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**USE OF FATTY ACID STORES BY LARVAE
AND ADULTS OF A NEBRASKA SALT MARSH
TIGER BEETLE, *Cicindela togata globicollis* Casey**

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ABSTRACT—Tiger beetles are common predators in open habitats throughout the Great Plains, including the eastern salt marshes. Adult tiger beetles are active searchers that attack and eat small insects. By contrast, their larvae are sit-and-wait predators that form permanent burrows and depend on prey moving within striking distance. We hypothesized that adults and larvae of the tiger beetle, *Cicindela togata globicollis* Casey, would differ in their utilization of lipid (fat) energy reserves, such as fatty acids, based on differences in the likelihood of starvation. To investigate this, we determined the fatty acid profiles from larvae and adult tiger beetles. We found that normally-feeding adults and larvae did not differ substantially in their fatty acid profiles. But, after fasting for a two-week period, larvae selectively used their lipid reserves while adults did not. Moreover, in contrast to all other insect species studied, we found that larval tiger beetles were not able to biosynthesize fatty acids from acetate. Our findings suggest that larvae optimize the use of fatty acids to allow for a lengthy larval developmental period in environments, such as the Great Plains, that provide unreliable and unpredictable food resources.

Introduction

While salt marshes occur in the coastal regions of most continents, the salt marshes of eastern Nebraska represent a localized and unique habitat in

the Great Plains. These inland salt marshes are characterized by high levels of soil salinity and low levels of soil moisture, except during seasonal periods of flooding from snowmelt and spring rains (Farrar and Gersib 1992). The eastern Nebraska salt marshes are relatively harsh environments that support salt-adapted plant and animal communities. During the last five decades, the area occupied by these salt marshes has declined by over 90%, to about 2,640 ha (1,200 acres), leaving a series of fragmented and threatened natural communities (Farrar and Gersib 1992). Although threatened, the eastern Nebraska salt marshes, and associated Salt Creek watershed, are home to a relatively diverse assemblage of ten species of tiger beetles (Spomer et al. 1997). These beetles are similar in size and predation habits, and it is not clear how these ten species of predators co-exist in a habitat with limited prey (Hoback et al. 1999).

The term "tiger beetle" is derived from the active style of predation exhibited by adults. Adult tiger beetles of saline habitats are very effective hunters, preying on small arthropods, such as insects and spiders (Willis 1967). While insect numbers are sparse, Hoback et al. (1999) documented the presence of more than 60 insect families in the salt marshes of the Arbor Lake Wildlife Management Area. Most of these insect groups are potential prey for adult tiger beetles (Willis 1967). Moreover, Hoback (1999) suggested that the tiger beetle community of the salt marsh is structured by intraguild predation, where the adults of some tiger beetle species prey upon adults of other tiger beetle species.

The work on prey abundance and intraguild predation has yielded useful insights into the biology of adults of the salt marsh tiger beetle community. However, much less is known about the larval forms of these species. Tiger beetle larvae are sedentary animals that live in one burrow, which they enlarge in preparation for each molt to a bigger size, until they pupate prior to becoming adults (Pearson 1988; Knisley and Schultz 1997). The larval phase of a tiger beetle life cycle can take one to three years, depending upon environmental conditions and the availability of food (Pearson and Knisley 1985). Larval death from starvation can be extremely high, accounting for 75% or more of tiger beetle mortality based on several thorough investigations (Mury-Meyer 1983; Knisley and Juliano 1988).

Larvae are especially susceptible to starvation because their episodic feeding events depend upon prey species, mostly small insects, coming within grasp of their open mandibles at the top of their burrows (Knisley and Juliano 1988). Even if starvation does not cause mortality, food limitation of larvae will result in smaller adults that have lower fecundity and shorter life



Figure 1. Tiger beetle larva, with insect prey item, near Gothenberg, NE. Photo courtesy of S.M. Spomer.

spans (Hori 1982; Knisley and Juliano 1988). Adult females usually select oviposition sites in shaded areas of the salt marsh, perhaps to increase the rate of contact of their larvae with prey (Hoback et al. in press). Despite microhabitat selection by females, larval feeding (Fig. 1) is a stochastic process. Since the larvae are sedentary, food acquisition cannot be enhanced by foraging behavior. Thus, tiger beetle larvae must be prepared to undergo frequent periods of starvation.

On the basis of these differences in predation style and potential food abundances for larvae and adults, we reasoned that larvae would differ from adult tiger beetles with respect to lipid (fat) metabolism during periods of starvation. The fat reserves of most organisms including insects are partitioned into several classes, each with a different function in the organism. For example, phospholipids are mostly involved in cell structures, serving as components of biomembranes. While phospholipid fatty acid profiles undergo substantial changes according to cell needs (Chilton and Murphy 1986; Gadelhak and Stanley-Samuelson 1994), the fatty acid components of phospholipids usually are not used for energy metabolism.



Figure 2. Adult tiger beetle, *Cicindela togata globicollis*, at Arbor Lake Wildlife Managment Area, Lancaster County, NE. Photo courtesy of L.G. Higley.

During times of abundant food, fatty acids are typically stored in the form of triacylglycerols (neutral lipids) for later use in energy metabolism. The fatty acids stored in triacylglycerols represent a very dense form of energy storage (Chapman 1998), and we hypothesized that tiger beetle larvae would draw upon their triacylglycerol reserves to support themselves during starvation. Because adults are more mobile than larvae, and thus less likely to face starvation, it seemed reasonable that utilization of triacylglycerol fatty acids would be more pronounced in tiger beetle larvae than in adults.

We tested our hypothesis by analyzing the fatty acid compositions of triacylglycerol and phospholipid fractions of whole adult and larval tiger beetles, *Cicindela togata globicollis* Casey (Fig. 2). Briefly, we found that the fatty acid compositions of fed adults and larvae were fairly similar. Alternatively, 14 d of fasting markedly altered the triacylglycerol fatty acid profile of larvae but not of adult tiger beetles. We infer from these data that the ability of larvae to draw on fatty acids during fasting is one metabolic adaptation to the inevitable periods of fasting associated with sit-and-wait



Figure 3. Salt marsh habitat of the tiger beetle, *Cicindela togata globicollis*, at the Arbor Lake Wildlife Management Area, Lancaster County, NE. Photo courtesy of S.M. Spomer.

predation. This adaptation may be particularly important in the prey-limited eastern Nebraska salt marshes.

Materials and Methods

Organisms

Larvae of *C. t. globicollis* in the third instar stage were collected in July 1997 from muddy salt flats at the Arbor Lake Wildlife Management Area (Fig. 3), north of Lincoln, Nebraska. Larvae were assigned randomly to one of two experimental groups. Sets of 12 larvae were placed into containers filled to a depth of approximately 20 cm with soil from their habitat and allowed to establish burrows. These containers with larvae were maintained in a growth chamber (35/25 °C, 16:8 light:dark) in the laboratory. One half of the larvae were fed *ad lib* every 2 d with apterous (wingless) fruit flies (*Drosophila melanogaster* Meigen) or larvae of the Confused Flour Beetle (*Tribolium confusum* Jacq. Du Val) while the other larvae

were not fed. After two weeks, all larvae were sacrificed (N = 12 per treatment), and fatty acid composition of the tissues was analyzed (methods below).

Adult *C. t. globicollis* were collected by aerial net in August 1997, from the same site as the larvae, described above. Adults were housed individually in 800 ml plastic cages, and they were provided paper towel substrate and a cotton-plugged water vial. Adults were subdivided into two groups, one group was fed adult flour beetles (*T. confusum*), while the other group was not fed for two weeks.

Tissue Preparation and Lipid Extraction

We used three individual larvae or adults, pooled for each analysis in order to have a sufficient quantity of material for analysis of tissue fatty acids. The animals were anesthetized by chilling them on ice, then the bodies were placed in tubes containing 500 ml of 0.1 M KH_2PO_4 buffer, 1 ml chloroform:methanol (2:1, v/v), and 50 ml 2% butylated hydroxytoluene to prevent autoxidation of polyunsaturated fatty acids. The tissues were homogenized using a mechanical grinder and then sonicated. We used routine protocols for lipid extraction and analysis of the fatty acid composition of phospholipid and triacylglycerol fractions (Howard and Stanley-Samuelson 1996).

Total lipid extracts and known lipid standards were applied to thin-layer chromatography plates (20 x 20 cm, 250 mm Silica Gel G: Sigma, St. Louis, MO). The plates were developed in petroleum ether-diethyl ether-acetic acid (80:20:1, v/v/v). Lipid fractions were visualized by exposing the plates to iodine vapors. The phospholipid and triacylglycerol fractions were scraped into 15 ml screw-cap reaction tubes and fatty acid methyl esters were formed by refluxing in acidified methanol for 90 min. The fatty acid methyl esters were extracted three times from the reaction tubes in petroleum ether and concentrated for analysis.

Our gas chromatographic and gas chromatographic-mass spectrometric techniques follow those of Howard and Stanley-Samuelson (1996). The fatty acid methyl esters were analyzed on a Hewlett-Packard 5890 gas chromatograph, equipped with a Supelcowax 10 capillary column (30 m x 0.32 mm i.d., 0.25 mm thickness: Supelco, Bellefonte, PA), a flame ionization detector, and a HP 3390A recording integrator. The gas chromatograph was operated with temperature programming at 2°C/min from 160 to 230°C. Injections were made in split mode (45:1), and separations were conducted with ultra-pure helium carrier gas at a rate of 0.6 ml/min fatty acid methyl

esters were identified by comparisons with the chromatographic behavior of known standards.

Identities of the fatty acid methyl esters were confirmed by capillary gas chromatography-mass spectrometry. Electron impact mass spectra were obtained using a Hewlett-Packard 6890 gas chromatograph coupled to a HP 5973 mass selective detector (Hewlett-Packard, Englewood, CO). In this process, the gas chromatograph was equipped with a Supelcowax 10 capillary column (30 m x 0.25 mm i.d.: Supelco, Bellefonte, PA). Chromatography was conducted in split mode (50:1) with temperature programming at 2°C/min from 150 to 230°C, with ultra-pure helium as a carrier gas. Mass spectra were scanned from m/z 50-400, and data were collected and analyzed using a HP Vectra Xm series 4 computer with HP Chemstation software. The structures of the fatty acid methyl esters were determined by analysis of the spectra and by comparison of spectra obtained from known standards.

While results of qualitative chromatographic analyses allow comparisons between proportions of particular kinds of fatty acids in tissue, they do not allow comparisons of absolute changes in tissue lipids. We addressed this point by recording the total areas of chromatograms of identified fatty acids, normalized to a standard injection volume. This is a convenient technique to register relative changes in the quantity of total fatty acid, although it can not be used to determine absolute quantities.

Fatty Acid Nomenclature

There are several ways to denote specific fatty acid components of tissue, either based on their chemical name or with a standard chemical short-hand notation (see Stanley 2000). In the shorthand method, the number to the left represents the number of carbons, and the number to the right represents the number of double bonds. And the n-3, or n-6, indicate the position of the first double bond, counting from the methyl end of the fatty acid. For example, linoleic acid in chemical short-hand is 18:2n-6, meaning that it has 18 carbons with two double bonds beginning at the sixth carbon from the methyl terminus of the fatty acid. We use the short-hand notation as a convenient method to characterize our fatty acid profiles.

Fatty Acid Biosynthesis from Radioactive Acetate and Linoleate

In the first experiment, we tested the ability of tiger beetle larvae to make complex fats (triacylglycerols and phospholipids) from acetate, which

is a chemical precursor of fats. Fourteen *C. t. globicollis* larvae in the third instar stage were used. Half were fed flour beetles *Tribolium confusum*, and half were not fed for two weeks. As a control on our techniques, we simultaneously tested fat bodies from 4 fifth instar Tomato Hornworms (*Manduca sexta* (L.)), an insect that we culture in the laboratory and one that is often used for studies of fatty acid synthesis. Whole tiger beetles ($n = 7$) or fat bodies from *M. sexta* were homogenized in 1.5 ml of 50 mM KH_2PO_4 at pH 8.0 and 2 μCi of [^{14}C]acetate was added to each vial. The radioactive acetate ([1,2- ^{14}C]acetate, 2.1 GBq/mmol) was purchased from New England Nuclear (Boston MA). The homogenate was incubated for 2 h at 28 °C in a shaking water bath. The reaction was stopped by adding 0.5 ml chloroform:methanol (v/v) and 100 ml concentrated HCl.

In the second experiment, we tested the ability of tiger beetle larvae to incorporate pre-made fatty acids, using radioactive linoleate (18:2n-6). Fourteen third instar *C. t. globicollis* were used. Half were fed Flour beetles (*T. confusum*), and half were not fed for two weeks. The larvae in this experiment were handled in precisely the same way that the adults were handled in the first experiment. Whole larvae ($n = 7$) were homogenized in 1.5 ml of 50 mM KH_2PO_4 at pH 8.0 and 0.6 μCi of [^{14}C]linoleate was added to each vial. The homogenate was incubated for 4 h at 26°C in a shaking water bath, and the reaction was stopped by adding 0.5 ml chloroform:methanol (v/v) and 100 ml concentrated HCl.

In our analyses of these experiments, three aliquots of the tissue homogenates were applied to thin layer chromatography plates, as described above. We measured R_f , the distance of spot from the starting point compared to the distance traveled by the solvent. Fractions that corresponded in R_f to phospholipids, free fatty acids, diacylglycerals, and triacylglycerols were visualized by exposure to iodine vapors. The location of radioactive bands was determined using an imaging scanner (BIOSCAN, Washington DC) for a 2 min period. The fractions were scraped from the chromatography plates into scintillation vials. Radioactivity from each fraction was estimated by adding 5 ml of Ecolite scintillation fluid (ICN Biomedicals, Irvine CA) and counting on a LKB Wallac 1209 Rackbeta liquid scintillation counter (Pharmacia, Turku, Finland) at 96% counting efficiency for ^{14}C .

Statistical Analysis

We analyzed the proportion of fatty acids within phospholipids and triacylglycerols for adult and larval tiger beetles using the analysis of vari-

ance in a general linear models procedure. Differences were compared using the least significant difference between means (LSD) test ($\alpha = 0.05$) (SAS Institute 1989). This procedure is designed to test whether the averages of more than two samples differ from each other. We limited our analysis to those fatty acids that accounted for $> 5\%$ of the total and which were also normally distributed, allowing analysis of percentages without transformation (Zar 1984). Because we were interested in fatty acid utilization during starvation, we did not analyze components that accounted for $< 5\%$ of the total and probably do not provide a significant source of energy during starvation periods.

Results

For larvae, the four predominant fatty acids of the triacylglycerol and phospholipid fractions isolated from total lipid extracts were: 16:0, 18:0, 18:1 and 18:2n-6 (Table 1). The proportions were similar to those commonly seen in analyses of insect tissue lipids (Stanley-Samuelson and Dadd 1983; Stanley-Samuelson et al. 1988). The quantitatively minor components were: 18:3n-3, 20:4n-6 and 20:5n-3.

Larval starvation for 14-days resulted in substantial changes in the triacylglycerol fatty acid pattern. In particular, the proportions of 18:1 declined significantly, from about 47% of the triacylglycerol fatty acids in fed larvae to about 36% in starved larvae. The larvae phospholipid fatty acid profiles were also altered during starvation, with the most significant change occurring in the proportions of phospholipid 18:1, which declined by about 6% from 43% to 37% of phospholipid fatty acids (Table 1). We infer from these data that the larvae were able to draw upon their triacylglycerol reserves to support metabolic activity during starvation.

For adults, the predominant fatty acid from total lipid extracts were similar, consisting of 16:0, 18:0, 18:1 and 18:2n-6 (Table 2). However, the key observation was that prolonged fasting by adults did not alter the triacylglycerol fatty acid patterns in a substantial way, unlike our findings with the larvae. Most changes were minor (Table 2). However, analysis did reveal a statistically significant reduction (from 31% to 25%) in the proportions of one component (18:2n-6) in starved adults. While our results indicated that fasting larvae used fatty acids in a way different from the fasting adults, they do not allow direct comparisons of absolute changes in tissue lipids. However, we recorded a significant 76% reduction in the triacylglycerol fraction from larvae, after 14 days of fasting (t-test, $P < 0.05$). The corresponding reduction for adults was also significant, 54% (t-test,

TABLE 1

Fatty acid composition, as percentages of total fatty acids, in triacylglycerols^a prepared from total lipid extracts of tissues from *Cicindela togata globicollis* larvae^b. Starved larvae were maintained without food for 14 d; fed larvae were given larval flour beetles (*Tenebrio confusum*) or adult apterous *Drosophila melanogaster* every other day.

Fatty acid ^c	Phospholipids		Triacylglycerols	
	Fed	Starved	Fed	Starved
Major Components				
16:0	15.4 (0.58)	15.8 (0.18)	18.7 (0.89)	21.7 (1.09)*
16:1	6.7 (0.51)*	5.3 (0.28)	11.7 (0.69)	12.2 (1.58)
18:0	10.3 (1.69)*	14.4 (1.18)	3.8 (0.57)	5.4 (0.31)*
18:1	43.3 (2.59)*	37.0 (1.21)	47.5 (2.46)	35.8 (1.96)*
18:2 _{n-6}	19.3 (3.33)*	17.2 (1.25)	15.2 (0.09)	17.5 (1.07)*
LSD		1.80		1.41
Minor components				
18:3 _{n-3}	1.8 (0.53)	1.8 (1.80)	2.4 (0.47)	3.3 (0.72)
20:0	Trace	1.2 (1.17)	Trace	0.2 (0.07)
20:4 _{n-6}	1.6 (0.54)	2.6 (0.49)	0.8 (0.27)	1.14 (0.37)
20:5 _{n-3}	1.5 (0.46)	4.5 (1.41)	1.5 (0.41)	2.78 (0.57)

^a Fatty acids stored in triacylglycerols represent a dense form of energy storage while phospholipids mostly serve in biomembranes and are not usually used for energy metabolism.

^b N = 3 per sample, 6 replicates. Values are means (\pm S.E.); Trace <0.1%; * Least Significant Differences Test, P < 0.05 for major components; minor components were not statistically evaluated.

^c Fatty acids short-hand names refer to number of carbons: number of double bonds and the position of the double bonds counting from the methyl end of the fatty acid.

P<0.05). Thus, both larvae and adults drew upon lipid reserves during fasting, as expected from the general background on animal energetics (Schmidt-Nielsen 1990).

We also considered the source of lipid reserves in larval tiger beetles. Larvae are predators, and their diets of insects and spiders should provide

TABLE 2

Fatty acid composition, as a mean percentage (\pm S.E.) of total fatty acids, in triacylglycerols^a prepared from total lipid extracts of tissues from *Cicindela togata globicollis* adults^b. Starved adults were maintained without food for 14 d; fed adults were given adult flour beetles (*Tribolium confusum*) daily

Fatty acid ^c	Phospholipids		Triacylglycerols	
	Fed	Starved	Fed	Starved
Major Components				
16:0	14.6 (1.05)	14.1 (1.65)	23.1 (0.83)	27.8 (0.83)*
16:1	3.9 (0.76)	2.5 (0.16)	8.8 (0.27)	6.3 (2.55)*
18:0	9.9 (0.26)*	12.8 (1.28)	4.2 (0.51)	4.9 (0.89)
18:1	34.1 (1.52)*	38.5 (0.75)	39.7 (1.58)	41.4 (1.03)
18:2 _n -6	30.9 (1.28)*	25.3 (1.84)	18.6 (2.05)	20.7 (2.59)
LSD		1.84		2.29
Minor components				
18:3 _n -3	2.6 (0.69)	1.1 (0.18)	3.3 (0.67)	0.75 (0.31)
20:0	0.5 (0.38)	1.4 (0.29)	Trace	Trace
20:4 _n -6	3.0 (0.44)	3.4 (0.26)	0.1 (0.12)	Trace
20:5 _n -3	0.4 (0.39)	0.9 (0.12)	Trace	Trace

^a Fatty acids stored in triacylglycerols represent a dense form of energy storage while phospholipids mostly serve in biomembranes and are not usually used for energy metabolism.

^b N = 3 per sample, 6 replicates. Values are means (\pm S.E.); Trace <0.1%; * Least Significant Differences Test, P < 0.05 for major components; minor components were not statistically evaluated

^c Fatty acids short-hand names refer to number of carbons; number of double bonds and the position of the double bonds counting from the methyl end of the fatty acid.

the major biomolecules, including proteins, carbohydrates, as well as lipids. Through digestion, the larvae would absorb the constituents of each of these major biomolecules, including amino acids, simple sugars, and fatty acids. The fatty acids would be directly incorporated into tissue lipids. Beyond this, the sugars and amino acids that were not used or stored potentially

could be converted into saturated and mono-unsaturated fatty acids, or these components also could be incorporated into complex lipids for storage (Chapman 1998). The enzyme, fatty acid synthase, a multi-enzyme complex known from virtually all animals (Vance and Vance 1991), is responsible for biosynthesizing fatty acids from acetate, a two-carbon substrate from sugars and amino acids. To test for the presence of this activity in tiger beetle larvae, we incubated homogenized larvae tissues in the presence of radioactive acetate. We recorded absolutely no fatty acid biosynthesis from these tissues. We repeated this experiment, this time including fat body from tobacco hornworms, *Manduca sexta*, to ensure that our technique was working. Again, the tiger beetle tissue showed no fatty acid biosynthesis, whereas the tissues of the tobacco hornworm control showed substantial biosynthetic activity. We conclude that third instar tiger beetles, whether fed or starved, do not routinely biosynthesize fatty acids from excess sugars or amino acids to store as lipids.

Because tiger beetle larvae were unable to synthesize fatty acids from acetate precursors, we hypothesized that they could incorporate the pre-formed fatty acids in digested foods directly into tissue lipids. We tested this hypothesis by incubating larval homogenates from starved and fed larvae in the presence of a radioactive polyunsaturated fatty acid, specifically 18:2n-6. We found that both fed and starved larval tissue incorporated the radioactivity and so the fatty acid. For fed larvae, we recovered most of the radioactivity from the phospholipid fraction (69%), and the remainder from the triacylglycerol fraction (Fig. 1). However, the situation was otherwise for starved larvae, in which about 99% of the radioactivity was associated with the phospholipid fraction with the only remaining 1% in the triacylglycerol fraction (Fig. 1). This low recovery of radioactivity from triacylglycerols suggest that the starving larvae metabolized some of the radioactive fatty acid for energy before it could be incorporated into the triacylglycerols. To ensure that the radioactive fatty acid was not incorporated into other lipid fractions, we assessed the radioactivity in other fractions, including free fatty acids and diacylglycerols. We detected no radioactivity in other fractions (Fig. 4).

Discussion

In our studies on tiger beetles, we have considered two biochemical adaptations for their sit-and-wait predation strategy in the eastern Nebraska salt marshes. First, we documented the ability of tiger beetle larvae to

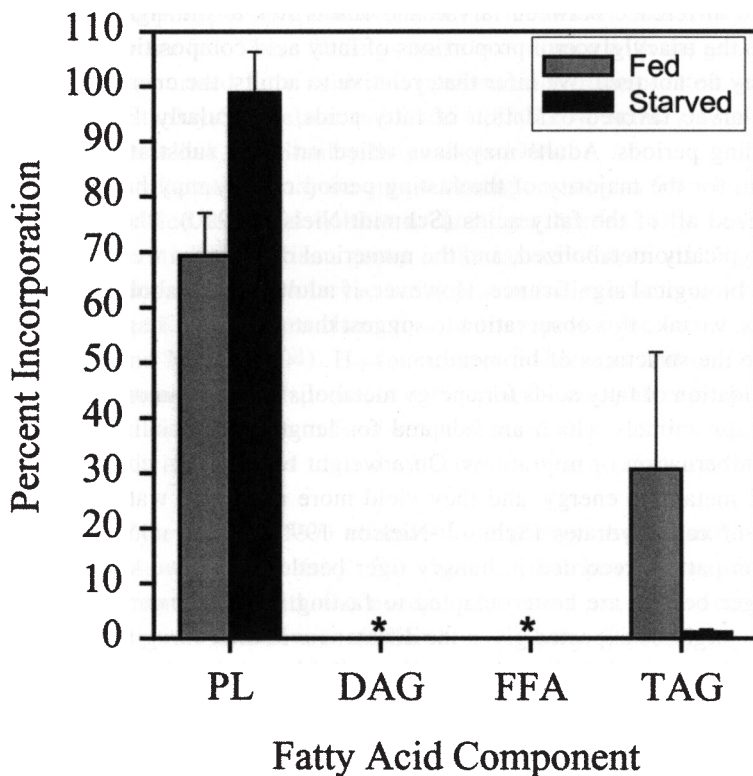


Figure 4. Mean percent (\pm S.E.) incorporation of radioactive fatty acid by homogenate of larval *Cicindela togata* ($N = 7$ per tissue sample, 3 replicates) into fatty acid components PL, phospholipid; DAG, diacylglycerol; FFA, free fatty acid; TAG, triacylglycerol. *=no radioactivity detected.

facultatively switch to anaerobic metabolic pathways during periods of flooding (Hoback et al. 1998). In this paper we considered the idea that the larvae optimize their fatty acid metabolism to allow for lengthy larval developmental periods in environments that provide unreliable and unpredictable food resources. Our findings suggest that young salt marsh tiger beetles are able to thrive in very tenuous environments through rather subtle modifications of basic cellular physiology and biochemistry.

One difference between larvae and adults lies in the significant decrease in the triacylglycerol proportions of fatty acid composition in larvae when they do not feed. We infer that, relative to adults, the energy metabolism of larvae favored oxidation of fatty acids, particularly 18:1, during non-feeding periods. Adults may have relied on other substrates, such as glycogen, for the majority of the fasting period or they may have equally metabolized all of the fatty acids (Schmidt-Nielson 1990). Phospholipids are not typically metabolized, and the numerical differences we found may not hold biological significance. However, if adults are metabolizing phospholipids, we take this observation to suggest that prolonged starvation may influence the structures of biomembranes.

Oxidation of fatty acids for energy metabolism is a common metabolic strategy for animals which are adapted for lengthy non-feeding periods, such as hibernation or migrations. On a weight basis, lipids contain more potential metabolic energy, and they yield more metabolic water, than do proteins or carbohydrates (Schmidt-Nielson 1990). Based upon the lipid utilization pattern recorded in hungry tiger beetle larvae, we suggest that larval tiger beetles are better adapted to fasting than their corresponding adults, as might be expected given the differences in their foraging patterns.

The unexpected finding that the tissues of larval tiger beetles did not synthesize storage fatty acids from acetate precursors can be postulated to be a special adaptation to sit-and-wait predation in the eastern Nebraska salt marshes, and possibly other harsh environments. While the burning (oxidation) of fatty acids yields a great deal of metabolic energy, the creation (biosynthesis) of fatty acids requires substantially more energy (Vance and Vance 1991). Hence, biosynthesis of fat reserves from carbohydrate and protein is only feasible in times of plentiful, reliable food availability. For example, the Sandhill cranes spend about 6 weeks in the Great Plains eating and accumulating substantial fat reserves (Folk and Tacha 1990; Krapu and Johnson 1990). The availability of food during these weeks far exceeds the cranes' capacity for processing food, and thus, the net cost of biosynthesizing lipids is likely to be negligible. The situation is considerably different for salt marsh tiger beetle larvae. The salt marshes have sparse populations of insects, and the larvae rarely, if ever, experience rich and reliable food availability (Hoback et al. 1999).

We hypothesize that the salt marsh tiger beetle larvae can not afford the energetic expenses involved in fatty acid biosynthesis from excess sugars and amino acids. Alternatively, tiger beetle larvae should be able to incorporate pre-formed fatty acids, taken directly from digested foods, into tissue lipid reserves. Indeed, we found that the larvae incorporated the majority of

18:2n-6 into phospholipid fraction and the remainder into triacylglycerol when they were fed. These findings are in accordance with the general view that polyunsaturated fatty acids are preferentially incorporated into phospholipids (Stanley-Samuelson and Dadd 1983, 1984; Stanley-Samuelson et al. 1988; Gadelhak and Stanley-Samuelson 1994).

These adaptations may be unique among insects, as few other species are as restricted to larval habitat as are tiger beetles. One ecologically similar, potential comparison group is the larvae of antlions (Neuroptera: Myrmeleontidae). Antlion larvae build pits in the substrate in which they trap prey, and antlions are characterized by a prolonged larval life cycle (Matsura and Murao 1994). However, tiger beetle and antlion life cycles vary. For example, many species of antlion larvae are quite mobile, relocating their pits in response to food-stress (Linton et al. 1991), while others remain in their initial pit until they starve to death (Matsura and Murao 1994).

Van Zyle et al. (1997) characterized energy use by antlion larvae feeding at low prey densities. For these antlion larvae, the amounts of extractable nutrients, with the exception of proteins, were recovered in direct proportion to amounts available in the prey. Lipids were the most important nutrients ingested by antlion larvae, and they were important in allowing antlions to survive starvation (Griffiths 1991). These results for antlion larvae are consistent with our findings for tiger beetle larvae. It appears that lipid storage and metabolization are important for survival in these two unrelated insects that share a sit-and-wait predation style.

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