

Evidence for Indirect Effects of Fish Predation on Maternal Lipid Investment in *Holopedium gibberum*

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We examined selected aspects of the reproductive dynamics of *Holopedium gibberum* in 25 lakes in Algonquin Park, Ontario. Using an index based on *Daphnia* size, we divided the lakes into two groups characterized as high and low fish predation. Overall, egg volumes were found to be a good predictor of energy reserves in stage 1 eggs of *Holopedium*. However, small eggs, which were associated with small females typical of the high predation lakes, had relatively less fat than large eggs, suggesting that fish predation indirectly reduces starvation resistance of *Holopedium* neonates. In addition, in high-fish lakes, mean adult body size, minimum size of ovigerous females, clutch size, and fat volumes of *Holopedium* were smaller than in lakes characterized as low fish predation. Maternal lipid investment (MLI) into eggs of *Holopedium* was compared between the two lake types. We found striking differences in the seasonal pattern of absolute MLI between the two lake groups, mostly mediated through differences in body size. The greatest difference in absolute MLI between the two lake types occurred in May; females from the low-predation lakes invested 4 times as much energy reserves into reproduction as compared with females from the high-predation lakes at this time. Relative MLI (energy reserves per microgram female weight), comparing only individuals which overlapped in size, was unaffected by lake type. Thus a constant proportion of an ovigerous female's weight is invested in reproduction with a seasonal component that matches standard algal abundance for many shield lakes, suggesting that relative MLI is determined more by the exigencies of cladoceran life histories than by the presence of fish. Nevertheless, absolute energy investment is depressed in the high-predation lakes and we suggest that clonal replacement mediated by size-selective predation by the fish can account for the differences between the lake types.

Les auteurs ont étudié certains aspects de la dynamique reproductive de *Holopedium gibberum* dans 25 lacs du parc Algonquin, en Ontario. À l'aide d'un indice basé sur la taille des *Daphnia*, ils ont réparti les lacs en deux groupes caractérisés par une prédation par les poissons élevée ou faible. De façon générale, le volume des oeufs s'est avéré un bon paramètre de prévision des réserves énergétiques des oeufs de stade 1 de *Holopedium*. Les oeufs de petite taille, des petites femelles typiques des lacs à forte prédation, contenaient relativement moins de lipides que les gros oeufs, ce qui porte à croire que la prédation par les poissons réduit indirectement la résistance au manque de nourriture des *Holopedium* nouvellement nés. De plus, dans les lacs contenant beaucoup de poissons, la taille moyenne des adultes, la taille minimale des femelles ovigères, les pontes et les volumes de graisse des *Holopedium* étaient inférieurs, comparativement aux lacs où la prédation par les poissons était faible. L'utilisation maternelle des lipides (UML) pour la production d'oeufs dans chacun des types de lac a été comparée. Les auteurs ont noté des écarts très apparents entre l'allure saisonnière de la valeur absolue de l'UML entre les deux groupes de lacs. Les écarts étaient surtout fonction des différences de tailles corporelles. La plus grande différence de UML absolue entre les deux types de lacs a été notée en mai, les femelles des lacs de faible prédation consacrant quatre fois plus de leurs réserves énergétiques à la reproduction, comparativement aux femelles des lacs de prédation élevée. La UML relative (réserves énergétiques par microgramme poids des femelles), qui ne compare que les individus dont les tailles se recouvrent, n'était pas fonction du type de lac. Une proportion constante de la masse des femelles ovigères est donc consacrée à la reproduction et l'on note une composante saisonnière fonction de l'abondance des algues communes, ceci dans bon nombre de lacs du Bouclier. Cela porte à croire que la UML relative est plus déterminée par les besoins du cycle vital des cladocères que par la présence de poissons. L'utilisation absolue d'énergie pour la reproduction est cependant réduite dans les lacs à prédation élevée et les auteurs formulent l'hypothèse qu'un remplacement clonal ayant pour cause la prédation sélective quant à la taille faite par les poissons pourrait expliquer les écarts entre les deux types de lacs.

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Body size is reduced by the action of size-selective fish predators (Hrbáček 1962; Brooks and Dodson 1965; Wells 1970; Hutchinson 1971; Warshaw 1972; Vanni

1987); however, the direct and indirect effects of these size reductions on the amount and seasonal pattern of lipid allocated to the offspring of parthenogenic cladocerans remain largely unexamined. If fish predation affects maternal lipid investment in zooplankton through reduction in body size, there will likely be important indirect consequences for early survival ability, and hence community structure, since early life stages of zoo-

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plankton are the most susceptible to starvation and are often a "bottleneck" to population growth (Neill 1975; Threlkeld 1976).

The eggs of *Holopedium gibberum*, as with most planktonic cladocerans, are carried in a transparent brood chamber and progress through five developmental stages (Threlkeld 1979). In the first developmental stage the eggs have a single spherical lipid droplet. In freshwater Cladocera these lipids have been characterized as neutral lipids belonging to the triacylglycerol family (Goulden and Henry 1984).

This lipid reserve provides energy to the embryo throughout early development. The fat remaining once the animal is released from the brood chamber is available to sustain the neonate through early periods of food shortage. Neonates of larger species are born with disproportionately more lipid than neonates of small-bodied forms, and this extra energy reserve is correlated with enhanced early survival (Goulden et al. 1982). Goulden and Henry (1984) found that neonates of *Daphnia magna* had relatively more triacylglycerols and survived starvation longer (Tessier et al. 1983) than neonates of the smaller *D. galeata mendotae*.

In this paper we explore this critical aspect of early life, namely the energy investment into eggs of *Holopedium*, by

measuring clutch size and lipid energy reserves allocated to eggs of *Holopedium* among lakes which vary in the intensity of fish predation.

Materials and Methods

We collected zooplankton from 22 lakes in May, an additional 3 lakes in June and August, and an additional 12 lakes in July of 1984 in Algonquin Provincial Park, Ontario (Table 1). The lakes, located on granitic or conglomerate and derived-metamorphic rock typical of the Canadian Shield, are oligotrophic and of circumneutral pH. Secchi disk transparency ranged from 1.3 to 6.6 m (mean 3.9 m), conductivity from 26 to 181 $\mu\text{mho/cm}$ (mean 45 $\mu\text{mho/cm}$), and dissolved organic carbon from 2.2 to 8.2 mg/L (mean 4.34 mg/L) (Table 1).

Holopedium were caught using a large, tapered tow net (1 m² opening with 0.5-mm mesh) (Nero 1982) which was hauled from bottom to surface at the center of the deepest basin of each lake. *Holopedium* were immediately preserved in a cold, sucrose-saturated solution of 5% formaldehyde and stored in a styrofoam chest. *Daphnia* were sampled with a Wisconsin-style tow net with 30- and 45-cm diameter hoops and 2 m of 53- μm

TABLE 1. Selected physicochemical characteristics and indexing criteria (low predation followed by high predation, see text) of the study lakes from Algonquin Park, Ontario.

| Lake | Lat. | Long. | Surface area (ha) | Secchi depth (m) | pH | DOC (mg/L) | Conductivity ($\mu\text{mho/cm}$) | <i>Daphnia</i> mean size (mm) | Mean % <i>Daphnia</i> >1.3 mm |
|-------------------------|---------|---------|-------------------|------------------|------|------------|-------------------------------------|-------------------------------|-------------------------------|
| Big Trout | 45°46'N | 78°37'W | 1333.4 | 3.0 | 6.48 | 4.0 | 32 | 0.86 | 8.0 |
| Billy | 45°38'N | 78°07'W | 94.9 | 5.3 | 6.81 | 4.0 | 40 | 1.07 | 42.1 |
| Brewer | 45°35'N | 78°19'W | 38.8 | 4.0 | 6.37 | 4.6 | 68 | 0.86 | 12.4 |
| Canisbay | 45°34'N | 78°35'W | 161.4 | 4.3 | 6.33 | 3.8 | 34 | 1.01 | 15.3 |
| Costello | 45°35'N | 78°19'W | 7.5 | 3.5 | 6.29 | 4.9 | 54 | 0.86 | 14.9 |
| Happy Isle | 45°45'N | 78°30'W | 536.2 | 6.4 | 6.75 | 2.2 | 35 | 1.11 | 24.8 |
| Heron | 45°25'N | 78°51'W | 26.0 | 4.3 | 6.77 | 2.8 | 57 | 0.81 | 5.2 |
| Kearney | 45°34'N | 78°26'W | 34.6 | 3.6 | 6.28 | 5.2 | 43 | 0.94 | 17.6 |
| Lamuir | 45°50'N | 78°35'W | 740.6 | 4.2 | 6.72 | 3.6 | 38 | 1.11 | 34.0 |
| Major ^a | 45°37'N | 78°06'W | 26.3 | 6.6 | 6.89 | — | 54 | 1.12 | 36.6 |
| Merchant | 45°46'N | 78°31'W | 443.3 | 5.5 | 6.48 | 2.8 | 34 | 0.98 | 17.0 |
| Mykiss | 45°40'N | 78°14'W | 23.9 | 5.4 | 6.60 | 3.8 | 38 | 0.97 | 17.4 |
| Provoking | 45°34'N | 78°30'W | 110.4 | 4.1 | 6.05 | 3.9 | 29 | 0.83 | 7.3 |
| Rock | 45°31'N | 78°24'W | 499.3 | 4.0 | 6.36 | — | 36 | 1.04 | 30.2 |
| Ryan | 45°41'N | 78°06'W | 48.1 | 5.2 | 6.79 | 2.7 | 33 | 0.92 | 15.6 |
| Two Rivers | 45°35'N | 78°29'W | 790.0 | 3.0 | 6.37 | 4.4 | 38 | 1.02 | 27.6 |
| Whitefish ^a | 45°33'N | 78°25'W | 312.2 | 3.9 | 6.52 | 4.3 | 39 | 0.80 | 6.2 |
| Big Crow | 45°49'N | 78°26'W | 439.4 | 3.1 | 6.56 | 5.4 | 40 | 0.75 | 0.5 |
| Booth | 45°39'N | 78°12'W | 538.6 | 4.0 | 6.66 | 3.9 | 40 | 0.78 | 0.9 |
| Bridle | 45°40'N | 78°09'W | 22.0 | 3.5 | 6.72 | 4.9 | 51 | 0.58 | 0.0 |
| Burnt Island | 45°39'N | 78°39'W | 982.6 | 3.9 | 6.28 | 3.3 | 32 | 0.90 | 0.0 |
| Catfish | 45°57'N | 78°33'W | 652.0 | 3.0 | 6.43 | 4.2 | 33 | 0.59 | 0.0 |
| Clarke ^a | 45°32'N | 78°16'W | 26.0 | 2.5 | 6.35 | — | 53 | 0.70 | 1.1 |
| Crotch ^a | 45°39'N | 78°06'W | 268.8 | 4.0 | 6.45 | — | 32 | 0.68 | 0.0 |
| Hogan | 45°52'N | 78°30'W | 1624.4 | 3.3 | 6.78 | 4.3 | 40 | 0.60 | 0.0 |
| Lavielle ^a | 45°52'N | 78°15'W | 2549.6 | 3.9 | 6.90 | — | 57 | 0.79 | 0.0 |
| Opeongo ^a | 45°43'N | 78°21'W | 6018.4 | 6.0 | 6.39 | — | 36 | 0.74 | 1.1 |
| Park | 45°25'N | 78°51'W | 44.9 | 1.8 | 6.29 | 6.1 | 37 | 0.58 | 0.6 |
| Poverty ^a | 45°29'N | 78°13'W | 23.5 | 1.5 | 6.10 | — | 51 | 0.63 | 2.7 |
| Proulx | 45°46'N | 78°24'W | 383.5 | 3.0 | 6.71 | 5.1 | 41 | 0.62 | 0.0 |
| Sasajewan | 45°35'N | 78°31'W | 35.9 | 1.3 | 6.30 | 8.2 | 40 | 0.75 | 2.6 |
| Smoke ^a | 45°31'N | 78°41'W | 598.4 | 4.8 | 6.47 | — | 26 | 0.75 | 1.9 |
| West Smith ^a | 45°34'N | 78°17'W | 18.0 | 3.0 | 6.28 | — | 181 | 0.69 | 0.0 |
| Wright | 45°45'N | 78°17'W | 64.9 | 2.8 | 6.59 | 6.0 | 39 | 0.69 | 0.0 |

^aData collected this study, 1984. Remaining data collected by the Fisheries Acidification Study Program, 1982, the Ontario Ministry of Natural Resources, Research Section, Fisheries Branch, Maple, Ont. L0J 1E0.

mesh, hauled from bottom to surface in the deepest basin. All collections were made between 0800 and 1800.

From each lake, and on each sampling date, we systematically removed, whenever possible, 50 gravid *Holopedium* from a Petri dish etched with a square grid (0.5 cm). If, after removing 50 animals, there were still fewer than 10 of these carrying stage 1 eggs, we continued to examine females until we obtained, where possible, at least 10 females carrying stage 1 eggs. Carapace length of *Holopedium* was measured from the dorsal margin of the carapace immediately above the eye to the posterior tip of the carapace for animals in the contracted (preserved) position (Yan and Mackie 1987).

Minimum length of ovigerous females (OL_{min}) was defined as the mean length of females in the lower 10th length percentile (after Culver 1980) of the size distribution of ovigerous individuals (all egg stages) from a lake on a particular date. This is a conservative measure designed to eliminate the possibility of reliance on a single anomalous female to characterize OL_{min} . What we measured is not strictly the minimum length at first reproduction (L_{min}), but rather, the minimum ovigerous female size typical of a lake at a particular time (OL_{min}). In other words, we cannot determine from our data whether the observed clutch of a particular female is the first, or some later clutch; only the body size at the time the first clutch is released into the brood chamber can be correctly identified as the minimum size at first reproduction.

Clutch size was measured for females carrying stage 1 eggs only, because possible egg loss through abortion might reduce apparent clutch size for older clutches. Stage 1 egg and fat droplet diameters were measured using a compound microscope fitted with an ocular micrometer and converted to volumes assuming a spherical egg and fat droplet shape.

In the eggs of some cladocerans, e.g. *D. magna*, the lipid is dispersed in the yolk as several small droplets instead of a single droplet. We tested this for *Holopedium* by staining stage 1 eggs from females preserved in 5% sugared Formalin, with a triacylglycerol specific stain (Sudan IV red) according to the method of Bjorkman and Shapiro (1986). In all cases the only area in the egg to take up stain was the single large lipid droplet.

As an aid to future studies on maternal energy investment, we estimated sample sizes needed to obtain 95% confidence intervals (C.I.) around the mean egg volume, no wider than 1 and 2 nL, roughly representing a fine and a coarse level of precision. For most lakes (>90%), on a given month, we estimated that 50 and 20 females are sufficient to obtain 95% C.I.'s around the mean egg volume of 1- and 2-nL width, respectively.

Regression analysis, nested analysis of variance, and analysis of covariance were conducted using SAS Institute Inc. (1982) on a VAX 8200 computer. Sample sizes for 95% C.I.'s of a specified width and level of assurance (95%) were obtained as in Zar (1984). We used *t*-tests for differences between monthly means of percent fat in stage 1 eggs on data which were initially transformed using the arcsine transformation in order to satisfy the underlying assumption of linearity (Zar 1984).

Results and Discussion

Natural Variation in Egg Volumes and Maternal Lipid Investment

There was a significant linear relationship between fat droplet volume (stage 1 eggs) and total egg volume when all months and lakes were combined (Fig. 1). This regression (model I)

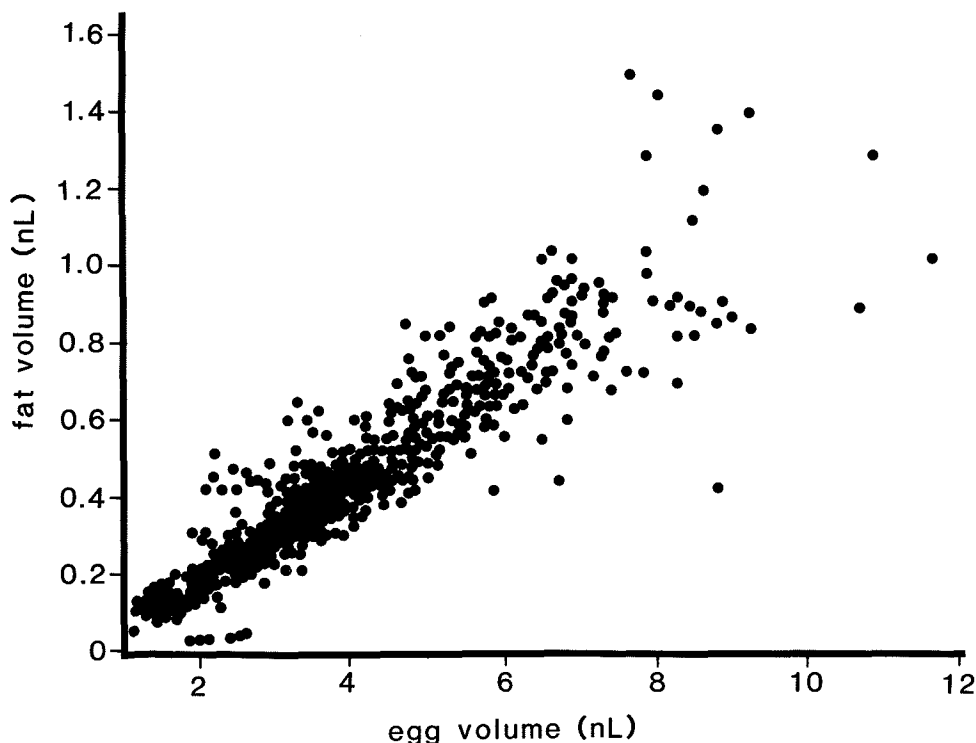


FIG. 1. Mean fat droplet volume versus mean egg volume of parthenogenic *Holopedium* carrying stage 1 eggs for all months and lakes combined. Each point is the mean fat volume and mean egg volume of a female. The regression equation is given by $Y = 0.126X - 0.0523$ ($n = 852$, $r^2 = 0.85$). The 95% C.I. for the slope is 0.126 ± 0.004 .

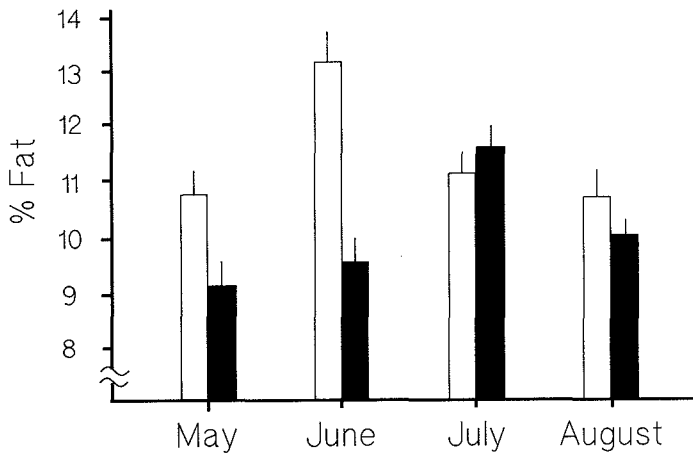


FIG. 2. Seasonal distribution of the relative proportion of fat (percent of total egg volume that is fat volume) in stage 1 eggs of *Holopedium* in low-predation (open bars) and high-predation (solid bars) lakes. Vertical lines above each bar are 95% C.I.'s

accounts for most of the variation in fat volume ($r^2 = 85\%$), and the standard error of the regression is small (0.90), indicating that egg volume is a relatively good predictor of the fat volume in the eggs of this species.

Stage 1 eggs from low-predation lakes contained relatively more fat, on average, than eggs from high predation lakes in May, June, and August (t -test, $p < 0.001$) (Fig. 2). In July, however, the reverse is true ($p < 0.05$) (Fig. 2). We did not

determine the exact magnitude of advantage that would accrue to an egg which possesses a relatively larger amount of fat; however, Goulden et al. (1987) have shown that eggs which have increased relative energy reserves (different *Daphnia* species) are able to resist starvation for longer periods. Also, neonates born from eggs with relatively more fat may benefit from increased buoyancy, since these neutral lipids float in water. Typically, absolute egg and fat volumes were larger in the low-predation lakes (Table 2).

In this paper we use the term "maternal lipid investment" (MLI) to describe the lipid energy reserves transferred from the mature female to the eggs at the time that they are deposited in the brood chamber. We defined MLI for a given female as the product of the average fat volume of the stage 1 eggs in her clutch and clutch size. Because *Holopedium* provides no parental care for its offspring beyond the egg stage, maternal investment in this species is represented largely by the energy content of its eggs (MLI) and the protection the brood chamber affords the developing embryo. We realize that by calculating means for MLI for each month across lakes that we are amalgamating energy investments from different individuals (perhaps different clones) which may differ in important attributes such as size, egg development times, or feeding success both within and between lakes. More work needs to be done under carefully controlled laboratory conditions, or in in situ enclosures, with respect to egg development times and survivorship of neonates of different clones of *Holopedium* in relation to MLI, both with and without fish. On the other hand, multilake surveys such as the one presented here may impart a realism unhampered by problems such as enclosure effects or artificial conditions inherent in laboratory culture studies.

In surveys of MLI in *Holopedium*, and possibly other Cladocera, most effort should be directed at sampling more lakes rather than collecting more females within a lake because although lake of origin and female within lake significantly

TABLE 2. Comparison of fat volumes, egg volumes, clutch size, and biomass-weighted MLI in low- and high-predation lakes. Sample size is the same for fat and egg volume and clutch. Significance was determined using the t -test (Zar 1984).

| | May | | June | | July | | August | |
|----------------------|-------------|------|-------------|------|-------------|------|-------------|------|
| | Low | High | Low | High | Low | High | Low | High |
| <i>n</i> | 114 | 69 | 106 | 110 | 138 | 128 | 116 | 71 |
| Fat volume (nL) | | | | | | | | |
| Mean | 0.46 | 0.13 | 0.63 | 0.29 | 0.53 | 0.49 | 0.44 | 0.33 |
| 95% C.I. | 0.05 | 0.01 | 0.05 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Prob. | $p < 0.001$ | | $p < 0.001$ | | $p > 0.05$ | | $p < 0.001$ | |
| Egg volume (nL) | | | | | | | | |
| Mean | 4.05 | 1.49 | 4.89 | 2.86 | 4.69 | 4.20 | 4.03 | 3.27 |
| 95% C.I. | 0.31 | 0.06 | 0.35 | 0.22 | 0.24 | 0.28 | 0.22 | 0.24 |
| Prob. | $p < 0.001$ | | $p < 0.001$ | | $p < 0.01$ | | $p < 0.001$ | |
| Clutch size | | | | | | | | |
| Mean | 8.8 | 6.32 | 3.35 | 3.27 | 3.18 | 1.89 | 3.40 | 2.13 |
| 95% C.I. | 1.19 | 0.72 | 0.64 | 0.52 | 0.37 | 0.20 | 0.35 | 0.18 |
| Prob. | $p < 0.005$ | | $p > 0.05$ | | $p < 0.001$ | | $p < 0.001$ | |
| <i>n</i> | 53 | 34 | 35 | 72 | 79 | 48 | 78 | 47 |
| Biomass-weighted MLI | | | | | | | | |
| Mean | 0.21 | 0.21 | 0.09 | 0.08 | 0.04 | 0.04 | 0.06 | 0.06 |
| 95% C.I. | 0.03 | 0.02 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Prob. | $p > 0.05$ | | $p > 0.05$ | | $p > 0.05$ | | $p > 0.05$ | |

TABLE 3. Nested analysis of variance on egg volumes for each of 4 mo in 1984. * $p < 0.001$.

| Source of variation | May | | June | | July | | August | |
|---------------------|---------|-------|---------|-------|---------|-------|---------|-------|
| | % total | F | % total | F | % total | F | % total | F |
| Among all females | 97.5 | | 98.7 | | 97.2 | | 96.2 | |
| Lakes | 79.5 | 42.2* | 55.5 | 12.2* | 82.4 | 52.4* | 63.6 | 19.0* |
| Females in lake | 18.0 | 55.5* | 43.2 | 83.2* | 14.8 | 8.7* | 32.6 | 18.9* |
| Error | 2.5 | | 1.3 | | 2.8 | | 3.8 | |

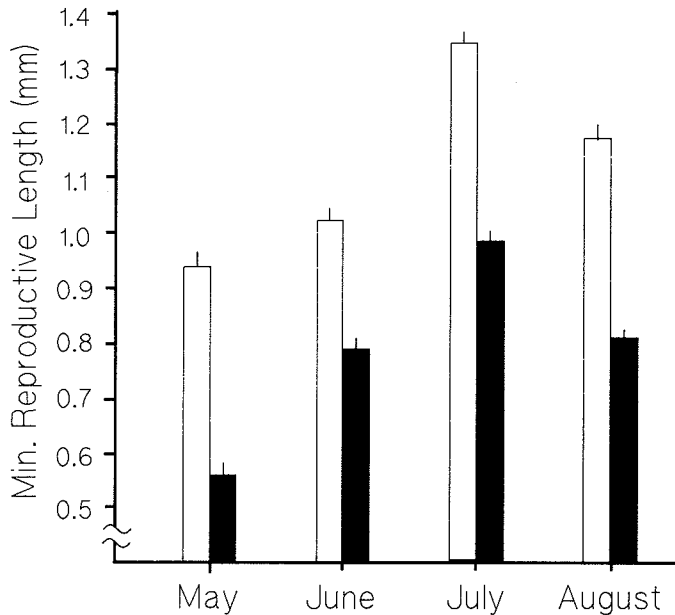


FIG. 3. Seasonal pattern of minimum reproductive length of *Holopedium* in low-predation (open bars) and high-predation (solid bars) lakes. Vertical lines above each bar are 95% C.I.'s. $p < 0.001$ for each comparison.

affected egg volume, lake of origin accounted for greater than 50% of the overall variation (Table 3). Also, less than 4% of the total variation in egg volume is attributable to variation among eggs within a brood pouch (Table 3). Thus, the product of a single egg's fat drop volume and clutch size of its mother should suffice to characterize the extent of a female's MLI, regardless of clutch size.

Daphnia as an Index of Predation Risk

Adult and larval fish planktivores are usually size-selective visual feeders, and thus manifest their presence by a reduction in frequency of large-bodied forms and especially slower moving conspicuous cladocerans such as *Daphnia* (Hrbáček 1962; Brooks and Dodson 1965). Despite widespread support (Galbraith 1967; Wells 1970; Hutchinson 1971; Warshaw 1972; Werner and Hall 1974; Vanni 1987), these empirical observations are rarely used to predict effects on particular zooplankton species when standing stocks of adult planktivores are unknown. In addition, larval fish biomass is often ignored and correlations between adult fish biomass and the biomass of their larvae are not always viable because cohort strength of larval fish in any particular year may be influenced by catastrophic wind, current, and/or temperature episodes (Clady 1976).

Galbraith (1967 and 1975) and Mills and Schiavone (1982) used the relative frequency of large Cladocera (principally *Daphnia* species >1.3 mm) as an index of piscivore quality

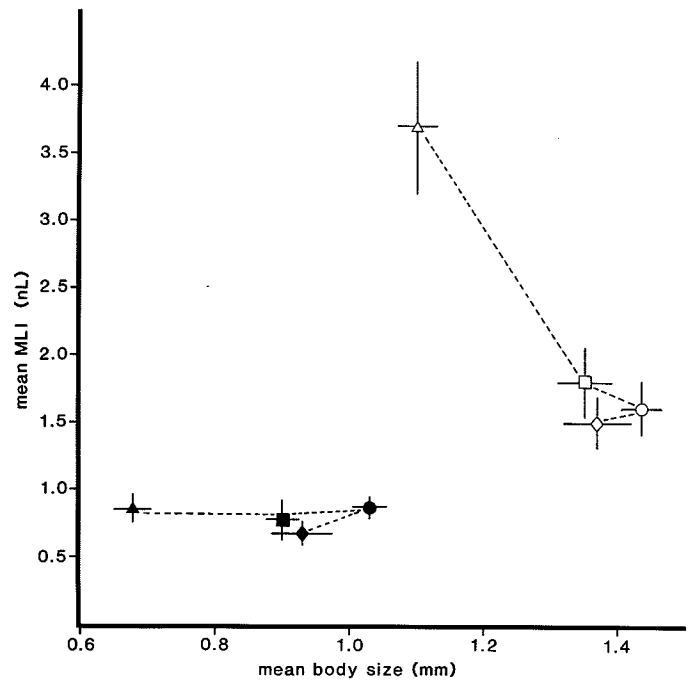


FIG. 4. Mean MLI and body length of ovigerous females in May (triangles), June (squares), July (circles), and August (diamonds). Closed and open symbols represent the high- and low-predation lakes, respectively. The broken lines connect the lakes in time. Solid vertical and horizontal bars are the 95% C.I.'s for mean MLI and mean body length, respectively. Sample sizes are 114, 106, 138, and 116 and 69, 110, 128, and 71 for the low- and high-predation lakes, respectively, from May to August.

and as a guide to management policy. Similarly, we used the relative frequency of large-bodied *Daphnia* in each lake to classify that lake as having a *Daphnia* size distribution characteristic of a lake with a relatively high or low risk from fish (hereafter referred to as high and low predation) (Table 1). We designated lakes in which more than 5% of the *Daphnia* exceeded 1.3 mm as "low-risk" communities and those in which fewer than 5% of the *Daphnia* were greater than 1.3 mm as "high-risk" communities (Table 1). Our use of 1.3-mm *Daphnia* size is somewhat arbitrary; however, essentially similar results were obtained when 1.1- or 1.2-mm sizes were used as a cutoff. Also, yellow perch (*Perca flavescens*), lake trout (*Salvelinus namaycush*), and brook trout (*Salvelinus fontinalis*), which are common planktivores in the Algonquin Park region, have demonstrated preferences (by July for young-of-the-year fish) for mid-sized *Daphnia* of approximately 1.3-mm body length or greater (perch: Galbraith 1967; Wong and Ward 1972; Hanson and Wahl 1981; Mills et al. 1984; trout: Galbraith 1967; Northcote and Clarotto 1975; Kitchell and Kitchell 1980; Konkle and Sprules 1986). We used 5%, a conservative measure in parallel with statistical convention, because even in lakes with

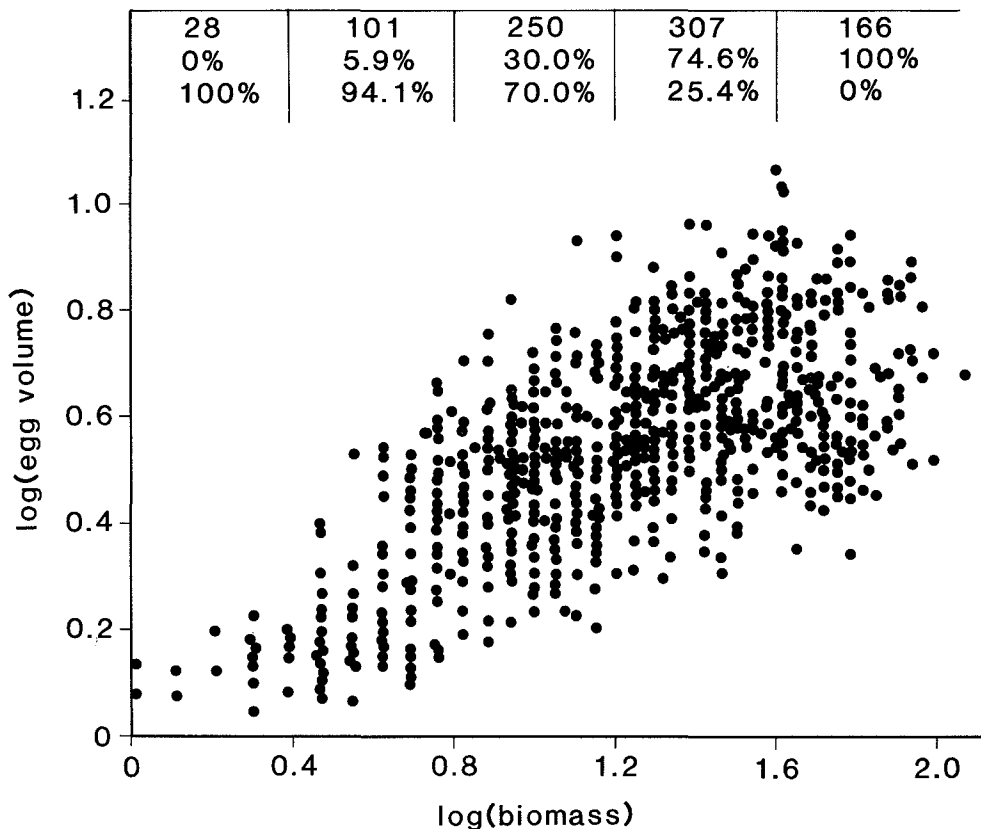


FIG. 5. Relationship between female size (gravid with stage 1 eggs) and egg size in *Holopedium* for all months pooled. The upper, middle, and lower numbers are the sample size and relative number of females from the low- and high-predation lakes, respectively, that fall within each equal logarithmic biomass interval. The model 1 regression ($n = 852$) is given by $Y = 0.3389 X + 0.1326, \pm 0.024$ (95% C.I. for coefficient). $r^2 = 0.47$.

high levels of planktivory there can be a small percentage of large *Daphnia* (Wells 1970; Evans and Jude 1986) that may have either escaped predation or recently immigrated. Although this index might be affected by the relative contribution of juveniles, for example if we had sampled immediately following a reproductive peak when large numbers of neonates could skew the size distribution, the fact that we reported a lake as high predation, only if less than 5% of the females were greater than 1.3 mm in any month (May to August), reduces the effect of this bias.

Predation Risk and Maternal Lipid Investment in *Holopedium*

Both Langford and Martin (1941) and Tessier (1986a) showed that *Holopedium* is vulnerable to predation by yellow perch, principally in July. Biomass of *Holopedium* was sharply reduced in the high-predation lakes in July, coincident with the increase in mouth gape (Arts and Evans 1987) and positive selection for *Holopedium* characteristic of the perch at this time (Arts and Sprules 1988). Perch occur in 34 of the 35 study lakes, are pelagic from June to early August, and have high fecundities (mean of 23 000 eggs/nest, range of 2000–90 000; Scott and Crossman 1973). Thus, it is likely that yellow perch have a

significant impact on *Holopedium* in these lakes, although in other lake systems the presence of different fish predators such as roach (*Leuciscus rutilus*) and Eurasian perch (*Perca fluviatilis*) (Stenson 1973), whitefish (Coregonidae) (O'Brien 1975), and golden shiner (*Notemigonus crysoleucas*) (Tessier 1986b) also reduced mean size of *Holopedium*.

It has been proposed that fish predation can reduce size at first reproduction (L_{min}) of some zooplankton (Dodson 1974; Culver 1980; Vanni 1987). The OL_{min} of *Holopedium* is significantly reduced in the high- versus low-predation lakes in any pair of months (t -test, $p < 0.0001$) (Fig. 3). This observation lends support to the hypothesis that fish predation acts to selectively eliminate large-bodied clones resulting in populations with reduced OL_{min} 's. Culver (1980) proposed that temperature and not predation was the proximate cause for the seasonal reduction in L_{min} . However, the high- and low-predation lakes occur in the same geographic area and are represented by similar numbers and size ranges of lakes (Table 1), so that summer epilimnetic temperatures are similar for each group (M.T. Arts, unpubl. data).

Mean body lengths of *Holopedium* and mean MLI were both significantly different between high- and low-predation risk lakes (t -test, $p < 0.001$ between any pair of months) (Fig. 4).

Females in high-predation lakes were smaller and allocated less energy to the average clutch than did females from low-predation lakes (Fig. 4). The seasonal pattern of MLI differed markedly between the two lake types (Fig. 4). In high-predation lakes, mean MLI was depressed and remained relatively constant throughout the season at roughly 1.0 nL/female. In contrast, MLI was initially elevated in the low-predation lakes in May, but dropped sharply from 4.0 nL/female to between 1.4 and 1.8 nL/female in the June to August period (still significantly greater than the highest mean investment in the high-predation lakes). Thus, the reduction in MLI proved to be a combined effect of absolute fat volume and clutch size, since both were reduced (although not significantly for clutch size in June and fat volume in July) in the high-predation lakes (Table 3).

Although absolute MLI differed between the two lake types between any given month (Fig. 4) the relative amount of fat (nanolitres of fat per microgram wet weight of gravid female) did not differ when the data set was constrained to compare only females in a range of overlapping body lengths in a given month (Table 3). The seasonal pattern of relative per capita MLI followed the same trends between the two lake types. In both lake types the relative MLI was elevated at roughly 0.21 nL fat/ μg tissue in May and then dropping abruptly to 0.09 and 0.04 nL fat/ μg tissue in June and July, respectively. This was followed by a slight, but significant, increase in August to 0.06 nL fat/ μg tissue. The similarity in these trends between the high- and low-predation lakes suggests that the basic dynamics of energy acquisition (relative MLI) are not substantially influenced by the presence of predators at a higher trophic level but rather may be a reflection of seasonal patterns in the underlying algal resource. Thus, the differences in the seasonal pattern of absolute MLI (Fig. 4) were largely the result of differences in body size.

Implications for Individuals

There are three important costs associated with being born to a smaller-bodied adult. First, the ability of zooplankton to allocate vital energy reserves to their offspring depends in part on the size range of ingestible particles and filtering rate which in turn depends on body size (Burns 1968 and 1969; Hall et al. 1976). Goulden et al. (1987), for example, found that females of large and small *Daphnia* species (interspecific) allocated fat to their eggs in proportion to their body size. In addition, respiration rates for small *Daphnia* embryos are relatively higher throughout development than for large *Daphnia* embryos, with the result that smaller neonates are born with relatively less fat and presumably reduced ability to withstand starvation (Goulden et al. 1987). We find, however, that large eggs of *Holopedium* (intraspecific), which are positively associated with large female size typical of the low-predation lakes (Fig. 5), typically have relatively more fat than small eggs (Fig. 2). Thus, in addition to the higher respiratory costs incurred by smaller developing embryos (Goulden et al. 1987), small eggs of *Holopedium* had relatively less fat than large eggs at the outset of development (Fig. 1 and 2). This suggests that in the high-predator lakes, *Holopedium* neonates would be less resistant to starvation. Starvation is likely to be increasingly important late in summer as the pulse of algal biomass generated by nutrients released during spring turnover decreases.

Second, invertebrate predators are constrained by the size of the feeding basket formed by their mouthparts to feed on smaller

zooplankton (Kerfoot 1977; Wong 1981). *Holopedium* is susceptible to predation by the invertebrate predator *Chaoborus*, particularly by later instars (Fedorenko 1975). Lynch (1980), in defining optimal body size, noted that ecological fitness (E_x) would be greatest at that body size (x) where the product of survival probability (P_x) and feeding efficiency (F_x) for an individual is maximized. In the high-predation lakes, therefore, small size at birth implies increased risk to invertebrate predators, in particular *Chaoborus*, because time required to grow to optimal body size is likely increased. Although *Chaoborus americanus* shows a strong negative association with fish, other species such as *C. flavicans* and *C. punctipennis* which are commonly found in both lake types (M.T. Arts, unpubl. data) are more successful at avoiding predation by fish through behavioral avoidance mechanisms such as diel vertical migration (Von Ende 1979).

The small size (stage 1 females only) at the beginning of spring in the high-predation lakes (Fig. 4), before predation by perch is intense, suggests a carry-over effect from the previous season (smaller adults in the fall presumably producing smaller overwintering eggs which hatch into small-bodied progeny) and further reinforces the hypothesis of clonal replacement. Although we did not measure the sizes of resting eggs and the resulting sizes of neonates of these resting eggs relative to the sizes of their mothers, it is possible that individuals hatching from resting eggs deposited by the larger-bodied *Holopedium* of the low-predation lakes would be larger. Larger neonates would be more effective at utilizing the algal bloom initiated by spring turnover because they would reach the higher assimilation and ingestion rates associated with larger body size more quickly (for example see Lynch 1986). The suggestion that larger hatching size from resting eggs results in larger juveniles and adults with higher feeding rates is supported because mean body size and MLI of gravid females in May in the low-predation lakes are significantly higher than mean body size and MLI of gravid females from the high-predation lakes (Fig. 4). This occurred even though juveniles were first observed at about the same time in both lake types after ice-off when lakes of similar size were compared.

Implications for Populations

Havel (1987) showed the existence of different clones of *H. gibberum* in a study of 25 populations in Michigan and Wisconsin. We suggest that small-bodied *Holopedium* clones may replace large-bodied morphs due to size-selective fish predation. The fact that OL_{\min} is reduced in the high- compared with the low-predation lakes suggests selection, over many generations, for smaller-sized females or clones. Lynch (1986) suggested that size at maturity has a strong regulatory influence on *Daphnia* life histories. All four *Daphnia* species in that study reached the species-specific L_{\min} at first reproduction at nearly the same time and, having reached this size, allocated most of their net carbon intake (roughly 90%) into reproduction. However, for each species of *Daphnia*, ingestion and assimilation rates and absolute clutch size each showed a positive increase with body weight (Lynch 1986). Thus, it seems likely that individuals of the same species attaining L_{\min} at relatively greater sizes should be able to allocate more energy (absolute) into growth and specifically reproduction (Lynch 1986). Although we did not calculate energy budgets for *Holopedium*, Lynch's (1986) findings raise the interesting possibility that the reduced minimum size of ovigerous *Holopedium*, through selection for

small-bodied clones in high- compared with low-predation lakes, may result in reduced energy allocation to reproduction at the individual level. The reduced MLI levels of individuals (Fig. 4) in the high- versus low-predation lakes support this.

Conclusions

Recently, there has been a proliferation of research on indirect effects of predators on their prey (Sih 1987). Chemically mediated morphological changes have been shown for invertebrate prey in the presence of their predators (Grant and Bayly 1981; Krueger and Dodson 1981; Stemberger and Gilbert 1984; Havel 1985). Complex behavioral interactions can exist between predators in two or more trophic levels and their common prey (Werner et al. 1983; Sih 1987). Predators, by their presence, may also disrupt the feeding of their prey (Folt and Goldman 1981). Here, we present another mechanism whereby predators can affect the growth and reproduction of their prey. *Holopedium* in high-predation lakes produced smaller eggs with relatively less fat, suggesting that starvation resistance of neonates is compromised in the presence of fish predators. Small body size (lower assimilation and ingestion rates) in the high-predation lakes was presumably carried over via resting eggs through the winter, resulting in a per capita MLI in the high-predation lakes that was 4 times lower than in low-predation lakes in May. In addition, the MLI pattern in the low-predation lakes remained about twice as high as that of the high-predation lakes for the remainder of the season. We hypothesize that in addition to direct consumption of prey, size-selective fish predation may result in subtle indirect effects on absolute maternal energy investment, and thus fitness, perhaps mediated through clonal replacement. Lake type had little effect on relative MLI (energy reserves per microgram female weight), suggesting that seasonal phytoplankton abundance rather than fish limits the relative capacity of *Holopedium* to allocate energy into reproduction.

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