

LIPID COMPOSITION RELATED TO SIZE AND MATURITY OF THE AMPHIPOD *PONTOPOREIA HOYI*

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ABSTRACT. Micro-gravimetric determination of the lipid content of amphipods (*Pontoporeia hoyi*) obtained from a 45-m-deep Lake Michigan sampling site indicated that the mean lipid content of adult females was 30% on a non-lipid dry weight (NLDW) basis, and that juveniles and adult males contained 21 and 10% lipid (NLDW basis), respectively. Thin layer chromatography-flame ionization detection (TLC-FID) analyses revealed that lipids of females were composed primarily of triacylglycerols (81%), the principal energy storage lipid of amphipods. Lipids of juveniles were composed largely of triacylglycerols (41%) and phospholipids (44%). Adult male *P. hoyi* lipids consisted mostly of phospholipids (64%) and, secondarily, of triacylglycerols (12%). The relatively low triacylglycerol concentrations in males may be associated with the minimal requirements for energy stores to support metabolic needs during the male's brief (10 day) life span. By contrast, the high lipid content and marked abundance of triacylglycerols in adult females represents an important energy store supporting subsequent egg development, particularly since females appear to halt all feeding upon maturation. In juvenile *P. hoyi*, increased individual size (NLDW) was accompanied by increased lipid dry weight, implying that juveniles accumulate lipids during growth. Overall, the results demonstrated the importance of considering *P. hoyi* size, life stage, and sex when describing a population's lipid content or composition. This consideration is particularly critical when evaluating the role of *P. hoyi* in the transfer of energy and/or organic contaminants within the Great Lakes food web.

KEYWORDS: Benthos, benthic environment, macroinvertebrate.

INTRODUCTION

The amphipod *Pontoporeia hoyi* is a prominent member of the upper Great Lakes benthic macroinvertebrate community where it often constitutes up to 60% of the resident biomass (Nalepa *et al.* 1985). The amphipod's abundance (e.g., a mean density of 7,000 animals m⁻² in Lake Michigan; Nalepa *et al.* 1985) coupled with its high energy content (ca. 5,000 cal g⁻¹ dry weight; Green 1971, Johnson and Brinkhurst 1971) suggest that this organism is potentially important as a food resource for Great Lakes fish stocks. Several studies have demonstrated that a variety of commercial and sportfish species rely on *P. hoyi* as an important prey item (Van Oosten and Deason 1938, Wells and Beeton 1963, Anderson and Smith 1971, Wells 1980). *P. hoyi*'s high energy content has been

attributed to the animal's unusually high lipid content which can constitute as much as one-half of its overall dry weight (Gardner *et al.* 1985a). A portion of these lipids exists as visible lipid droplets within the amphipod's body cavity (Green 1971, Johnson and Brinkhurst 1971). Moreover, *P. hoyi*'s high lipid content and intimate association with sediments appears to be closely linked with its demonstrated bioaccumulation of lipophilic organic contaminants (Landrum 1982, 1988; Landrum *et al.* 1985). Thus, *P. hoyi* plays an integral role in the cycling of both energy and organic contaminants between benthic and pelagic components of the upper Great Lakes.

Although previous studies have described overall *P. hoyi* lipid content (Green 1971, Whittle and Fitzsimons 1983, Gardner *et al.* 1985a), the relationship between lipid content or composition and

individual size and/or maturity has not been documented.

Lipids are well-known compounds that play important structural, hormonal, and energetic roles in life histories of aquatic invertebrates (Giese 1966, Hadley 1985). Because lipids represent up to twice the potential metabolic energy per unit weight, compared to proteins or carbohydrates, lipid reserves offer the most efficient means of storing energy to support metabolic needs during low food availability or to meet reproductive needs (Prosser and Brown 1966).

The objective of our study was to examine lipid content and composition among different *P. hoyi* sizes and life stages. Such information is essential for understanding the energetic importance of lipids in *P. hoyi* survival and reproduction. Moreover, changes in lipid content and composition throughout the animal's life history may strongly affect its uptake and/or elimination of lipophilic organic contaminants. For example, Landrum (1988) noted that depuration rate constants of benzo(a)pyrene and phenanthrene were inversely proportional to *P. hoyi* lipid content, and identified lipids as being extremely important in the storage of organic xenobiotics.

MATERIALS AND METHODS

In December 1986, *P. hoyi* were obtained by Ponar grab at a 45-m-deep sampling site in southeast Lake Michigan (43° 1' 6" N Lat., 86° 19' 35" W Long.). In the laboratory, individuals (≥ 5 mm) were isolated in 20-mL linear polyethylene liquid scintillation vials at a temperature of 5.5°C. The vials were submerged in trays of mildly aerated lake water and were covered on top with 500- μ m Nitex screen to confine animals. A 1-cm-deep layer of coarse (500–550 μ m) pre-combusted sand was placed in the bottom of the vials. Animals readily burrowed into this substrate and appeared to rapidly adjust to laboratory conditions. Individuals were held in vials for up to 40 days and were fed detritus rations (from seasonal sediment trap collections) every 10 days at rates ranging from 0 (controls) to 3.2 mg of detritus animal⁻¹ day⁻¹. Feeding rates for different groups corresponded to particle accumulation rates measured by sediment traps deployed 15 m above the sampling site, at various intervals during March-December 1986. Throughout the experiment, periodic inspection of individuals in vials revealed that upon the onset of sexual maturity, both males and females perma-

nently halted all feeding activity and had uniformly empty guts. Among juveniles, 56% had empty guts, while another 46% had guts that were half-full or less. These laboratory results were similar to the widespread incidence of empty and partially full guts noted among field-collected *P. hoyi* from Lake Michigan (Quigley 1988, Evans *et al.* In Press) and Lake Ontario (Dermott and Corning 1988). Since most animals had little or no gut contents, individuals were immediately processed for lipid analysis with no time allotted to allow animals to completely void their guts.

P. hoyi were removed from vials at intervals of 10, 20, 30, and 40 days. Immediately following removal, the dorsal outline of all individuals was sketched under a dissecting microscope equipped with a camera lucida, noting location of both rostrum and base of telson. When gut contents were present, the outline of the material was also sketched. These tracings were later scanned with a computer-linked digitizer to derive total length and percent gut fullness (gut contents length divided by an individual's total length) (Quigley and Lang 1989). Individuals were also inspected to determine state of maturity. Mature females were readily identified by ovary development characterized by the presence of an opaque white mass of tissue in the dorsal portion of the thorax. Mature males exhibited elongation of antennae and modification of the urosome for swimming (Seegerstrale 1971). Males were highly active and spent virtually all of their time swimming in water above the sand substrate.

A total of 280 animals were individually isolated in vials. Three animals were not found at the end of the experiment and may have escaped around the screen at the tops of the vials. Of the remaining 277 animals, 23 died over the 40-day course of the experiment (mortality = 8.3%). Of the 254 animals remaining, 37 animals successfully molted into mature females (14.6% of all animals), and 19 others molted into mature males (7.5% of all animals). Among dead animals, 4 of the 23 (17.4% died during molts into males, and 3 others (13.0%) during molts into mature females. Thus, percentages of females among dead and surviving animals (13.0 and 14.6%) were similar, while the percentage of males among dead animals (17.4%) was twice the percentage of males among surviving animals (7.5%).

Following measurement and inspection, individual *P. hoyi* were rinsed in distilled water, blotted dry, and placed in pre-weighed glass micro-culture

tubes, where they were dried under nitrogen at 50°C. Lipid content as a percentage of dry weight was determined with a micro-gravimetric extraction procedure (Gardner *et al.* 1985b). Relative concentrations of major lipid classes were determined on a portion of the final lipid extracts using thin layer chromatography with flame ionization detection (TLC-FID) (Parrish 1987, Parrish *et al.* 1988). Extracts for TLC-FID were stored frozen under nitrogen until lipid-class analysis could be performed.

RESULTS

P. hoyi Total Length and Dry Weight

Male *P. hoyi* mean total length [9.3 ± 0.2 (SE) mm, $n = 16$] was greater than that of females [8.9 ± 0.1 (SE) mm, $n = 33$]. However, mean female dry weight [1.7 ± 0.1 (SE) mg] was considerably higher than that of males [1.2 ± 0.1 (SE) mg]. All 16 of the adult males were of the neotenic *brevicornis* form (Seegerstrale 1971) and had antennae that were characteristically shorter than half of the overall body length. Juvenile mean total length [7.0 ± 0.1 mm] and mean dry weight [0.8 ± 0.02 (SE) mg] were lower than males or females. On a dry weight (mg) per length (mm) basis (W/L ratio computed from mean dry weight and mean total length), females had a W/L value of 0.19 ± 0.01 (SE); males, 0.12 ± 0.01 (SE); and juveniles, 0.10 ± 0.01 (SE).

Lipid Content

Figure 1 displays mean percent lipid content based on total and non-lipid dry weight (NLDW) of females, males, and juveniles. On a total dry weight basis, females had the highest mean lipid content, 22.7 ± 0.9 (SE) % ($n = 30$); followed by a juvenile mean of 16.4 ± 1.0 (SE) % ($n = 189$); with males having the lowest mean lipid content, 8.7 ± 0.9 (SE) % ($n = 14$). When mean lipid content was calculated on a non-lipid dry weight basis (total dry weight - dry weight of lipids), means of females and juveniles were considerably higher (29.7 ± 1.5 (SE) % and 20.7 ± 1.4 (SE) %, respectively) compared to dry weight-based lipid percentages. Due to the relatively low lipid content of males, mean percent lipid based on non-lipid dry weight [9.7 ± 1.1 (SE)%] increased only slightly from the previous dry weight-based mean of 8.7%. Overall, non-lipid dry weight-based lipid percentages were not biased by the amount of lipid

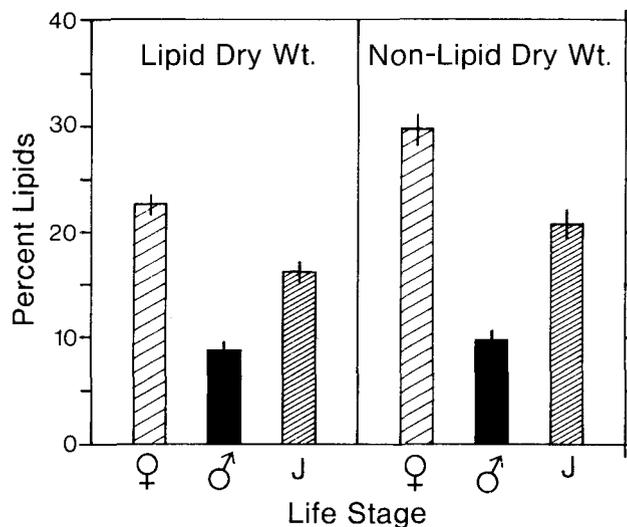


FIG. 1. Mean percent lipid content (\pm SE) of female, male, and juvenile *P. hoyi*. Mean percent lipid content, calculated on a dry weight basis, is shown at left and mean percent lipid content, calculated on a non-lipid dry weight basis, is shown on the right.

present and therefore provided a more uniform basis for comparing lipid content among individual *P. hoyi* in all subsequent comparisons.

Lipid Composition

TLC-FLD analysis detected seven major lipid classes in *P. hoyi* extracts. They are listed below (from least to most polar).

- 1) Hydrocarbons—Hydrocarbons are known to be important in the structure of the exterior cuticle of arthropods (Hadley 1981). They have also been found in small concentrations internally although their function in this area remains unknown (Kolattukudy 1976). High hydrocarbon levels may also indicate possible pollution sources (Stegeman and Teal 1973, Mackie *et al.* 1974).
- 2) Sterol and Wax Esters—Sterol esters / wax esters are well-known as energy stores for aquatic animals, particularly marine copepods (Chapman 1969, Sargent *et al.* 1977). Sterol esters are a storage form of free sterols and have been identified as important components of cell membranes (Parrish 1986).
- 3) Triacylglycerols—Triacylglycerols are best known as chemical stores of energy, but

they may also play a role in buoyancy or thermal insulation (Gagosian and Lee 1981).

- 4) Fatty Acids—Fatty acids, though commonly viewed as the functional “building blocks” composing more complex lipids, have been found to exist freely, in small quantities, as normal components of an animal’s overall lipid pool (Chapman 1969).
- 5) Sterols—Sterols are widespread in animal tissue and have important functions in cell membranes and lipoproteins of blood plasma (Hadley 1985). Despite the importance of these compounds, crustaceans are incapable of *de novo* sterol synthesis and must depend on dietary sterol sources (Goad 1976, O’Rourke and Monroe 1976).
- 6) Acetone-Mobile Polar Lipids—Acetone-mobile polar lipids represent that part of the polar lipid fraction that is acetone-extractable. Included in this class are pigments (e.g., chlorophyll *a*) (Parrish 1986). Glycolipids are also an important component of the AMPL class. They are common in chloroplasts and also occur in trace amounts in animal tissue (Hadley 1985).
- 7) Phospholipids—Phospholipids are important constituents of membranes (Lehninger 1975). They are particularly important in crustaceans where they also constitute the principal circulating (hemolymph) lipid (Gilbert and O’Connor 1970).

In terms of overall lipid composition (Fig. 2), female *P. hoyi* lipids consisted primarily of triacylglycerols [80.9 ± 1.5 (SE) % (n = 15) of all lipids], and secondarily, of phospholipids [11.6 ± 1.6 (SE) %]. Hydrocarbons and sterol esters/wax esters were present in only trace (<1%) amounts in female, male, and juvenile lipid fractions. In females, free fatty acids, sterols and acetone-mobile polar lipids occurred in roughly equal (2%) quantities.

Lipids of male *P. hoyi* consisted mainly of phospholipids [64.0 ± 6.7(SE) % (n = 8)], while triacylglycerols were second in abundance and highly variable [12.3 ± 7.8(SE) %]. Overall, lipids of males had higher percentages of free fatty acids [5.7 ± 1.0(SE) %], sterols [9.6 ± 2.0(SE) %] and acetone-mobile polar lipids [6.7 ± 1.6(SE) %], compared to females. However, these higher percentages are probably due to the relatively lower

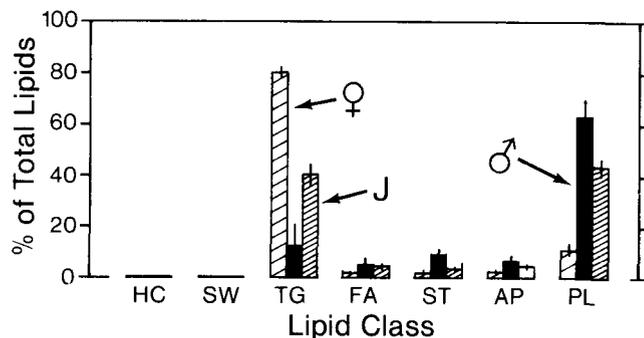


FIG. 2. Mean percentages (\pm SE) of lipid classes within overall lipids of female, male, and juvenile *P. hoyi*. HC = hydrocarbons, SW = sterol esters/wax esters, TG = triacylglycerols, FA = free fatty acids, ST = sterols, AP = acetone-mobile polar lipids, and PL = phospholipids.

amount of triacylglycerols in males compared to females and juveniles.

Juvenile *P. hoyi* contained lipids consisting of nearly equal portions of triacylglycerols [41.1 ± 3.8(SE) % (n = 69)] and phospholipids [43.7 ± 3.3(SE) %]. Percentages of free fatty acids [4.9 ± 0.6(SE) %], sterols [4.0 ± 0.4(SE) %], and acetone-mobile polar lipids [4.7 ± 0.8(SE) %] were lower than levels found in males, but greater than percentages found in females.

Lipid Class Concentration (Non-Lipid Dry Weight Basis)

Results of calculation of lipid class concentration (μg) based on mg non-lipid dry weight (NLDW) are shown in Figure 3. On this basis, the triacylglycerol content of female *P. hoyi* [238.6 ± 13.7(SE) $\mu\text{g mg}^{-1}$ NLDW] is over twice that of juveniles [114.5 ± 14.2(SE) $\mu\text{g mg}^{-1}$ NLDW] and 14 times higher than that of males [16.6 ± 10.8(SE) $\mu\text{g mg}^{-1}$ NLDW]. Phospholipid concentrations in males and juveniles were similar [58.3 ± 5.9(SE) and 67.2 ± 4.3(SE) $\mu\text{g mg}^{-1}$ NLDW, respectively], and both were higher than phospholipid concentrations in females [35.7 ± 0.5(SE) $\mu\text{g mg}^{-1}$ NLDW]. On a non-lipid dry weight basis, concentrations of free fatty acids, sterols, and acetone-mobile polar lipids were approximately equal among females, males, and juveniles and ranged from 5–9 $\mu\text{g mg}^{-1}$ NLDW. Only trace amounts of hydrocarbons and sterol esters/wax esters [0.4 – 1.7 $\mu\text{g mg}^{-1}$ NLDW] were found in females, males, and juveniles.

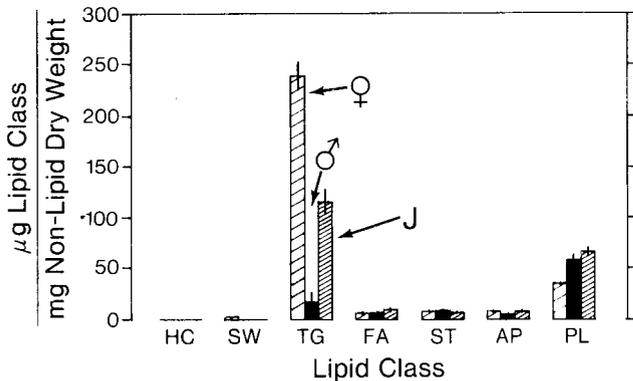


FIG. 3. Mean μg (\pm SE) of a lipid class per mg non-lipid dry weight among female, male, and juvenile *P. hoyi*. HC = hydrocarbons, SW = sterol esters/wax esters, TG = triacylglycerols, FA = free fatty acids, ST = sterols, AP = acetone-mobile polar lipids, and PL = phospholipids.

Variation in Lipid Content Versus Size

To examine possible relationships between female, male, and juvenile *P. hoyi* lipid content (mg lipid dry weight (LDW) and size (as non-lipid dry weight (NLDW)), we plotted these variables against each other (Fig. 4) and calculated corresponding linear regression equations. Overall, there was a significant ($P < 0.05$) linear increase in lipid dry weight with size (NLDW) among females, males, and juveniles. Additionally, the increase in lipid weight relative to NLDW was similar in females and juveniles, but lower in males.

An analysis of covariance F test for coincident regression lines (Zar 1984) indicated that signifi-

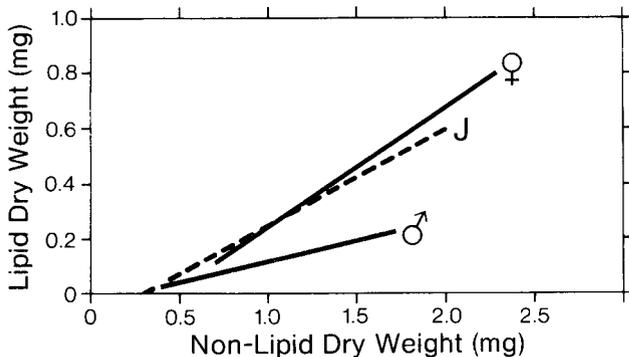


FIG. 4. Lines calculated from regressions of lipid dry weight on non-lipid dry weight for female, male, and juvenile *P. hoyi*.

cant differences ($P < 0.05$) existed among slopes and/or intercepts of the three (LDW versus NLDW) regression lines. Subsequent Tukey multiple comparison tests (Zar 1984) revealed no difference between slopes of female (0.42) and juvenile (0.33) regressions ($P < 0.05$) while the slope of the male regression line (0.14) was significantly different from both female and juvenile slopes. Multiple comparisons among intercepts indicated that intercepts of female (-0.18) and juvenile (-0.07) regression lines represented common intercepts while the intercept of the male regression line (-0.03) differed significantly from female and juvenile intercepts.

Since juvenile and female NLDW versus LDW regression lines were coincident, these data were pooled within a common regression where; $\text{LDW} = 0.35 \text{ NLDW} - 0.09$. When the smallest (juvenile, 0.25 mg) and the largest (female, 2.31 mg) NLDW values were entered into this equation, and the NLDW-based lipid percentage calculated (using regression-predicted LDW's), lipid percentages ranged from < 1 to 31%. Thus NLDW lipid percentage showed an overall increase with size (NLDW) within the pooled juvenile/female group.

For males ranging in size from 0.38 mg to 1.7 mg NLDW, calculated NLDW-based lipid percentages using predicted LDW's exhibited a far narrower range (from 7 to 12%).

Triacylglycerol and Phospholipid Weight Versus Lipid Weight

Figure 5 displays triacylglycerol weight plotted against overall lipid weight in female, male, and juvenile *P. hoyi*. In all three cases, increased LDW was accompanied by a corresponding and significant ($P < 0.05$) linear increase in triacylglycerol weight. Analysis of covariance and an F test for coincident regression lines indicated that one or more differences existed among slopes and/or intercepts of lines for females, males, and juveniles. Tukey multiple comparison tests revealed no significant differences ($P < 0.05$) among slopes of the three lines and only the intercept of the male regression line was significantly different from the intercept for juveniles. Overall, increases in triacylglycerol weight accompanying increased lipid dry weight appeared similar among female, male, and juvenile animals.

Phospholipids were not significantly correlated with overall lipid weight among females and males.

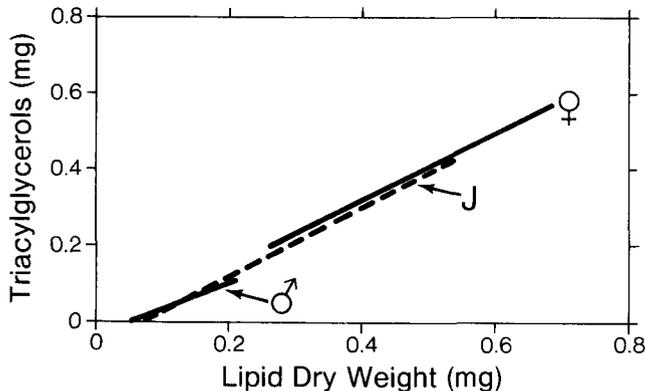


FIG. 5. Lines calculated from regression of triacylglycerol dry weight on lipid dry weight for female, male, and juvenile *P. hoyi*.

In juveniles, however, phospholipid weight increased significantly ($P < 0.05$) with increased lipid weight.

DISCUSSION

Length, Weight, and Lipid Content

Females

Females, while slightly smaller than males in overall length, had greater dry weights and were correspondingly more robust. Mean female length (8.9 mm) was considerably larger than a mean female total length of 6.4 ($n = 11$) previously noted at a 42-m-deep Lake Michigan station (Winnell and White 1984). However, Winnell and White also cited a wide (6.75 – 11 mm) size range for *P. hoyi* females in the literature, so it appears that the size differences in females at the two stations are not unusual. In addition, the small sample size of females obtained during our feeding experiments may not accurately reflect actual female total length at our 45-m station. On a dry weight basis, females from our 45-m Lake Michigan sampling site had an overall mean (1.7 mg) that was lower than the 1.9 mean noted for Winnell and White's 42-m station. Higher mean dry weight, combined with a smaller total length, produced a larger (0.30) W/L ratio at their 42-m station compared to a W/L ratio of 0.18 of females in our study.

Within the present study, females (W/L = 0.18) were more robust than males (W/L = 0.11) or juveniles (W/L = 0.10). Moreover, higher W/L ratios and correspondingly greater robustness of females

also coincided with a higher mean (NLDW-based) lipid percentage in females compared to males and juveniles (Fig. 1).

Given the important structural, regulatory, and energetic roles of lipids, females can be expected to have high lipid content at the onset of reproduction. Additionally, since females halt feeding upon maturation (Moore 1979, Quigley 1988), all lipid supplies needed to support development of young must be present at this time.

Female *P. hoyi* also showed the highest rate of increase in lipid weight relative to increased individual non-lipid dry weight (Fig. 4), implying that higher fecundity associated with larger size of females (Moore 1979) may be accompanied by proportionally larger lipid supplies.

Males and Juveniles

Mean total length (9.3 ± 0.2 mm (SE)) of male *P. hoyi* recovered during our experiments was above the 5–8.5 mm size range reported by Segerstrale (1971) for a variety of Great Lakes locations. Although males and juveniles of our study had similar W/L ratios, mean percent lipid content of juveniles (20.7% on a non-lipid dry weight basis) was over twice the mean percent for males (9.7%). Juvenile lipid content (mg dry weight) increased with individual non-lipid dry weight (Fig. 4) at a rate (slope = 0.33) that was close to (and not significantly different from) that noted in females (0.42). By contrast, male lipid content increased less rapidly with non-lipid dry weight. In fact, the LDW versus NLDW regression line has a slope (0.14) in which predicted NLDW-based percent lipid changes very little (7–12%) over the range of actual NLDW values for males.

Since increases in juvenile lipid dry weight with increased size (NLDW) followed trends similar to females, it appears that growth throughout juvenile life is characterized by a progressive increase in lipid supplies. This lipid buildup is most apparent in juveniles maturing into adult females which have uniformly high lipid content. Although the lipid increases accompanying juvenile growth may largely explain the eventually high lipid content of adult females, this trend does not account for the lower lipid content of adult males. Unfortunately, we were not able to distinguish between sexes among juveniles, and to our knowledge, this is presently not possible. Within our juvenile lipid data, however, there was no evidence that two sub-populations of individuals exhibited different sex-

related trends in lipid content with increasing NLDW. While such differences could exist, it seems more likely that juveniles, regardless of eventual sex as an adult, follow a strategy of lipid accumulation throughout growth. Upon maturation, however, this increase is readily apparent only in high lipid content of females and less discernible in individuals developing into males. Because juveniles undergo extensive morphological changes during maturation into males, to facilitate swimming and mate location during their brief pelagic existence (Wells 1960, 1968; Marzolf 1965; Segerstrale 1971; Lubner 1979), these changes may constitute a substantial energetic cost. Moreover, the energetic costs associated with development and mobilization of sperm, coupled with elevated metabolic demands of sustained swimming may further deplete lipids to low levels.

Lipid Composition

Females

The predominance of triacylglycerols in the lipid composition of *P. hoyi* females is characteristic of the storage role of this lipid class in amphipod reproduction. Triacylglycerols are regarded as the major energy storage lipid in benthic amphipods (Clarke *et al.* 1985). In the arctic marine amphipod *Onisimus* (= *Boeckosimus*) *affinis*, an overall elevation in lipid content coincided with a peak in reproductive activity (Percy 1979). However, Clarke (1977) observed little seasonal variation in lipid content except for changes associated with reproduction in the antarctic decapod *Chorismus antarcticus*. In crustacean zooplankton, reliance on lipid supplies during reproduction has been well documented (Meyers and Strickler 1984). For example, *Calanus cristatus* and *Calanus plumchrus* relied primarily on lipid (wax ester) reserves to support egg development during adult life, when females lacked feeding mouth parts (Lee *et al.* 1972, Benson and Lee 1974). Additionally, Herring (1973) estimated that over 60% of all lipids of pelagic oceanic decapods is directed toward egg development. In cladocerans, stored lipids (triacylglycerols) are transferred to the ovaries during late stages of oocyte development to support embryonic growth, and to supplement energy needs during earliest stages of neonate life, when young may not be able to feed efficiently (Goulden and Henry 1984).

In the marine amphipod, *Gammarus oceanus*, linkages between lipid content increases and repro-

duction was attributed largely to lipid accumulation in the ovaries (Clarke *et al.* 1985). Lipids in *G. oceanus* spring eggs consisted chiefly of triacylglycerols (63%) and phospholipids (27%), while winter eggs contained nearly equal portions of triacylglycerols (52%) and phospholipids (43%). Female lipid composition appeared to largely reflect egg composition and the higher triacylglycerol content in spring eggs was attributed to better feeding conditions in the spring. In another marine gammarid (*Echinogammarus marinus*), lipids in spring eggs consisted almost entirely of triacylglycerols with little or no phospholipids. In both species, wax esters were a minor component, while sterols and hydrocarbons constituted up to 12% of all lipids. Overall reproductive output of individual females appeared to depend largely on food availability during the period of ovary maturation, when females continued to feed. Differing patterns of lipid utilization observed during egg development reflected a wide degree of flexibility in the overall reproductive biology of these two gammarid species. Since both species breed repeatedly, this reproductive strategy is consequently far more complex than the one-time reproductive strategy employed by *P. hoyi*.

Our results suggest that *P. hoyi* juveniles tend to accumulate lipids through life with progressively greater amounts of this lipid supply being composed of triacylglycerols. This trend can be readily seen within the regression equation (Fig. 4) calculated to compare triacylglycerol weight as a portion of juvenile LDW where; $TG = 0.853 LDW - 0.038$ (significant $P < 0.05$). When minimum (0.017 mg) and maximum (0.531 mg) juvenile LDWs are used in this equation and percent triacylglycerol calculated based on predicted triacylglycerol weights from the equation, percent triacylglycerols among all lipids are found to range from <1 to 78%. The 78% upper value is particularly noteworthy since it approaches the 81% triacylglycerol mean value we observed in female *P. hoyi* females. Thus, the emerging prominence of triacylglycerols in juvenile lipid supplies is a trend seen throughout juvenile life and this trend extends to include higher triacylglycerol levels in *P. hoyi* females. The lipid composition we observed in *P. hoyi* males differed most widely from the composition of females and juveniles in terms of percent triacylglycerols and phospholipids. Phospholipids constituted 64% of all lipids in males compared to females (12%) and juveniles (44%). Males also had

distinctly lower triacylglycerol percentages (12%) compared to females (81%) and juveniles (41%).

In terms of overall lipid content (8.7% dry weight), male *P. hoyi* had only slightly more than the 5.2% lower limit commonly used to define the presence of lipid reserves in invertebrate tissue (Giese 1966). The fact that triacylglycerol levels were highly varied among individual males implies that this energy storage lipid is being depleted during male adult life. Furthermore, when male (NLDW-based) percentages of triacylglycerols (1.7%) and phospholipids (5.8%) are adjusted upward to values comparable to those seen in juveniles (11.5% for triacylglycerols, 6.7% for phospholipids) and the overall NLDW-based lipid percentage recalculated using the new values, the resulting value for NLDW-based lipid percentage of 20.2% comes remarkably close to the 20.7% value noted in juveniles. Thus, it appears that the energetic cost of a juvenile's transition to, and existence as, an adult male may be supported largely by energy stored as triacylglycerols.

From the standpoint of previous data on *P. hoyi* lipid content (as % dry weight), mean lipid content of juveniles (16%) and females (23%) was consistently lower than values reported in previous field studies for unsexed individuals. These values range from 24% (dry weight) (Whittle and Fitzsimons 1983, Lake Ontario) to 33% (dry weight) (Gardner *et al.* 1985a, Lake Michigan). While lower mean juvenile lipid content may be an artifact of our laboratory feeding regimes, recent lipid content determinations of field-collected females from our 45-m-deep Lake Michigan site indicated that the mean lipid content [$23.8 \pm 1.4\%$ (SE), $n = 6$] was not significantly different from the mean for females determined in the present study [$22.7 \pm 0.9\%$ (SE)]. Additionally, we recently determined lipid content in adult males collected in the water column (30 m above the bottom at an 80-m-deep Lake Michigan sampling station) and found a mean (dry weight) lipid content of $16.0 \pm 1.5\%$ (SE), ($n = 11$) that was nearly twice the mean of $8.7 \pm 0.9\%$ (SE) for males we noted in the present study. Field-collected males were similar, in total length and dry weight, to males molting to adults during our feeding experiments. Overall, field-collected males were more robust with a higher mean W/L ratio of 0.14 ± 0.01 , compared to 0.11 ± 0.04 for males molting to adults in the laboratory. In addition to their higher lipid content and more robust condition, field-collected males all had antennae that were considerably longer than

their overall body length, indicating that these individuals were normal adult males classified as the form *filicornis* (Seegerstrale 1971). By contrast, males molting to the adult stage in the laboratory all had second antennae that were less than half the overall body length, implying that these males were the neotenic *brevicornis* form. Despite the immature appearance of *brevicornis* males, these animals are fully functional reproductive adults. Coexisting populations of *filicornis* and *brevicornis* forms have been observed in large lakes of North America (Seegerstrale 1971). Another widespread adult male form (f. *intermedia*) has also been identified among *P. hoyi* populations of North America, and has second antennae that are approximately 60% of overall body length (Seegerstrale 1971). To date, the underlying reasons for multiple adult male *P. hoyi* forms are not understood. However, the fact that we observed differences in lipid content between laboratory *brevicornis* males and field-collected *filicornis* males implies that the two forms may also utilize different energetic strategies during reproduction in addition to having obvious morphological differences.

In Lake Michigan, *P. hoyi* feeding and lipid accumulation appears to be closely linked with seasonal phytoplankton-derived detritus inputs to sediments (Quigley 1988, Gardner *et al.* In press). Thus, lipid storage may provide a means by which *P. hoyi* populations can fully exploit temporarily available food sources to support future metabolic needs.

Given the differences we found in lipid content and composition among *P. hoyi* life stage and sex, we expect that the transfer of energy and organic contaminants to fish stocks may depend in part on the amount of differential predation on a particular *P. hoyi* size, life stage, or sex. For example, recent gut analysis of sculpin collected at a southeastern Lake Michigan site indicated that male *P. hoyi* constituted 46% of all prey in July compared to 7.6% in September and 0% in May (Evans *et al.* In press). These results suggest that transfer of energy and/or contaminants from *P. hoyi* to fish stocks is highly seasonal and dependent on what *P. hoyi* life stages or sex are exposed to predation.

Finally, the results demonstrate the importance of considering *P. hoyi* size, life stage, and sex when describing mean lipid content or composition of a population. This consideration is particularly critical when evaluating the role of *P. hoyi* in the trans-

fer of energy or organic contaminants within the Great Lakes food web.

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