalgamation, Spex CertiPrep 5100 grinding mill; www.spexsampleprep.com). Fish <120 mm L_S were ground whole, while those ≥ 120 mm L_S were first coarse ground in a Waring (www.waringproducts.com) stainless steel blender and a random aliquot was then selected for grinding to a fine powder. Eighty individuals 25–182 mm L_S (43, 21 and 16 in size classes I, II and III, respectively) collected from the four marsh creeks between August and October 1999 were utilized to compare the distribution of TAG among juvenile *C. regalis* tissues. Partially thawed fish were dissected to isolate liver, viscera (including mesenteries) and remaining carcass. Each component was separately freeze-dried, weighed [M_{DC} , where C = component, L (liver), V (viscera) or R (remaining carcass)] and ground to a fine powder. An exception was liver and viscera from smaller fish which were not ground and underwent biochemical analysis whole to avoid significant loss of material. All samples were stored in pre-combusted glass vials with acid-washed polyethylene caps and purged with N_2 gas to exclude airborne oxygen and prevent lipid peroxidation prior to storage at -80°C .

A 50 mg, range 49–55 mg sub-sample, if available, of ground amalgamated fish or component (liver, viscera or remaining carcass) was folded into a 70 mm diameter Whatman 541 ashless filter paper (www.whatman.com) and extracted three times, first in 4.0 ml of 2:1 (v/v) dichloromethane: methanol for 15 h at 5°C (vial headspace purged with N_2), then in 3.0 ml of fresh solvent mixture for 3 h, followed by a final 3 h extraction in 2 ml of fresh solvent

mixture. The three sequential extracts were combined and stored at -80°C under N_2 for no more than 4 days to avoid esterification of fatty acids by methanol in the extraction solvent (Parrish, 1987). To each extract, $50\text{ }\mu\text{g }\mu\text{l}^{-1}$ nonadecane ($\text{C}_{19}\text{H}_{40}$: 1.000 mg total) internal standard (for 50 mg samples reconstituted to 0.5 ml or adjusted to preserve the concentration of standard relative to the final sample volume) in chloroform was added and back extraction in 0.1 M aqueous KCl solution at 5°C (Folch *et al.*, 1957) was used to remove non-lipid materials. Back-extracted samples were concentrated by evaporating to dryness in a 38°C water bath under a stream of N_2 gas and then reconstituted in 0.5–1.5 ml of dichloromethane, depending on sample size and lipid content.

Lipid classes in these extracts were separated using a modification of the method of Lochmann *et al.* (1995) in which 1–2 μl aliquots of lipid extracts were spotted on Iatron Chromarod III silica-coated rods (www.iatronscan.com) and partially developed in three successive HPLC grade solvent systems. Modifications included the placement of a desiccant into the first two development tanks to lower humidity, extending development duration to 45 min for better definition of polar classes and drying Chromarods for 3 min at 90°C prior to analysis. Flame ionization detector (FID) scanning using an Iatronscan Mark V with a Hewlett Packard HP3690 integrator was used for data acquisition. Blanks, a combined synthetic standard that included hydrocarbon (nonadecane), WE (palmitic acid stearyl ester), triacylglycerol (tripalmitin), FFA (palmitic acid), fatty alcohol (1-hexadecanol = cetyl alcohol), sterol (ST) (cholesterol), monoacylglycerol (1-monopalmitoyl-rac-glycerol), phospholipid (PL) (phosphatidylcholine), and one replicate for every seven samples were utilized to determine lipid class concentrations and ensure accuracy. Conversion of FID peak areas to extract lipid concentrations was performed using a segmented third-order polynomial calibration model with separate curves generated for each standard and each Chromarod set used. Because the standards did not represent all lipid classes present in fishes, peaks not corresponding to standards were converted to concentrations using a model representing the average response for the eight specified classes. Conversion of all peaks provided a measure of all classes, and therefore total lipid concentration, when summed.

STATISTICAL ANALYSIS

The dynamics of individual lipid class concentrations in juvenile *C. regalis* were investigated for WE, TAG, FFA, ST, PL, as well as total lipids, all expressed as mg g^{-1} dry *M*. ST and PL are major structural lipids classes with unambiguous FID peaks while TAG and WE represent potential long-term energy stores. FFA, a metabolic intermediate and potential short-term energy source, was considered the sum of the FFA plus methyl esters (ME) from the analysis because ME most likely originates from the methylation of FFA in the extraction process (Parrish, 1987). Differences and variability in the concentrations of the five lipid classes, total lipid concentration and per cent of total lipid concentration in the form of TAG for individual YOY *C. regalis* were examined over the entire range of L_S via fitting a non-parametric locally weighted smoothing function (LOWESS; tension = 0.5; Cleveland, 1981) ignoring size class boundaries.

To assess the patterns and distribution of storage lipids within juvenile *C. regalis* across liver, viscera and the remaining carcass, component TAG concentration ($[T]_C$), allometries of component dry *M* and component TAG content (in mg, T_C) were considered. TAG content in a given component was estimated: $T_C = [T]_C M_{DC}$, where C = component, L , V , R .

An allometric model was used to characterize the changes of all three measures across the size range of YOY *C. regalis* analysed:

$$[T]_C, M_{DC} \text{ or } T_C = \alpha_C L_S^{\beta_C}, \text{ where } C = \text{component, } L, V, R \quad (1)$$

where α_C and β_C are the scale and rate parameters that were fitted by performing least-squares linear regression on $\ln [T]_C$, M_{DC} or T_C and $\ln L_S$ (Bagenal & Tesch, 1978). Dry *M* was examined over the entire size range ignoring class boundaries while for $[T]_C$ and T_C a stage-specific approach was utilized where separate models were fitted for each size class.

Differences in accumulation patterns and distribution of biomass (M_{DC}), $[T]_C$ and T_C among components were assessed *via* comparison of rate parameters (general linear models, GLM) and ANCOVA with natural logarithm of L_S as the covariate.

To compare estimates of individual juvenile *C. regalis* TAG concentration from different methods, *i.e.* extraction of samples from amalgamated whole individuals ($[T]_W$) *v.* those from separate components, a whole body estimate of TAG concentration ($[T]_E$) was calculated from the analysis of components:

$$[T]_E = (T_L + T_V + T_R)(M_{DL} + M_{DV} + M_{DR})^{-1} \quad (2)$$

Individuals used to compare the extraction methods were sub-sampled from the same collection (sampling location and date) in order to eliminate possible spatio-temporal effects. A stage-specific allometric model (equation 1) was used to characterize the distribution of estimated individual TAG concentrations from both extraction methods and differences among the methods evaluated *via* ANCOVA with $\ln L_S$ as the covariate. All statistical analyses were performed in SYSTAT 12.0 (www.systat.com).

RESULTS

While there was substantial variability in the concentration of the five lipid classes and the overall concentration of lipids for a YOY *C. regalis* of a given size, definite trends within the data were apparent (Fig. 1). Concentrations of total lipids

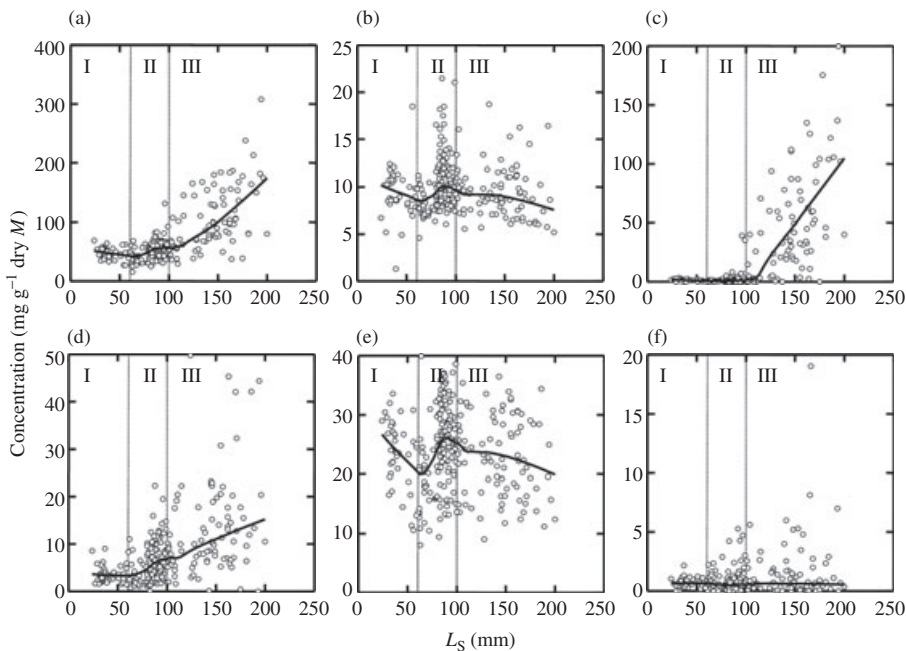


FIG. 1. Changes in the concentration of (a) total lipid, (b) sterol, (c) triacylglycerol, (d) free fatty acids, (e) phosphatidylcholine and (f) waxy esters with standard length (L_S) for juvenile *Cynoscion regalis* over stages I to III. Lines represent a locally weighted smoothing function (LOWESS; tension = 0.5; Cleveland, 1981) fit over the L_S range, regardless of stage.

varied widely over the juvenile period, up to 20-fold, but generally increased with individual L_S with the preponderance of the accumulation occurring >100 mm L_S (Fig. 1). Total lipid concentrations of YOY *C. regalis* in size classes I and II averaged 52.1 ± 14.6 mg g⁻¹ (mean \pm s.d.) and, with one exception, were <100 mg g⁻¹ while individuals within the third size class exhibited a pattern of rapid lipid accumulation to >300 mg g⁻¹ among the largest fish analysed. Phospholipid was the predominant lipid class in YOY *C. regalis* in size classes I and II, beyond which TAG concentrations exceed that of PL. Both PL and ST showed moderate but variable concentrations, 8.1–40.0 and 1.3–21.5 mg g⁻¹, respectively, throughout the YOY period, declining slightly over the largest L_S class. Concentrations of WE were low, c. 1 mg g⁻¹, and exhibited the least amount of variability among lipid classes. Triacylglycerols and FFA had similar dynamics, with relatively low concentrations in the smaller L_S classes followed by a rapid increase in larger juveniles. For FFA, concentration in size classes I and II generally varied between 2 and 15 mg g⁻¹ and increased upwards of five-fold in *C. regalis* >100 mm L_S . TAG concentrations were at or near zero (*i.e.* below detection level) in smaller fish, began to increase between 60 and 100 mm L_S then increased rapidly to almost 200 mg g⁻¹ in size class III. In the largest size class, however, there was considerable variability in the measured concentrations of TAG (0.0–199.7 mg g⁻¹) and FFA (0.0–49.7 mg g⁻¹). When viewed as a percentage of total lipid concentration, the predominant role of TAG in large juveniles is highlighted, increasing rapidly in the third size class and reaching upwards of 75% (Fig. 2). Because of its probable role as the predominant lipid class utilized by juvenile *C. regalis* for long-term energy storage, subsequent analysis focused on TAG.

Generally, carcasses, livers and viscera of juveniles in size classes I and II contained <10 mg g⁻¹ TAG [Fig. 3(a)], with 36% of the individuals components having

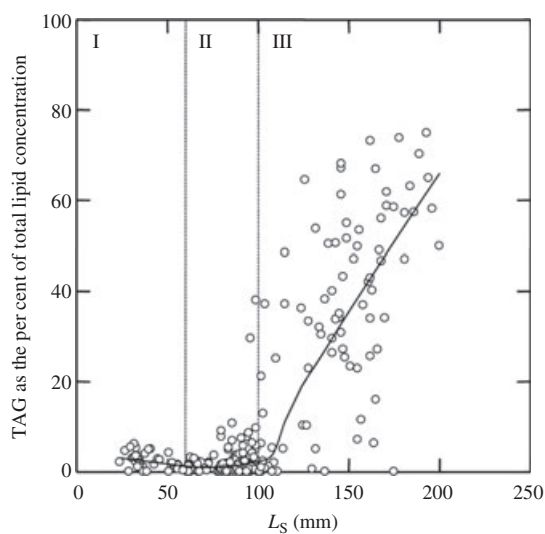


FIG. 2. Triacylglycerol (TAG) as the percentage of total lipid concentration as a function of standard length (L_S) in juvenile *Cynoscion regalis* over stages I to III. Line represents a locally weighted smoothing function (LOWESS; tension = 0.5; Cleveland, 1981) fit over L_S , regardless of stage.

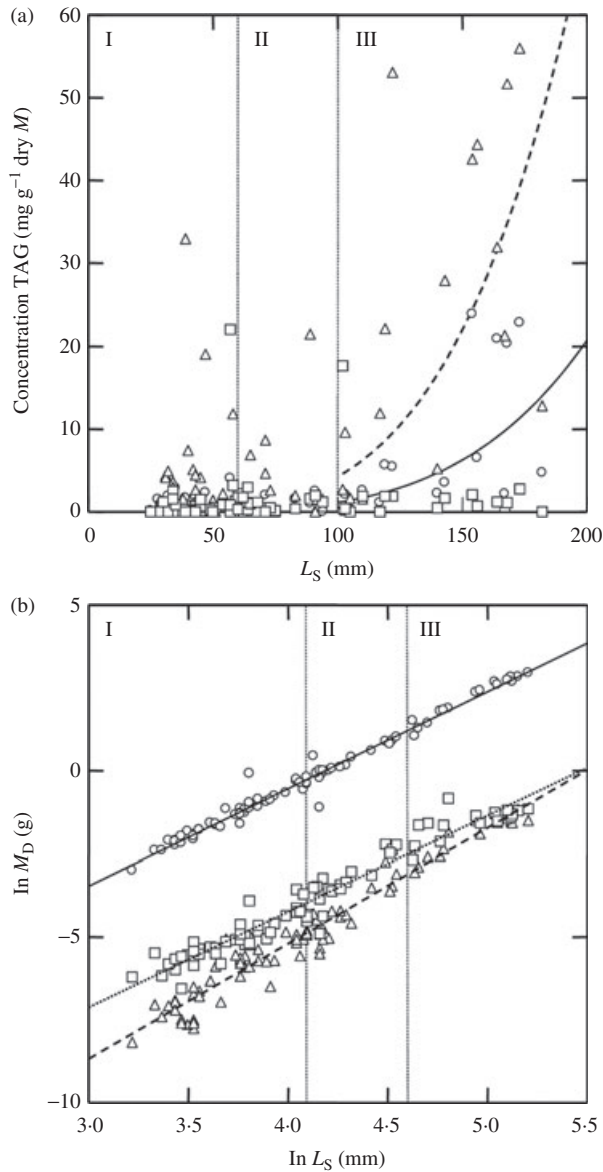


FIG. 3. (a) Concentration of triacylglycerol (TAG) in carcasses (O), livers (Δ) and viscera (\square) and (b) dry mass (M_D) of each component as a function of standard length (L_S) in juvenile *Cynoscion regalis* from stages I to III. (a) Stage-specific and (b) full L_S range allometric model fits [see Table I(a), (b) for coefficient estimates, respectively] for carcasses (—), livers (---) and viscera (.....).

no measurable TAG [Table I(a)]. As the lack of TAG content could reflect poor condition, individual components with no measurable TAG were excluded when fitting allometric models. Of the components sampled from *C. regalis* in size classes I and II with measurable TAG, none exhibited a significant pattern of increase with L_S [Table I(a)] ($P > 0.05$). Above 100 mm L_S , TAG concentrations in viscera

TABLE I. Coefficient estimates for the most parsimonious allometric models describing stage-specific changes in (a) component triacylglycerol concentration ($[T]$) and content (T) and continuous changes in (b) component dry mass (M_D) with standard length (L_S) (equation 1) for livers, viscera and remaining carcasses in juvenile *Cynoscion regalis* from stages I to III

Stage	<i>n</i>	Carcass						Liver						Viscera						
		<i>n</i> _{[<i>T</i>]_{>0}}	α	β	S.E. β	<i>P</i> (β)	<i>r</i> ²	<i>n</i> _{[<i>T</i>]_{>0}}	α	β	S.E. β	<i>P</i> (β)	<i>r</i> ²	<i>n</i> _{[<i>T</i>]_{>0}}	α	β	S.E. β	<i>P</i> (β)	<i>r</i> ²	
(a)	I	43	[<i>T</i>]	29	1.1×10^{-1}	NS	—	—	—	—	—	—	—	25	3.7×10^{-2}	NS	—	—	—	
			<i>T</i>		3.9×10^{-7}	3.6	0.6	<0.001	0.55	—	—	—	0.020	0.19	2.8×10^{-8}	3.2	1.3	0.024	0.20	
	II	21	[<i>T</i>]	10	9.6×10^{-5}	NS	—	—	—	—	—	—	—	19	3.2×10^{-1}	NS	—	—	—	
			<i>T</i>		9.2×10^{-10}	4.9	2.2	0.003	0.69	—	—	—	—	—	7.1×10^{-11}	4.6	1.5	0.007	0.35	
(b)	III	16	[<i>T</i>]	15	5.5×10^{-9}	4.2	1.3	0.006	0.45	16	3.3×10^{-8}	4.1	1.2	0.004	0.45	2.4×10^3	NS	—	—	—
			<i>T</i>		8.3×10^{-15}	7.3	1.3	<0.001	0.71	—	7.1×10^{-15}	6.8	1.3	<0.001	0.66	1.5×10^{-1}	NS	—	—	—
	All (I to III)	80	<i>M</i> _D	—	4.8×10^{-6}	2.9	0.0	<0.001	0.98	—	5.0×10^{-9}	3.5	0.1	<0.001	0.95	1.4×10^{-7}	2.9	0.1	<0.001	0.94

n , sample size; $n_{[T]>0}$, the number of samples for a given component and size class with $[T] > 0$ which were utilized to estimate model parameters for $[T]$ and T .

remained low and did not increase with L_S ($P > 0.05$) while concentrations in both livers and carcasses increased with L_S at a similar rate [Table I(a)] (GLM, $P > 0.05$) with livers containing higher TAG concentrations relative to carcasses (ANCOVA, $P < 0.01$). As in YOY analysed whole, however, TAG concentrations in livers and carcasses of fish >100 mm L_S exhibited a great deal of variability [Fig. 3(a)]. Unlike TAG concentrations, the relationship between dry M and L_S [Fig. 3(b)] was tight across the entire size range of YOY *C. regalis* carcasses, livers and viscera [Table I(b)]. While the carcasses represents the majority of biomass across all L_S (mean 96%), the rate of increase with L_S was slightly higher in livers relative to carcasses and viscera (GLM, $P < 0.01$). The relative distribution of stored TAG among components was a function of the increasing concentration of TAG in livers and carcasses of juveniles >100 mm L_S and the allometries of component dry M (Fig. 4). In juveniles <100 mm L_S , significant increases in TAG content were generally comparable to increases in component biomass [Table I(a)]. Among individuals in size class III, TAG content in viscera exhibited no discernable increase with L_S ($P > 0.05$), while content in both livers and carcasses increased with L_S at a similar accelerated rate (Table II), (GLM, $P > 0.05$). This reflects the interaction of increasing concentrations and allometric growth in both components from YOY >100 mm L_S [Table I(a), (b)], with carcasses containing higher TAG content relative to livers (ANCOVA, $P < 0.005$). Irrespective of size class or the factors (relative biomass or TAG concentration) driving the relative distribution, the proportion of TAG stored within the carcasses of juvenile *C. regalis* was high, with a mean \pm s.d. of $94.8 \pm 6.2\%$ in the 54 individuals with carcass TAG concentrations >0 (Fig. 4).

The TAG concentrations from extraction of 107 whole YOY *C. regalis* 24–200 mm L_S (39, 46 and 22 in size classes I, II and III, respectively), collected from the

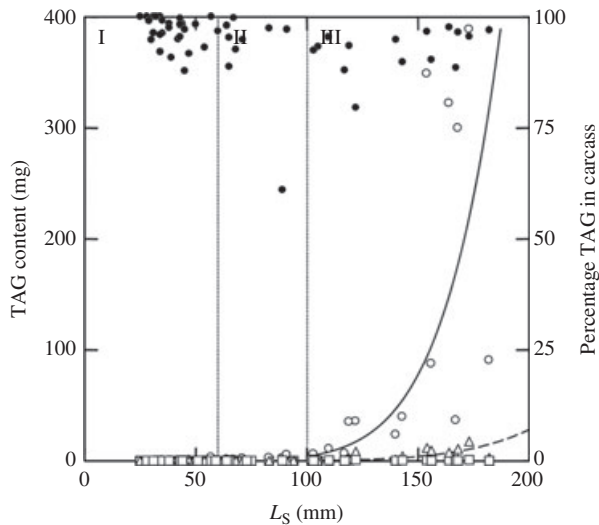


FIG. 4. Triacylglycerol (TAG) content of carcasses (○), livers (△) and viscera (□) and percentage of TAG located in the carcass (●) as a function of standard length (L_S) in juvenile *Cynoscion regalis* from stages I to III. Stage III specific allometric model fits [see Table I(a) for coefficient estimates] for carcasses (—) and livers (---).

TABLE II. Coefficient estimates for the most parsimonious stage-specific allometric models describing changes in triacylglycerol content (T) estimated from extraction of samples from amalgamated whole individuals and separate components (carcasses, liver and viscera; see equation 2) with standard length (L_S) (equation 1) in juvenile *Cynoscion regalis* from stages I to III

	Extraction of amalgamated whole individuals							Extraction of components						
Stage	n	$n_{[T]>0}$	α	β	S.E. β	$P(\beta)$	r^2	n	$n_{[T]>0}$	α	β	S.E. β	$P(\beta)$	r^2
I	39	34	2.1×10^3	-2.1	0.5	<0.001	0.36	43	41	4.9×10^{-2}	NS	—	—	—
II	46	31	1.6	NS	—	—	—	21	21	1.7×10^{-7}	NS	—	—	—
III	22	21	1.3×10^{-13}	6.5	1.0	<0.001	0.71	16	16	2.1×10^{-10}	4.8	1.2	0.001	0.53

n , sample size; $n_{[T]>0}$, the number of samples for a given analysis type and size class with TAG concentrations >0 which were utilized to estimate model coefficients for T .

same systems as those used in the component analysis, were used to compare the extraction approaches. Again, as the lack of TAG content could reflect poor condition, individuals analysed whole with no measurable TAG (*i.e.* $[T]_W = 0$) and individuals with a whole body TAG concentration estimate derived from component analysis that was zero (*i.e.* $[T]_E = 0$) were excluded when fitting allometric models. Both extraction methods revealed a similar pattern of TAG accumulation (Fig. 5), with low concentrations and no significant increase with L_S in size classes I and II followed by a rapid increase in larger individuals (Table II). In the largest individuals, however,

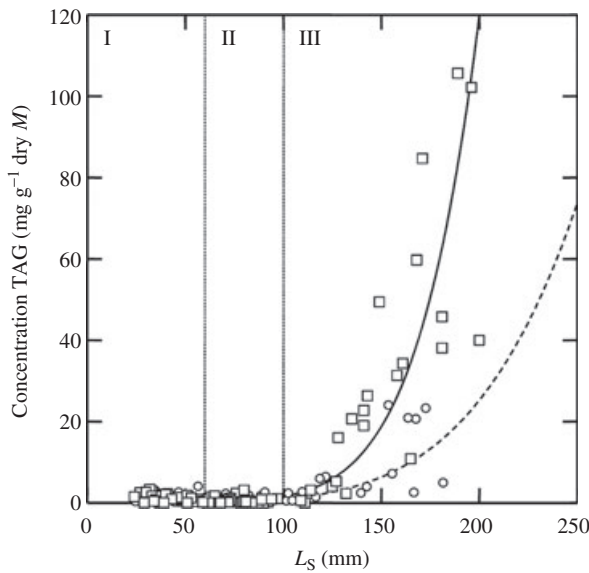


FIG. 5. Concentration of triacylglycerol (TAG) estimated from separate extractions of carcasses, liver and viscera (○) and from extraction of amalgamated whole fish samples (□) as a function of standard length (L_S) in juvenile *Cynoscion regalis* from stages I to III. Stage III allometric model fits (see Table II for coefficient estimates) for estimation from separate tissues (---) and extraction of whole fish samples (—).

while TAG concentrations from both methods increased with length at similar rates (GLM, $P > 0.05$), the estimate of TAG concentration at length calculated from whole body extractions was higher than from component analysis (ANCOVA, $P < 0.005$).

DISCUSSION

By analysing YOY *C. regalis* sampled over several seasons, from a range of habitats and throughout their period of estuarine residency, it was possible to determine the general patterns of lipid class dynamics and the location and patterns of energy storage during their period of juvenile estuarine residency. As shown for other species (Norton *et al.*, 2001; Eldridge *et al.*, 2002; Hurst & Conover, 2003; Berkeley *et al.*, 2004), an understanding of lipid class dynamics and changes in energy stores is needed for comparing condition among individuals across a range of sizes or ontogenetic life stages. For juvenile *C. regalis*, differences in energy storage associated with size class were expected because changes in diet and energetic demands are related to growth and seasonal period, *i.e.* larger individuals collected later in the YOY period would be expected to invest in long-term energy reserves in order to avoid mortality due to overwinter starvation (Nemerson, 2001; Hurst & Conover, 2003; Hurst, 2007). While the changes in lipid class distribution and energy dynamics associated with ontogenetic changes are expected to be continuous, the use of a size class-specific model for TAG provided an accurate representation of lipid class and energy storage dynamics across the three ontogenetic classes represented in this study, suggesting that there may be rapid shifts in lipid metabolism associated with growth during the first year of life.

The increase in total lipid concentrations in YOY *C. regalis* indicates greater allocation of energy to reserves with size, a pattern seen in a wide variety of species (Shulman & Love, 1999). While total lipid concentration or content is often used as an indicator of condition, the analysis of individual lipid classes has been demonstrated to be a more sensitive indicator of biochemical condition in juvenile and anadromous fishes (Jobling *et al.*, 1998; Norton *et al.*, 2001; Lochmann & Ludwig, 2003). Among the classes, TAG most often varies with feeding success and environmental factors and is often cited as a direct measure of individual condition (Håkanson, 1989; Weber *et al.*, 2003; Heintz *et al.*, 2004; Naesje *et al.*, 2006; Amara *et al.*, 2007; Weinstein *et al.*, 2009, 2010). While low or absent in the majority of fish < 100 mm L_S , TAG accounted for up to *c.* 75% of the total lipids in larger individuals, comparable with the highest reported values for juvenile fishes. Concentrations of FFA also rose with L_S , however, FFA are produced by enzymatic hydrolysis of phospholipids or TAG and therefore may have evolved from the utilization of endogenous sources or have accumulated post-mortem or while frozen (Parrish, 1987; Heintz *et al.*, 2004). Also, FFA concentrations were low relative to TAG over the range of sizes where TAG accounted for a significant proportion of total lipid content. For these two reasons, concentrations of FFA do not present a strong candidate as the primary form of lipid stores in juvenile *C. regalis*. The unlikely role of FFA, consistently low concentrations of wax esters (another possible form of storage lipids) and the correlation between the rapid increase in TAG and total lipid concentrations indicate that TAG is the primary form of storage lipids in YOY *C. regalis*.

Both sterols and phospholipids have been utilized as an indicator of mass change and to standardize lipid energy reserves for comparisons among individual larval fishes (Fraser *et al.*, 1988; Håkanson, 1989). *Cynoscion regalis*, however, are similar to juveniles of other species, in that concentrations of both classes exhibited fluctuations over their size range and may decline in individuals >100 mm L_S , therefore making both unsuitable as indicators of changes in M (Norton *et al.*, 2001; Lochmann & Ludwig, 2003; Heintz *et al.*, 2004). While a decline in PL may reflect catabolism due to starvation and PL has been used as an indicator of condition in larval fishes (Håkanson, 1993), it is improbable that the decline seen in this study is due to poor condition as TAG increased rapidly over the size class where PL fell (Norton *et al.*, 2001).

While the variability in TAG concentrations due to feeding or environmental conditions was not directly addressed, its dominant role in the accumulation of lipid energy reserves indicates its utility as a measure of biochemical condition in juvenile *C. regalis* (Norton *et al.*, 2001). Because of its relationship with L_S and the inability to use ST or PL to standardize for differences in M among individuals, caution must be taken to normalize TAG concentration with L_S when comparing condition among individuals >100 mm L_S (Lochmann *et al.*, 1995; Lochmann & Ludwig, 2003).

Many species utilize the liver as their primary lipid reserve (Shulman & Love, 1999), *e.g.* the gobies *Gobius niger* L. 1758 and *Zosterisessor ophiocephalus* (Pallas 1814) (Fiorin *et al.*, 2007). Similar to many salmonids (Jobling *et al.*, 1998; Morgan *et al.*, 2002), however, analysis of probable depots of TAG revealed the vast majority (*c.* 95%) was stored in the carcass, indicating that YOY *C. regalis* liver and viscera play a minor role in long-term storage of lipid reserves. While few studies have investigated the distribution of specific lipid classes among tissues in fishes (Sheridan *et al.*, 1983; Phleger *et al.*, 1995; Jobling *et al.*, 1998; Rodriguez *et al.*, 2004), the dominant role of TAG in juvenile *C. regalis* lipid stores validates comparisons with studies where storage among specific tissues was determined *via* analysis of total lipids.

While analysis of separate components was necessary to address the location of long-term storage depots in juvenile *C. regalis*, results from the analysis of components and from samples derived from amalgamated individuals revealed a similar pattern of rapid TAG accumulation in juveniles $> c.$ 100 mm L_S . This indicated that the extraction of amalgamated individuals was adequate for assessing juvenile *C. regalis* condition. Although both methods predicted a similar rate of increase in the concentration of TAG in juveniles >100 mm L_S , however, whole body analysis provided a significantly higher estimate of TAG concentration at a given L_S . There are two probable explanations for this discrepancy. First, a 50 mg sub-sample from an amalgamated individual juvenile *C. regalis* may consistently underrepresent tissues with lower concentrations of TAG. More probably, however, the average condition of the largest individuals selected for the component analysis was relatively low. Three of the seven *C. regalis* >150 mm L_S selected for component analysis had estimated whole body TAG concentrations lower than the lowest measurement from any amalgamated individual in this same size range. Because liver, viscera and remaining carcass for a given individual were not extracted or analysed together, if these low estimates of whole body TAG concentration resulted from methodological issues, the estimated distribution of TAG stores among the components would be affected. The mean proportion of TAG stored in the carcasses of these three fish was 92%, however,

near the overall mean of 95%, and well within the variability exhibited across all juveniles used in the component analysis. Furthermore, the mass of components from these individuals (>13 g for carcasses and >200 mg for livers and viscera) did not require any methodological differences relative to amalgamated individuals, *i.e.* each component was ground to a fine powder and a 50 mg sub-sample selected for extraction and analysis. While analysis of an amalgamated whole juvenile *C. regalis* may be the preferred method to assess their condition, caution must be taken not to confuse this approach with the analysis of only muscle tissue. Studies on salmonid species have demonstrated that >35% of TAG stores are located in the remaining tissue after the removal of viscera, liver and muscle tissue (only the head, fins, skeletal tissue and skin remain), with upwards of 13.5% specifically located in the skeletal tissue (Phleger *et al.*, 1989; Jobling *et al.*, 1998). Therefore, samples containing solely muscle tissue may underestimate the TAG stores in juvenile *C. regalis*.

Cynoscion regalis recruits settle throughout the marshes and open waters of Delaware Bay from the late spring into the early autumn with stage I–III juveniles found across the system from July through to October (Paperno *et al.*, 2000). Generally YOY *C. regalis* exhibit ‘saltatory’ behaviour with growth (Litvin & Weinstein, 2004), exiting their settlement habitats then moving down estuary varying distances and residing in open waters for extended periods before arriving at the mouth of Delaware Bay prior to emigration (Chao & Musick, 1977; Weinstein & Brooks, 1983). Across this mosaic of habitats, juvenile *C. regalis* may experience a wide range of turbidity and illumination levels, physicochemical conditions, feeding success and predation levels (Lankford & Targett, 1994; Grecay & Targett, 1996b), which drive variable growth and mortality rates (Paperno *et al.*, 2000; Nemerson & Able, 2004). Like other YOY temperate fish species that occur in complex and dynamic ecosystems, juvenile *C. regalis* face competing energetic demands for growth and energy reserves to avoid both size-selective predation and overwintering starvation. The relative importance of these two forces is thought to shift energy allocation from growth to storage over the juvenile period and may be associated with ontogenetic changes in diet and the impending winter season (Sogard, 1997; Post & Parkinson, 2001; Hurst & Conover, 2003). The absence of significant TAG reserves in smaller *C. regalis* (stage I and II) indicates that they may have invested primarily in growth to balance the high predation rates they probably encounter early in the juvenile period (Paperno *et al.*, 2000). Later in the juvenile period, two factors may have driven the rapid accumulation of energy reserves stored as TAG, in juvenile *C. regalis* >100 mm L_S (stage III). First, their increase in piscivory (Chao & Musick, 1977; Nemerson, 2001) represents an important expansion in the ontogenetic niche of juvenile *C. regalis* and probably increases the magnitude of energy they can acquire for storage or growth (Werner & Gilliam, 1984; Sogard, 1997; Post, 2003). Second, larger fish from later in the YOY period who are preparing to emigrate (Chao & Musick, 1977; Weinstein & Brooks, 1983) would be expected to allocate a greater proportion of energy to stores in order to avoid mortality due to starvation while overwintering (Hurst, 2007), a pattern exhibited by several species of temperate YOY fishes (Hurst & Conover, 2003; Borchertding *et al.*, 2007; Berg *et al.*, 2009).

In conclusion, the present study has demonstrated that the analysis of lipid class dynamics, in the context of growth of juvenile *C. regalis* collected over their YOY period and across a range of estuarine habitats, can provide a useful indicator of individual condition. While measures of total lipid content may be easily acquired,

as has been demonstrated for other fish species, the quantification of individual lipid class, particularly TAG, allows for a more accurate metric of condition. The elevated TAG concentrations in stage III juveniles indicate that this approach is best suited for assessing condition in larger individuals and is not applicable to fish <60 mm L_S . The results also indicate that ST or PL cannot be used to account for changes in M or to standardize TAG reserves. Therefore, the use of TAG as a metric of condition must be normalized with L_S when comparing among individuals. Finally, liver and viscera (including mesenteries) do not represent a significant depot for TAG stores in juvenile *C. regalis*, hence extraction of samples derived from amalgamated whole fish provides an accurate estimate of condition while minimizing effort and errors associated with analysis of separate tissues or organs.

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