

## Fat cycling in the mosquitofish (*Gambusia affinis*): fat storage as a reproductive adaptation

D. N. Reznick<sup>1</sup> and B. Braun<sup>2</sup>

<sup>1</sup> Department of Biology, University of California, Riverside, CA 92521, USA

<sup>2</sup> Department of Biochemistry, University of Pennsylvania, Philadelphia, PA 19104, USA

**Summary.** We argue, based on reviewed literature covering reptiles, amphibians, birds, and fish, that fat storage may represent a life history adaptation because it enables an organism to shift in time when resources are allocated to reproduction. We applied these arguments to fat and population cycles in three populations of the mosquito fish, *Gambusia affinis*. For males, there appeared to be a constant size at maturation during the reproductive season. Mature males became scarce late in the summer. At the same time, immature males delayed maturity and attained much larger sizes; they matured in large numbers in the fall. The amount of stored fat tended to be equal for immature and mature males at all times except in the late summer. In the August samples, when mature males were relatively rare, they also had the lowest level of fat reserves. It appears that the older generation of mature males did not store fat and did not overwinter. At the same time, immature males registered a two to three fold increase in fat reserves. These differences in fat content between mature and immature males disappeared by September, probably because of the recruitment of a new generation of mature males. The reserves were gradually utilized during the winter. Females reproduced from the late spring through mid- to late-summer. They stopped reproducing in the late summer, when there was ample time to produce an additional litter of young. There was an inverse relationship between resources devoted to reproduction and fat reserves. As reproductive allotment decreased in the late summer, fat reserves increased. The magnitude of the change in fat reserves was similar to that displayed by males. The reserves were depleted over the winter. Significant reserves remained at the beginning of the reproductive season the following spring. Reproducing females utilized the remaining reserves significantly more rapidly than non-reproducing females. An analysis of resource availability revealed an overall decrease in food availability in the late summer, coincident with the increase in fat reserves. These cycles are therefore not attributable to changes in resource availability. They instead indicate a change in how resources are allocated by the fish. The trends in the data indicate that fat reserves are used to shift investment in reproduction from the late summer to the following spring. In males, deferring maturity, rather than maturing in August, allows them to store the necessary reserves to survive the winter so that they can mate the following spring. In females, a subset of the fat reserves

is intended for producing the first clutch of eggs the following spring. The female pattern corresponds to those reported for a diversity of organisms. The possible advantages of shifting reproductive effort from the fall to the following spring include higher fecundity and higher offspring fitness. The limitations of the methodology and potential directions for future research are discussed.

**Key words:** *Gambusia affinis* – Fat storage – Life history strategy – Reproductive cycles – Comparative life histories

Beginning with Fisher (1930), the role of resource allocation in life history evolution is most often viewed as the trinity of growth, maintenance, and reproduction. The process of evolution is believed to select for the optimal allocation of resources to these three functions. A fourth, equally important option for resource allocation is storage, usually as lipids. Storage is important because it represents a way of buying a degree of independence from the environment. Individuals can establish a 'savings account' when resources are abundant for use when resources are scarce. In vertebrates, such storage is frequently considered in the context of hibernation or migration, where it is an adaptation which enhances individual survival. Our interest is instead in how storage might represent a reproductive adaptation, as hypothesized by Fitzgerald (1976) and Derickson (1976). One example of the use of fat as a reproductive adaptation occurs when the season that is best for parturition is not the same as the time when resources are most readily available to adults. By storing resources, an adult could then shift them to a time when it is advantageous for the young to be born.

Hahn and Tinkle (1965) and Tinkle (1967) presented an example in the lizard *Uta stansburiana* where fat reserves were used to shift the timing of resource allocation to reproduction. This species produces multiple clutches per season. Lipid reserves increase in size in August, when reproduction ceases, then decrease in the spring as the first clutch is produced. Hahn and Tinkle demonstrated experimentally that the lipid reserves are necessary for the early production of the first clutch. Fat storage was therefore used to shift the allocation of resources to reproduction from the late summer until the following spring.

Why wait until the spring to produce a clutch of eggs when it could have been produced the previous fall? Hahn

and Tinkle (1965) asserted that the first clutch of the year is the most important and that fat storage allows the production of a large clutch early in the season, when food availability is low. Their argument for the importance of the first clutch, as opposed to a hypothetical last clutch produced the previous season, is that the fitness of young born early in the season is greater than young born later in the season. The young from the first clutch reached adult size in the year they were born and produced a full complement of clutches the following year. The young from later clutches matured sometime during their second season and were therefore not able to produce a full complement of clutches (Tinkle 1961, 1967; Tinkle et al. 1962). Since over 90% of the lizards which reached maturity only reproduced for one year, the young from the first clutch appeared to have a substantial reproductive advantage over young born later in the season. There is thus a premium placed on reproducing early in the season and fat storage makes this possible. Many authors have described similar cycles for temperate populations of snakes and lizards and have interpreted them in a similar fashion. Some recent examples include Telford (1970), Gaffney and Fitzpatrick (1973), Derickson (1974, 1976a, b), Goldberg (1974), Jameson (1974), Ballinger and Congdon (1981), and McKinney and Marion (1985). Similar studies and arguments have also been presented for amphibians (e.g., Fitzpatrick 1972, 1976; Morton 1981).

A second example where fat storage appears to allow parents to manipulate reproductive timing involves migratory, arctic breeding birds (e.g., Alisaikas and Aukney 1985; Krapu 1981; Krapu et al. 1985; McLandress and Raveling 1981). In all species studied, the adults arrived at the breeding grounds with substantial fat reserves, which helped sustain the birds during pair formation, nest site selection, and egg production. Krapu et al. (1985) proposed that fat and protein stores enable the birds to breed early in the spring, when resource availability is either low or unpredictable. Breeding early is important because the time available for the young to grow may be limited. Early breeding therefore enhances the probability that parents will successfully fledge young.

The common theme in both examples is that storage enables organisms to shift in time their allocation of resources to reproduction. The advantage of such shifts generally involves differences between the optimal time to produce young and when resources are available to adults. In *Uta stansburiana*, possibly many other species of amphibians and reptiles, and in arctic birds it is advantageous to produce young early in the season, thus allowing a longer interval of time for the young to grow during favorable weather. There is incomplete proof for these arguments, leaving room for alternative explanations; however, it is clearly plausible that fat storage is used to shift the timing of reproduction in an adaptive fashion.

This sort of phenomenon initiated our interest in fat cycling in the mosquito fish, *Gambusia affinis*. Such fat cycles are well known in fish, having been described mostly in pelagic, salt water species or species of commercial value (e.g., many examples are presented by Love 1970 and Nikolskii 1969). They have not been considered in smaller, short-lived species. Previous authors have found that the storage cycles of fish were generally associated with the reproductive cycle, as in the organisms discussed above, but Eliassen and Vahl (1982) demonstrated that the argu-

ments for a causal relationship between storage cycles and reproduction were often weak. *G. affinis*' pattern of reproduction suggests that reproduction may be tied to fat storage in a fashion similar to *U. stansburiana*. For example, many populations stopped reproducing early in the season, such as in late July or early August, when there still appeared to be ample time to produce an additional litter of young. Reproduction was initiated the following spring, when food reserves were potentially scarce (Barney and Anson 1921b; Hildebrand 1927; Krumholz 1948; Hughes 1985a). In addition, concurrent studies of life history patterns revealed strong differences between populations in life histories (summarized in part in Reznick, 1981). It was of interest to see if there were corresponding differences in fat cycles.

The main goal of our study was to determine if there was a cycle of fat storage which was inversely related to reproduction. We estimated the usual suite of variables associated with reproduction, plus considered additional dependent variables, particularly fat content. We also sampled from different localities and different years from the same locality to establish the generality of our observations.

## Materials and methods

*A. Natural history.* *Gambusia affinis* (family Poeciliidae) is a livebearer and is found along the Atlantic Coast as far north as New Jersey, the Gulf Coast, and the Mississippi Drainage as far north as Missouri, Illinois, Indiana, and Ohio. It has also been introduced throughout the world as a biological agent for mosquito control (Krumholz 1948). It is well-described as a fugitive species, generally occupying habitats that are inaccessible to potential predators. These include the shallow, weedy margins of ponds and streams plus ephemeral habitats, such as sloughs or ditches that contain water for part of the year. Its life cycle is well adjusted to such circumstances. It is capable of rapid growth and maturation, frequent reproduction (every three to four weeks at 25–30° C), and produces large broods. The generation time may be as short as seven to ten weeks (Krumholz 1948; Hughes 1985a; unpublished work). Reproduction is seasonal in the native portion of the range, with young generally produced from the late spring through the late summer.

*B. Field localities and sampling.* We report here on collections from Shallotte, Brunswick County, North Carolina (*G. a. holbrooki*); West Cape May, Cape May County, New Jersey (*G. a. holbrooki*); and the Ferne Clyffe State Park, near Goreville, Johnson County, Illinois (*G. a. affinis*). (Precise locality information is available on request.) All three localities are within the native range of *Gambusia*. The North Carolina locality is a still-water slough adjacent to the Shallotte River. The New Jersey locality is Pond Creek, where it crosses through the Hidden Valley Ranch. The Illinois locality is a small (appx. 1/2 acre) pond on the grounds of Ferne Clyffe State Park.

Collections were made at approximately monthly intervals. Fewer samples were collected during the winter because the localities were generally icebound. Seven collections were made in New Jersey between July 1976 and April 1977. Four additional collections were made in August 1977, and June through August 1980. Eleven collections were made in Illinois between July 1977 and July 1978.

Three collections were made in North Carolina between June 1978 and August 1978. The results are presented as four separate data sets: NJ '76, NJ '80, Illinois '77, and NC '78, respectively. The fish were collected with dipnets, then immediately preserved in five percent formaldehyde. We always concentrated on collecting a large sample of adults and subadults, plus a few representatives of smaller size classes. Juveniles were therefore generally not represented in proportion to their abundance in the population.

*C. Processing of preserved material.* The methods follow those of earlier publications (Reznick 1981, 1983; Reznick and Endler 1982). Briefly, collections were first sorted as females, mature males, immature males, or "too small to sex" (approximately 14 mm or less, standard length). Sorting was based on the morphology of the anal fin, as described by Turner (1941). Standard length measurements were made for the entire collection. Because male *G. affinis* have a determinate growth pattern, the size of mature males is an accurate measure of the size at maturity. The fish intended for fat extraction were then dissected and the contents of the gastrointestinal tract were removed. If the fish was an adult female, we also removed and counted all ova and developing embryos, and staged the embryos (as in Reznick 1981; Milton and Arthington 1983). The somatic and reproductive tissues were dried separately overnight in a drying oven at 60° C, then weighed to the nearest 0.1 mg. The dissection involved the removal of the caudal peduncle and a midline incision of the abdomen. The fish were dried with the sides of the abdomen well separated, facilitating contact with the solvent in subsequent fat extractions. The carcasses were then placed in individually labeled scintillation vials and covered with anhydrous diethyl ether. They were allowed to sit overnight, then the ether was poured off and replaced. The procedure was repeated until the fish attained a constant weight (up to four extractions were required for some samples). The carcass was dried, reweighed, and fat content was estimated as the percentage of weight lost. In females, estimates of fat content were based solely on somatic tissues, while in males they were based on the entire carcass. Dobush et al. (1985) recently demonstrated that non-polar solvents, such as diethyl ether and petroleum ether, are preferable to polar solvents like chloroform-methanol because the former remove only neutral (i.e., storage) lipids while the latter remove significant amounts of non-lipids.

*D. Resource availability and diet.* Patterns of fat storage may be driven by trends in resource availability. For example, an increase in fat content may be observed simply because of an increase in food resources. Since we are concerned here with determining if there is a causal relationship between fat storage and reproduction, it is critical to consider the possible influence of resource availability. We sampled prey abundance and food choice during the 1980 field season in New Jersey to see if trends in fat storage were associated with trends in food availability.

Diet was estimated on 20 May and 28 July 1980 by examining the stomach contents of 30 and 26 formalin preserved adult *G. affinis*, respectively. Prey were counted, identified at least to order, then classified into four broad categories: planktonic, benthic-littoral, windblown, or unidentified.

Our stomach content analyses revealed that most prey

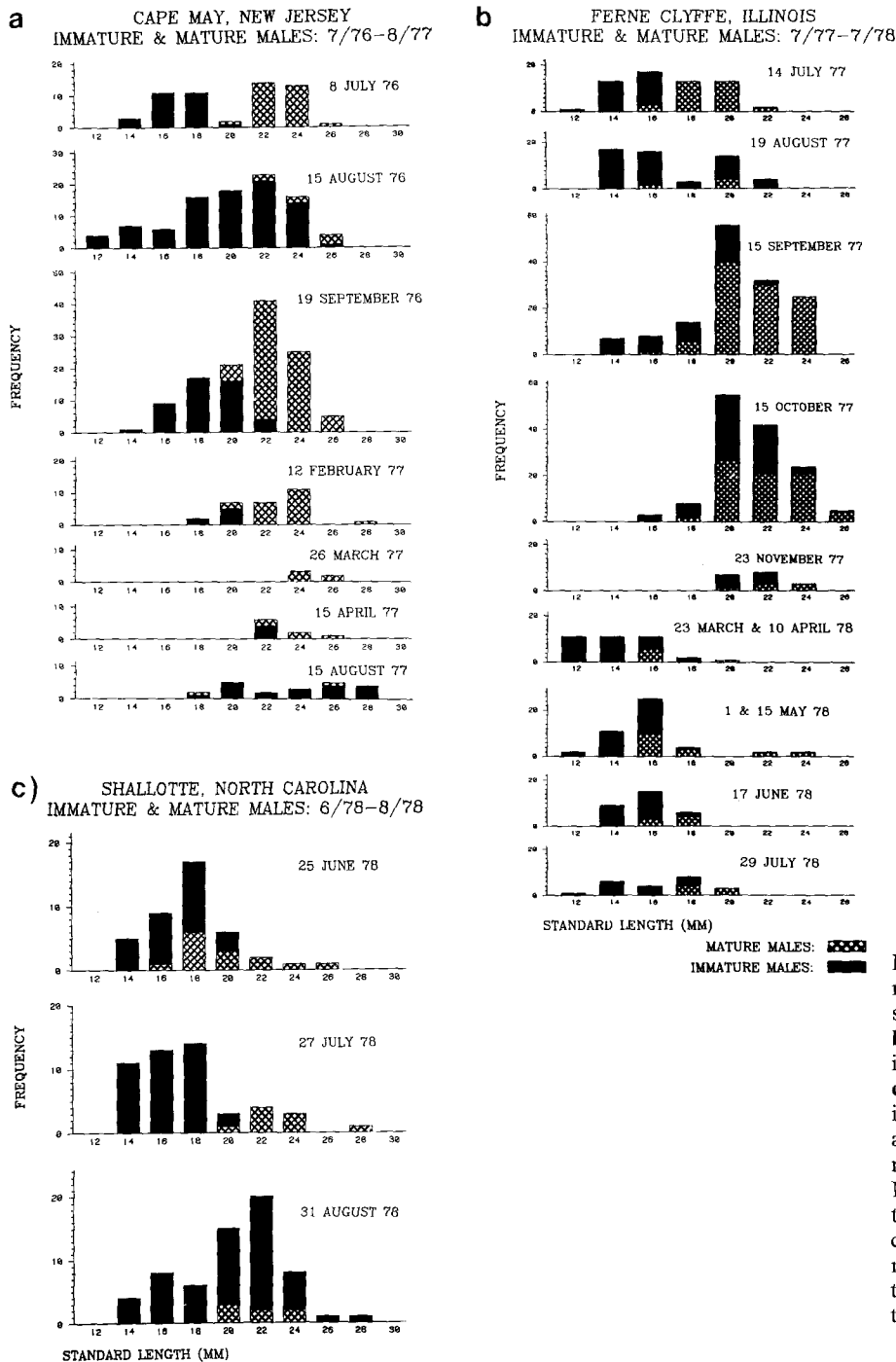
were either windblown insects or planktonic invertebrates, similar to the diets reported by previous investigators (Barney and Anson 1921 a; Hess and Tarzwell 1942; Harrington and Harrington 1961; Maglio and Rosen 1969; Hubbs 1971; Stearns 1975; Hurlbert and Mulla 1981). Plankton abundance was estimated at the NJ site at weekly intervals between 6/15/80 and 9/6/80 using a standard silk tow net (314.2 cm<sup>2</sup> cross-sectional area at the mouth) with a medium-fine mesh (18 per cm<sup>2</sup>). This mesh size included the preferred range of prey sizes (generally 1 to 5 mm; Hess and Tarzwell 1942; Harrington and Harrington 1961). The net was towed for a standard distance perpendicular to the shoreline. Samples were washed into a dated and numbered "Whirlpac" and stored in 2% formalin. For counting, samples were strained through a no 200 U.S. standard sieve then resuspended in 50 ml distilled water. We determined the number and size of prey in five 2 ml aliquots from each sample. Plankton abundance is reported as the number of plankton per 10 ml.

If there were a substantial increase in the lipid stores of the prey, these could generate similar changes in their predators. To see if this might be the case, we evaluated the lipid content of Cladocera, the most abundant plankton eaten by *G. affinis*. Cladocera deposit lipids as triacylglycerols in large fat bodies which are easily observed under a light microscope (Goulden and Horning 1980). We estimated the fat content of 25 Cladocera from each sample date following the methods of Goulden and Horning (1980).

Aerial insects were surveyed using an exposed-cone type suction trap manufactured for the U.S. Army. Insects, attracted by a light at the top of the trap, pass first through a fan and subsequently into a copper gauze cone (10 meshes per cm), before being collected in a plastic jar containing 2% formalin. Traps were set at one to two week intervals from 23 July until 18 September, 1980. Four traps were placed along the shoreline and set, with a time delay setting, to sample for 12 hours beginning at 8:30 P.M. Diurnal samples were collected twice but failed to catch anything. Hess and Tarzwell (1942) report "...a peak in feeding activity soon after daylight, a decline to a minimum in late afternoon, and a lesser peak in the evening" (p. 144). Our sampling time thus included the two peaks. Samples were later strained, suspended in distilled water, then the entire sample was counted and classified.

*E. Statistical analyses.* The chief dependent variable for *Gambusia* was the percent dry weight which was soluble in anhydrous ether ((1 - somatic lean dry weight/somatic dry weight)\*100). In females, we also considered the "reproductive allotment", which was the percentage of total dry weight that consisted of reproductive tissues.

All analyses of variance were executed with the SAS General Linear Models or the SAS NPAR1WAY procedures (SAS Institute Inc., 1985). The reported levels of significance for the GLM results were based on the Type III sums of squares. The assumptions of the analyses of variance were evaluated with the residuals from the model. The normality of the residuals was tested with the Proc Normal procedure on SAS. The homogeneity of variances were first evaluated visually with plots of the residual versus predicted values, then statistically with Bartlett's test using the Statistical Package for the Social Sciences (Nie et al. 1984). The Student-Neuman-Keuls test (Sokal and Rohlf 1969) was used for the *a. posteriori* comparison of means. We specify



**Fig. 1. a** Size-frequency distribution of mature and immature males from the NJ '76 series.

**b** Size-frequency distribution of mature and immature males from the Illinois '77 series. **c** Size-frequency distribution of mature and immature males from the NC '78 series. In all three cases, the y-axis represents the number of individuals in a given size class. Note that entire collections were included in these figures, while only a subset of each collection was processed for fat content. The number of individuals in these figures will therefore often exceed the sample sizes in the analyses of variance

in the Results where the assumptions of normality and equal sample variances have been violated. If violations of the assumptions of an analysis are not specified in the text, then all of the assumptions were satisfied.

There was a drastic reduction in the abundance of *Gambusia* in the late winter and early spring at the Illinois and New Jersey localities. For this reason, the sample sizes were also reduced. The spring samples from Illinois were so small that we lumped the 23 March with the 10 April and 1 May with the 15 May collections for statistical analyses. Too few females were present in the 17 June and 29 July samples from Illinois for inclusion in statistical analyses.

We considered length as a potential covariate in all analyses involving the percent fat or reproductive allotment.

We found no regular relationship between length and either fat content or reproductive allotment, so it is not included in any results.

## Results

The results for males and females are presented separately. In each case, we first describe the population cycles in our populations, then the fat cycles. The purpose for first considering population cycles is that their association with fat cycles provides the clues for the potential adaptive significance of fat storage. Population cycles also provide a basis for comparing our results with earlier reports.

PERCENT FAT IN MATURE VS. IMMATURE MALES

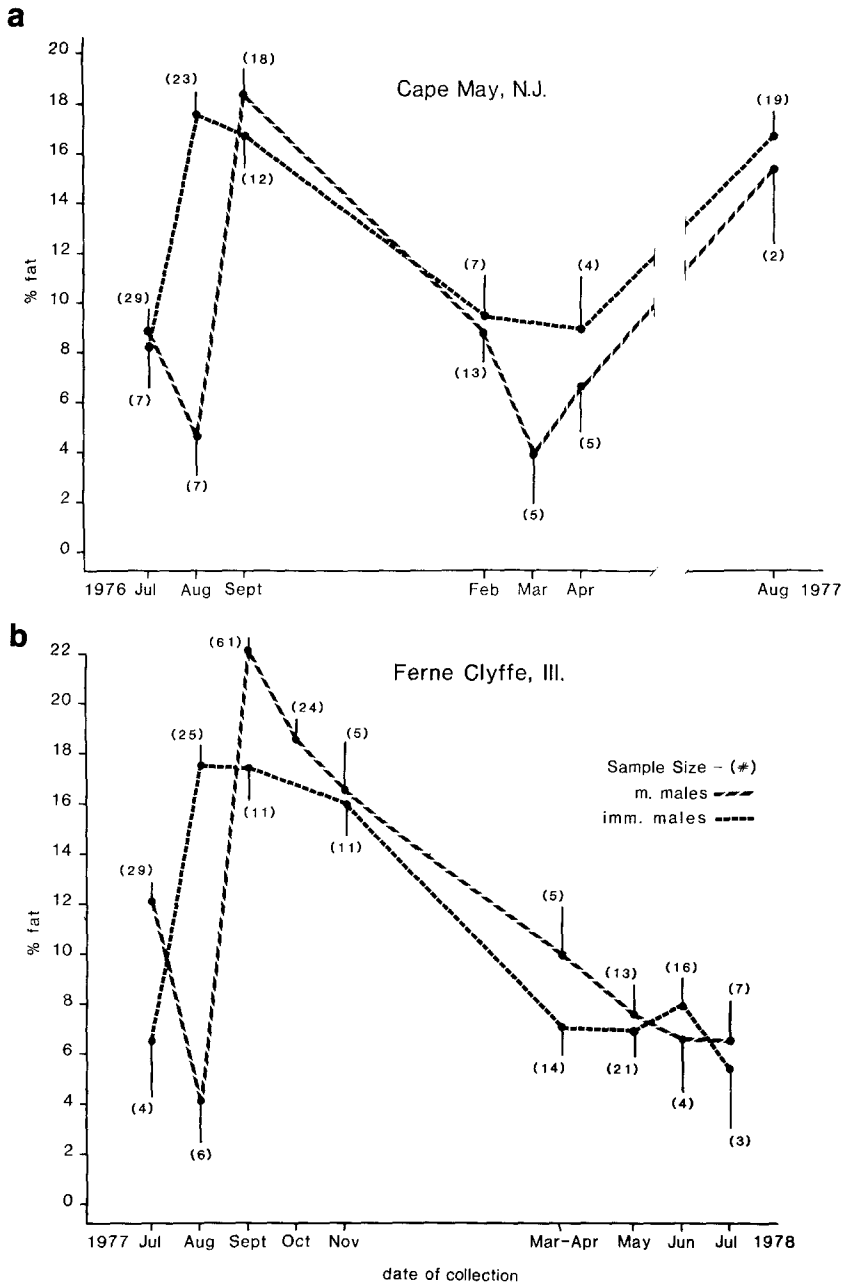


Fig. 2a, b. Percent fat in mature and immature males. The corresponding analysis appears in Table 1. See text for details. **a** NJ '76 series; **b** Illinois '77 series. Vertical lines equal one standard error

A. Males

Data are not presented here for New Jersey in 1980 because the mature males were being used for a separate series of experiments. We therefore present only three series of data.

*Pattern of maturation.* Males tended to mature by upon reaching a critical size during the reproductive season (May through July). This pattern was most apparent in the July samples from each locality (Fig. 1), in which most or all males larger than a critical size were mature, while all smaller individuals were immature. The localities differed in this critical size. Other collections made during the reproductive season (e.g. 6/78 from NC, or 5/78 and 6/78 from Illinois) showed a similar pattern, but a greater degree of overlap in the size distribution of mature and immature males.

Maturity was delayed and the mortality rate of mature males appeared high at the end of the summer. Delayed maturity appeared as a temporary increase in the size of immature males and a broader overlap in the size distribution of mature and immature individuals (see all four August collections in Fig. 1). For example, we found immature males as large as 28 mm in the 8/77 sample from NJ and the 8/78 sample from NC; the largest immatures observed earlier in the season were 20 mm. At the same time as the increase in size in immature males, there was a sharp decline in the relative abundance of mature males. For example, in NJ '76 the frequency of mature males declined from 79% to 23% between July and August. This decline was followed by a significant increase in the frequency of mature males in September. For example, in the NJ '76 series the frequency of mature males increased from 23% to 60% from August to September. We interpret the decline in the

**Table 1A, B.** Statistical analysis of male fat content

**A** Summary of analyses of variance of % fat in males. The reported F-ratios are based on the Type III sums of squares from the SAS GLM procedure. The numbers in parentheses next to each F-ratio are the degrees of freedom. Rsq = the proportion of the total variance accounted for by the model.

**B** The reported means are the LS Means from the SAS GLM procedure for the NC '78 analysis only. The sample size associated with each mean is reported in parentheses. The corresponding values for NJ '76 and Ill '78 appear in Fig. 2. Means which do not differ significantly are joined by a horizontal or vertical line. These paired comparisons were made with the Student-Newman-Keuls test. The only paired comparisons dealt with in this table are between immature and mature males within a collection and between consecutive collections within just the mature or just the immature males

**A. Results of analyses of variance:**

	NJ '76 <sup>a</sup>	Ill '77	NC '78 <sup>a</sup>
Collection Date-F value (df)	19.29*** (5,133)	36.30*** (7,219)	6.71** (2,113)
Mature vs Immature-F value (df)	8.16*** (1,133)	0.01 (1,219) ns	5.21* (1,113)
Interaction-F value (df)	9.79*** (5,133)	10.69*** (7,219)	2.05 ns (2,113)
Residual S.S.	0.2101	0.3579	0.4000
Rsq	0.60	0.72	0.35

**B. Mean fat content (sample size in parentheses) for NC '78 analysis:**

Date:	6/25/78	7/27/78	8/31/78
Mature:	4.8 (14)	4.9 (9)	7.2 (7)
Immature:	5.8 (26)	6.4 (9)	14.2 (54)

\* 0.01 < P < 0.05; \*\* 0.001 < P < 0.01; \*\*\* P < 0.001

<sup>a</sup> Residuals not normally distributed; significant heterogeneity of variance

abundance of mature males as mortality and the later increase as the recruitment of newly matured individuals. We observed identical trends in the relative abundances and sizes of mature and immature males in the NC '78 and Ill '77 series of collections.

**Fat storage.** With one notable exception, the trends in fat content of mature vs. immature males parallel one another (Fig. 2, Table 1). Fat reserves were at relatively low values during the spring and summer, then increased dramatically during the late summer and autumn. For example, they increased from 8.4% to 17.6% from July to August in immature males from New Jersey. They declined gradually through the winter. The statistical summaries (Table 1) support the significance of these trends.

August was the only month when mature and immature males differed in fat content (Fig. 2); this is also the time when mature males were relatively scarce. The simultaneous changes in fat content and relative abundance suggests that mature males were dying in late summer and being replaced by a new cohort in the fall. To illustrate these trends, immature males in the 8/76 NJ collection were 17.6% fat while

mature males were only 4.9% fat. There were no differences in fat content in the September collection at the same time that mature males increased in frequency, presumably because of recruitment from the pool of immature individuals. The significance of this trend can be illustrated statistically by considering the magnitude of the interaction term in a two way analysis with and without the August collection. In the NJ '76 data, the interaction term with the complete data set was highly significant and accounted for 24.9% of the variance attributable to the model (Table 1). When the August sample was removed, the interaction became nonsignificant ( $F_{4,105} = 0.65$ ;  $p = 0.6280$ ) and accounted for only 2.2% of the variance attributable to the model. The interaction term in the Illinois '77 series also became nonsignificant with the removal of the August sample ( $F_{6,190} = 1.96$ ;  $p = 0.0733$ ) and the percentage of the model variance attributable to interaction dropped from 13.2% to 2.1%. In each case, the significant interaction can thus be attributed to the difference in fat content between mature and immature males in the August collections. We observed one exception to the August difference between mature and immature males in our 8/77 collection from NJ (Fig. 2). There were only two mature males in this sample (Fig. 1), one with a very low fat content, and one large individual with a high fat content, similar to the immature males. Such an exception, based on so few observations, is not inconsistent with the rest of the data. For example, if the large male were newly recruited, it would generate this result.

There were significant deviations from normality and heterogeneity of variance in the NJ '76 and NC '78 analyses. The main consequence of such violations of the assumptions of the analysis is that the reported levels of significance will tend to be too high. Given the high levels of significance in the key comparisons ( $P < 0.001$  in most cases) and the repeatability of the patterns of fat storage, particularly in Ill '78 where the assumptions of the analysis were satisfied, the nature of our conclusions remain valid.

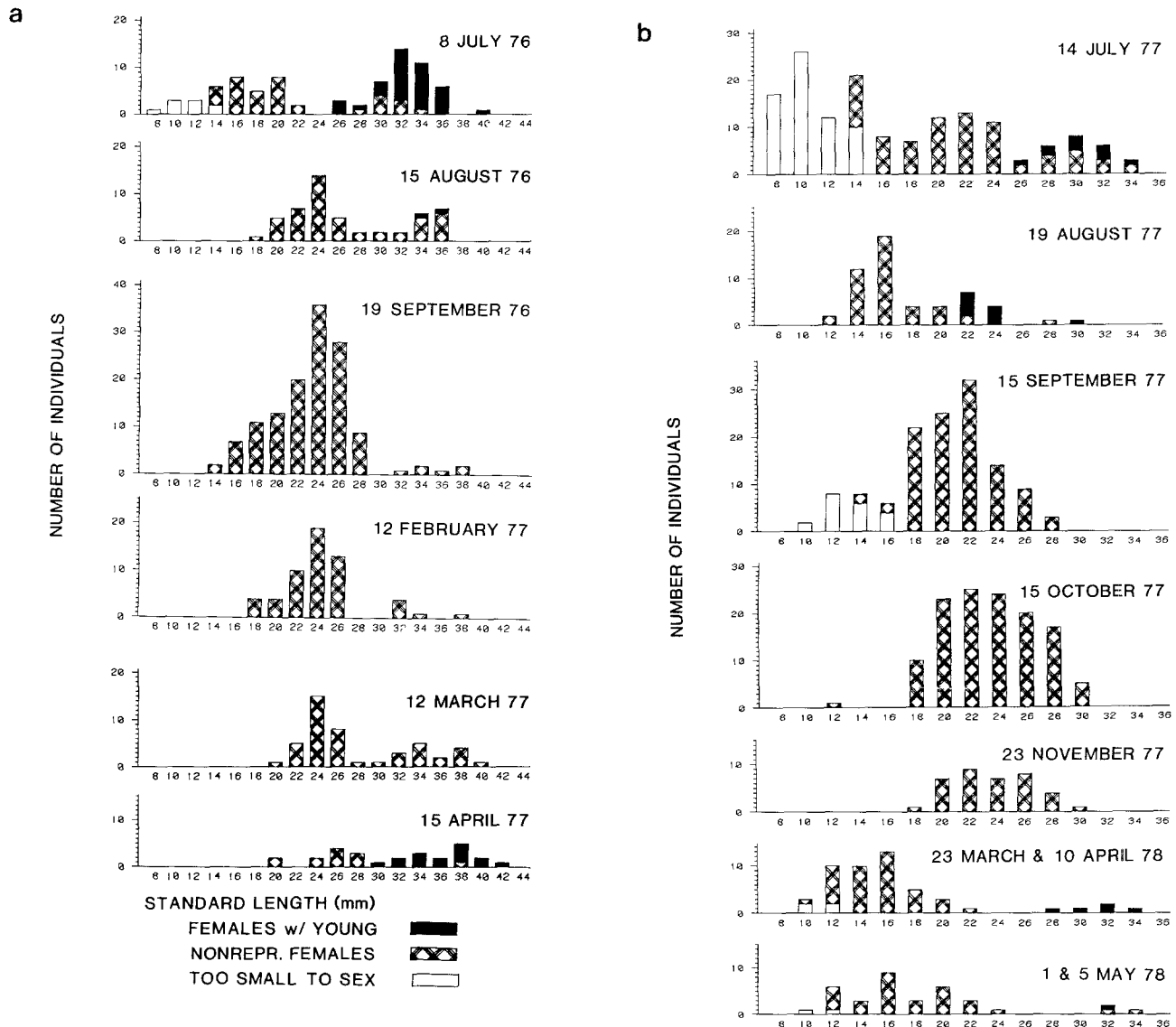
**B. Females**

**Population cycles.** Beginning with our late spring or early summer samples (7/77 in Illinois, 7/76 in NJ), we observed a bimodal size distribution (Fig. 3). Large, reproductively active females comprised one mode and small, reproductively inactive females, or individuals too small to sex, comprised the second mode. The Illinois series was actually trimodal, with two modes of non-reproducing individuals. These probably represent the first and second broods produced by the largest size class. This multi-modality is consistent with earlier observations in this part of *G. affinis*' range (Krumholz 1948; Hughes 1985a) and is generated by the nearly synchronous onset of reproduction in the late spring followed by brood production at regular intervals. These same trends were replicated in our samples from NJ '80 and NC '78 (not illustrated).

Reproduction and growth virtually ceased by late July or mid-August and began again in April in all series (Fig. 3). The few reproducing females in some August and September collections had very small broods, relative to their body size, in comparison with fecundities observed earlier in the season (D. Reznick, unpublished work). The only notable difference between series of collections was in the survivorship of large females in Ill '77 vs. NJ '76. In Illinois,

CAPE MAY, NEW JERSEY - LENGTH DISTRIBUTIONS  
FEMALES AND IMMATURES: 7/76 - 4/77

FERNE CLYFFE, ILLINOIS - LENGTH DISTRIBUTIONS  
FEMALES AND IMMATURES: 7/77 - 5/78



**Fig. 3.** a Size-frequency distribution of females and immatures from the NJ '76 series; b Size-frequency distribution of females and immatures from the Illinois '77 series. See Fig. 1 caption for details

the larger size class disappeared by September while in NJ a subset of these individuals survived to reproduce for a second season (Fig. 3).

*Fat cycles.* The large and small size classes of females were lumped in most of the following analyses. Lumping the data simplifies the analysis to a one way analysis of variance. This lumping accurately characterizes the fat cycle because, with few exceptions (see below), there was generally no difference in the means for the two size classes or else no interaction between collection date and size class means. The analyses for reproductive allotment included only the reproductive size classes of females. Therefore, the results for reproductive allotment (summarized in Fig. 4 and Table 2) included only a subset of the sample from the percent fat analyses. Since the fat cycles of reproducers are generally congruent with those of non-reproducers, the

comparisons between fat content and reproductive allotment accurately depict trends within just the reproducing females.

There was an inverse relationship between fat storage and reproductive allotment in all four data sets (Fig. 4, Table 2). The percent fat was relatively low (generally 3 to 8%) while the females were actively reproducing, then increased as reproduction ceased. For example, in the 7/76-9/76 NJ collections (Fig. 4a), the reproductive allotment dropped to nearly zero while the percent fat increased from 10.3 to 18.4% of the total dry weight. The opposite trend was seen from February through April in this series; the percent fat declined steadily while the reproductive allotment increased. We obtained the same results in the Illinois series (Fig. 4b); however, the results for the spring were more erratic. These irregularities are largely attributable to small sample sizes and the uneven representation of the

FEMALES  
REPRODUCTIVE ALLOTMENT & FAT STORAGE

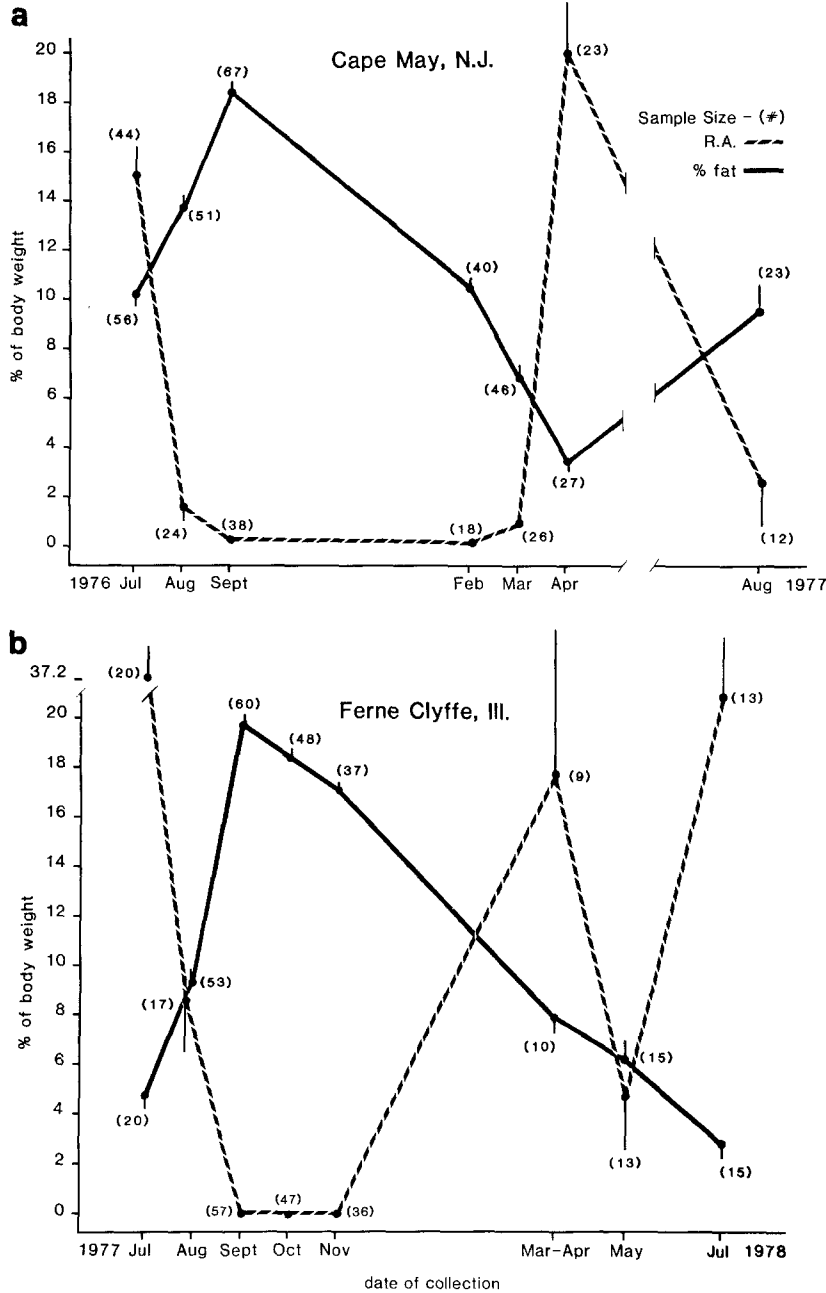


Fig. 4. Percent fat and reproductive allotment in females. The corresponding analyses appear in Table 2. See text for details; a NJ '76 series; b Illinois '77 series. Vertical lines equal one standard error

larger, reproductively active females. For example, the 3-4/78 samples contained a few large females, while the 5/78 samples contained none. The corresponding statistics (Table 2) demonstrate the significance of these patterns.

The results thus far are consistent with the idea that fat storage at the end of the reproductive season is causally related to reproduction. Some of the fat is clearly intended for individual maintenance during the winter, as indicated by the storage of fat by all size classes and by the gradual decline in percent fat from September through March in NJ '76 and Illinois. However, some reserves remain when reproduction begins again in the spring; these may be used to produce the first clutch of young. Such trends suggest, but do not prove, a causal relationship. One way to streng-

then the argument for this relationship is to compare the patterns of fat storage in reproducing versus non-reproducing females as reproduction begins in the spring. Our hypothesis was that, if the remaining reserves are used for reproduction, then we should see a more rapid decline in the % fat of reproducers than nonreproducers.

We found that reproducing females utilize fat reserves more rapidly than non-reproducing females, thus supporting our hypothesis (Fig. 5, Table 3). The comparison between reproducers and non-reproducers was equivalent to a comparison between the different size classes; large females were reproducing while small females were not. A two way analysis of variance revealed significant differences among collection dates and size classes, plus a significant



**Table 2A–C.** Percent fat and reproductive allotment in females

A	Fat $\bar{x}(n)$	RA $\bar{x}(n)$
NJ '76	194.5*** (6)	111.76*** (6)
Ill '77 <sup>a</sup>	203.59*** (8) <sup>a</sup>	46.69*** (5) <sup>b</sup>
NC '78	43.54*** (2)	15.78** (2)
NJ '80	31.53*** (2)	34.54*** (2)

B	NC '78	Fat $\bar{x}(n)$	RA $\bar{x}(n)$
	6/25/78	3.5 (34)	11.6 (34)
	7/27/78	4.9 (32)	14.0 (23)
	8/31/78	8.5 (66)	6.3 (49)

C	NJ '80	% Fat $\bar{x}(n)$	RA $\bar{x}(n)$
	6/23/80	3.8 (21)	29.3 (17)
	7/28/80	7.4 (32)	17.3 (13)
	8/22/80	11.4 (22)	1.4 (15)

\*\* 0.01 < P < 0.001; \*\*\* P < 0.001

**A** Results of non-parametric analyses of variance (Kruskal-Wallis test) of percent fat and reproductive allotment in females. Values reported are chi-square values, with degrees of freedom in parentheses. **B** and **C** Means reported only for NC '78 and NJ '80, respectively. The corresponding values for NJ '76 and Ill '77 appear in Fig. 4. The sample sizes appear in parentheses next to each mean

<sup>a</sup> Executed with and without sample collected on 6/17/78. Results are qualitatively the same in both cases

<sup>b</sup> Only zero values were recorded for all individuals in 3 winter samples. The actual dry weight of the reproductive tissues was generally less than 1 mg, which was beyond the effective resolution of our balance. These data were not included in the analysis

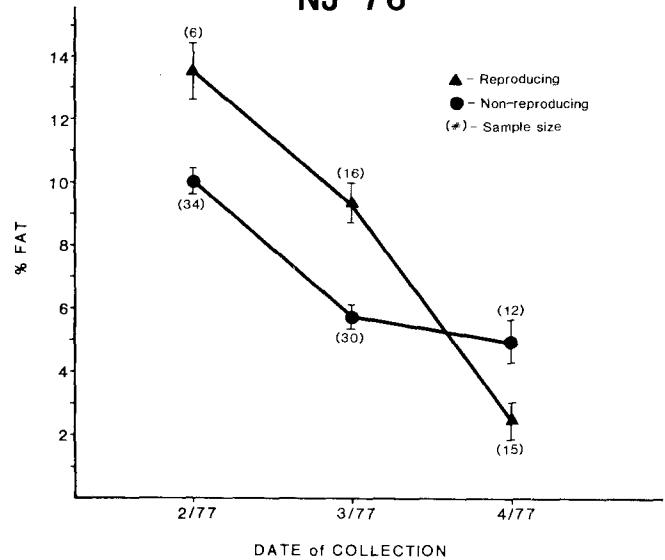
interaction. The interaction is of greatest interest here. It was generated because large females had significantly more fat than small females in February and March, then significantly less fat in April, when they began to reproduce. This more rapid decline in reproducers than non-reproducers suggests that the reserves were used to provision the first brood of young.

We recognize the confounded nature of this observation, since reproduction was associated with the inherent differences between age groups. For example, the age difference alone could alter the utilization of fat reserves independently of reproduction.

### C. Resource availability

What is the association between fat cycles and trends in resource availability? At one extreme, it was possible that the increase in fat reserves reflected an increase in food availability or the quality of food, while the cessation of reproduction was an independent response to environmental cues. Another possibility was that the end of the reproductive season corresponded to a decline in food availability to the adults and to prospective offspring. In this case, the increase in fat was likely the result of energy saved in not reproducing. We therefore quantified food choice and resource availability for the NJ '80 series.

## % FAT -- REPRODUCING VS. NON-REPRODUCING FEMALES NJ '76



**Fig. 5.** Percent fat in reproducing vs. non-reproducing females from the NJ '76 series. The Figure and corresponding analysis (Table 3) were restricted to late winter and spring to focus on the initiation of reproduction. See text for details. Vertical lines equal  $\pm 1$  standard error

**Table 3.** Analysis of fat content in reproducing (large) vs. non-reproducing (small) females from the February, March and April samples in the NJ '76 series. The corresponding means, standard errors, and sample sizes appear in Fig. 5

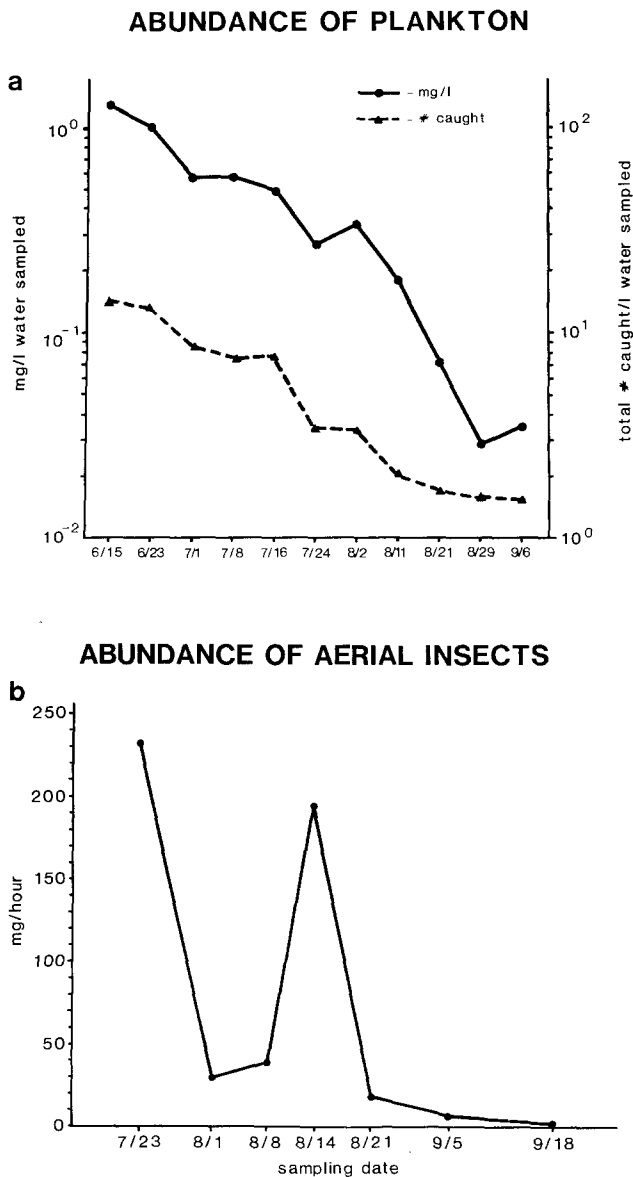
	d.f.	SS	F	pr > p
Collection date	2	0.0759	72.52	0.001
Size mode	1	0.0049	9.29	0.0029
Interaction	2	0.0165	15.80	0.001
Residual	107	0.0560		
			Rsq = 0.65	

The primary source of prey in our samples was plankton, followed by windblown insects (Table 4). Planktonic invertebrates were most abundant early in the season, then declined steadily through the summer (Fig. 6a). There were no trends in the lipid content of the planktonic prey and no significant differences among the mean values for lipid content over the course of the season ( $F_{10,247} = 0.09$ ). The trends in the abundance of flying insects were less regular (Fig. 6b). Abundance was highest in our first sample (23 July), declined through early August, attained a second peak on 14 August, then declined in the remaining samples. The second peak was associated with rainfall. The general trend was still a decline in abundance from late July through early September.

Our results therefore support the proposition that the increase in fat reflects a change in how resources were allocated by the fish, rather than an increase in resource availability or the fat content of the prey. We conclude this because the increase in fat reserves observed in the NJ '80 *G. affinis* was associated with a general decline in resource availability.

**Table 4.** *Gambusia* stomach contents for NJ '80 collections. See text for details

Sample Date (n)	5/20/80 (30)	7/28/80 (26)
no food items	413	476
<i>Diet composition</i> (based on no of prey items)		
Planktonic species	55.8%	85.1%
Benthic-littoral species	11.0%	0.0%
Windblown insects	26.1%	8.4%
Unidentified	7.1%	6.3%



**Fig. 6.** **a** Abundance of planktonic organisms, based on standard tows of a plankton net. These collections correspond with the NJ '80 series of *G. affinis*. Abundance is expressed as the number individuals caught per liter and as the biomass (mg) per l. See text for additional details. **b** Abundance of aerial insects caught in vacuum traps. These collections correspond to the NJ '80 series of *G. affinis*. Abundance (y-axis) is expressed as the number of mg collected per hour, averaged across four traps. See text for further details

## Discussion

The patterns of fat storage were similar in males and females. Such patterns were unexpected under the hypothesis that fat storage is a reproductive adaptation because of the expected differences between males and females in energy budgets. These similarities are in part due to a large portion of the fat stores being used for overwinter survival, which applies to both sexes. In addition, the arguments for the role of fat storage as a reproductive adaptation are fundamentally different for males and females. Since females conform to the arguments presented in the Introduction, their results are discussed first. Also, because the adaptive arguments are closely tied to the population cycle, the first goal is to find the similarities in the population cycles reported in this paper and in those reported by previous authors. Strong similarities in population cycles argue for corresponding similarities in fat cycles.

### A. Females

The patterns of growth and reproduction observed for females conform to observations reported by previous authors. The length of the reproductive season (generally June through mid-August) was similar to other observations made at similar latitudes (Hildebrand 1927 – North Carolina; Krumholz 1948 – Illinois; Hughes 1985a – Indiana). These localities were also similar in the fairly synchronized onset of reproduction in the spring and the subsequent separation of age classes as different size modes. Longer reproductive seasons, up to nine months, have been reported in less seasonal habitats (e.g. Hildebrand 1917 – Key West, Florida; Hubbs 1971 – Menard County, Texas; Milton and Arthington 1983 – Queensland, Australia). The patterns of growth, with rapid growth from May through late July, then little or no growth at other times of the year, also conform to earlier observations. Finally, although not detailed in this study, all authors observed relatively high size-specific fecundity in the spring with a decline through the reproductive season (Hildebrand 1917, 1927; Barney and Anson 1921b; Krumholz 1948; Milton and Arthington 1983; Hughes 1985a). Our observations on growth and reproduction are therefore typical of the species.

*G. affinis* female fat and reproductive cycles are similar to those reported for other vertebrates (e.g., Hahn and Tinkle 1965 and other references in the Introduction). These patterns are consistent with the proposition that fat storage is in part a reproductive adaptation. Evidence in favor of this interpretation includes: i) the inverse relationship between reproductive allotment and fat storage (Fig. 4), ii) only a portion of the fat was used during overwintering (Fig. 4), iii) the reserves remaining the following spring were rapidly utilized by reproducing, but not by non-reproducing, females (Fig. 5, Table 3), and iv) other investigators' finding that fecundity tended to be highest for the first litter of the season. The same seasonal trend in fecundity was also reported for lizards (e.g., Ballinger 1977; Nussbaum 1981) and is possibly caused by the contribution of nutrient reserves to the first litter.

Our observations on resource availability also support the interpretation of fat storage as a reproductive adaptation. Fat stores increased as resource availability decreased in our NJ '80 observations. This trend refutes the possibility that the fat cycles were a passive by-product of seasonal

trends in resources. These cycles were thus at least partly attributable to endogenous changes in *G. affinis*, rather than being driven by changes in food availability.

The advantages to the female of such cycles are expected to be similar to those hypothesized for other vertebrates. These include: i) the production of the first litter of young early in the season, presumably before they could produce a litter fueled only by current resource availability, ii) higher fecundity in the first litter, iii) possibly increased fitness of offspring born early vs. late in the season. Offspring fitness can be enhanced in three ways: i) our results (Table 6) imply higher levels of resource availability in the spring, ii) early born offspring can mature and reproduce in the same season (Krumholz 1948; Hughes 1985a), therefore generation time is reduced, and iii) these young will also be larger at the beginning of the following season. In females, increased size generally means higher fecundity. In males, increased size has been associated with increased mating success (Hughes 1985b). In both sexes, increased size translates into a higher probability of being sexually mature at the onset of the next season. In support of this last statement, note that only the larger males and females from the NJ '76 series matured or reproduced early in 1977 (Figs. 1, 4). Size might also affect the probability of overwinter survival. The influence of time of birth on fitness has been proposed by previous authors (see Introduction) but never adequately investigated. It remains a fruitful area for research in this and other species.

### B. Males

The main features of the population cycles we observed were similar to most previous studies of *Gambusia affinis*. The primary pattern was the relative scarcity of mature males at the end of the reproductive season followed by a substantial increase in relative abundance in the autumn (Hildebrand 1917; Barney and Anson 1921a; Krumholz 1948; Brown and Fox 1966; Hubbs 1971; Hughes 1984). Previous investigators also observed a relatively small size at maturity through the reproductive season, then a much larger size at maturity late in the season (Krumholz 1948; Hughes 1984). The most detailed report, by Hughes on a natural population in Indiana, precisely replicated our observations.

The added information from the fat cycles provides a plausible explanation for these trends. The time when mature males were relatively scarce (all August samples – Fig. 1) was coincident with the time when their fat reserves were at their nadir. At the same time, immature males had high reserves, similar to the values recorded for females. These strong differences in fat content disappeared in the September samples, at the same time that mature males became relatively more abundant (Table 1, Fig. 2). One interpretation for these trends is that the older generation of mature males dies out in August, then is replaced by newly recruited individuals. A second explanation is that the older generation of mature males rapidly stored fat between the August and September samples and caught up to the younger generation. We consider this possibility far less likely because of the rapid decline in the relative abundance of mature males at the end of the summer.

The trends in fat storage also potentially explain why immature males delayed maturity in the late summer and early fall. Some fat reserves were required to survive the

winter, given the gradual overwinter decline in fat in both sexes in the NJ '76 and Illinois '77 series (Figs. 2 and 4). If being mature precludes fat storage, it would be necessary to first build up reserves, then mature. Delaying maturity also means maturing at a larger size. Hughes (1985b) demonstrated that increased male size was associated with increased success in obtaining mates.

Why would the size at maturity change from mid-summer, when males all matured at relatively small sizes, to late summer or early fall, when males mature at relatively large sizes? This question is of interest because the size advantage would apply at all times of year, yet one only consistently observes the delay in maturity and corresponding increase in the size at maturity at the end of the season. One possible answer is that this delay represents a shift in when resources are allocated to reproduction. The consequence of growing, storing fat, and delaying maturity is that males are shifting their reproductive effort to the following spring. High female fecundity early in the season versus low fecundity late in the season (Hildebrand 1917, 1927; Barney and Anson 1921b; Krumholz 1948; Milton and Arthington 1983; Hughes 1985a; D. Reznick, pers. obsvn.) would favor delaying maturity until the following year because a given amount of reproductive effort by the males potentially results in more offspring. In addition, there are potential differences in the fitness of young born early vs. late in the season, as already discussed.

A second reason for the observed pattern of maturation, suggested by Hughes (1984), is that the delay in maturity is caused by mature males inhibiting the development of immature males. His argument follows the work of Borowsky (1973) and Sohn (1977a, b), who demonstrated in other species of Poeciliids that when immature males are kept with mature males, they matured at a later age and larger size than controls reared in isolation. We consider this possibility unlikely because the delay in maturity correspond with a time when mature males were becoming increasingly rare and were thus less likely to affect the immature individuals.

Why mature in September or October when reproduction will not begin until the following spring? One possibility relates to the observed patterns of reproduction. Geiser (1924) and Maglio and Rosen (1969) report a burst of mating activity in the spring, with only sporadic mating through the summer. By maturing in the fall, males will be ready to participate in this spring burst of mating activity. Females can retain sperm and reproduce for an entire season on a single insemination (Hildebrand 1917), so mating in the spring can result in the siring of offspring throughout the summer.

### C. Differences between populations

One feature of the life history which varied between localities from similar climates was the number of reproductive seasons. We found in our Illinois series (Fig. 3) that the females were annual, like the males. The NJ '76 males were also annual, but some adult females overwintered and reproduced in a second year (Fig. 3). Hughes' (1985a) population was similar to our Illinois series. Krumholz's results were split; in one population a component of the largest size class of females appeared to overwinter while in the other two few or no females from the largest size class survived beyond August. This variation in female survival

was at least partly environmental in origin because all of Krumholz's stocks were derived from the same source population. It would be of interest to know if there is also a genetic component to this difference in survival.

A second difference was in the male size at maturity. During the summer, Illinois '77 males tended to mature at smaller sizes than NC '78 and NJ '76 males. Reznick (1981) reported a similar trend for the average size of reproducing females from these populations. The same pattern was observed for the minimum size of reproducing females (Reznick, unpublished work). In guppies (*Poecilia reticulata*) such differences are an index for the age at maturity (Reznick and Endler 1982; Reznick 1982) and imply that the Illinois fish matured sooner than the NJ '76 or NC '78 fish. Reznick (1981) also reported that the Illinois females had higher reproductive allotments and tended to produce more and smaller offspring in each brood.

We observed no differences between localities in fat cycling or the patterns of male maturation at the end of the season; however, the resolution for comparing females at the beginning of the reproductive season was quite low because of the small samples in the Illinois '77 series.

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