



Influence of sex, body size, and reproduction on overwinter lipid depletion in brook trout

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Expressed as percentages of total fresh body weight, lipids of brook trout *Salvelinus fontinalis* declined between October and April: reproductive males from 2.89 to 1.22%, reproductive females from 3.19 to 1.84%, and non-reproductive males and females from 2.75 to 2.08%. The absolute and proportional overwinter reduction in lipids among reproductive trout was more than twice that of non-reproductive trout, with reproductive males losing significantly more lipids than reproductive females. Larger reproductive individuals lost more lipids during winter, relative to body size, than smaller individuals, although such an effect was not evident among non-reproductive trout. The average overwinter reduction in lipids for reproductive males (58%), females (42%), and non-reproductive trout (24%) was negatively associated with mark-recapture estimates of overwinter survival probabilities of 0.27, 0.36, and 0.58, respectively, providing support for the hypothesis that energy is allocated to reproduction to the detriment of post-reproductive survival. Our emergent hypothesis that reproductive costs differ between sexes, and the life history consequences thereof, merit further study.

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Key words: brook trout; reproductive cost; lipids; sex; overwinter mortality.

INTRODUCTION

Age at maturity reflects an evolutionary compromise between the benefits and costs to fitness of reproducing comparatively early or late in life. Benefits associated with early maturity include increased probability of surviving to reproduce and reduced generation time (Roff, 1992). Early reproduction can, however, effect reduced survival and fecundity at later ages. Such a cost of reproduction, i.e., a trade-off between present reproductive effort and future age-specific reproductive success (Williams, 1966; Gadgil & Bossert, 1970; Bell, 1980), can be generated by energetic losses (red deer, *Cervus elphaus* L.; Clutton-Brock *et al.*, 1982), ecological risks (increased predation experienced by the frog *Physalaemus pustulosus* Fitzinger; Tuttle & Ryan, 1981), and genetic constraints (antagonistic pleiotropy; Rose, 1991).

In fishes, loss of energy concomitant with gonad depletion and pre- and post-reproductive behaviour is associated with reductions in body mass (Rijnsdorp & Ibelings, 1989; Dufresne *et al.*, 1990) and growth rate (Roff, 1983; Hutchings, 1993). These changes in mass and growth can probably be attributed to the initial allocation and subsequent loss of lipids utilized for reproduction (Rijnsdorp & Ibelings, 1989; Jonsson *et al.*, 1991; Griffiths & Kirkwood, 1995),

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notwithstanding the effects of foregone feeding opportunities (e.g., one cost of providing parental care; Smith & Wootton, 1995). Any influence of decreased lipids on post-reproductive mortality would be expected to be strongest among fishes for whom reproduction immediately precedes an energetically-stressful period, e.g., winter. Such an association has been hypothesized to exist in Newfoundland populations of the autumn-spawning brook trout *Salvelinus fontinalis* Mitchell, for which reproduction appears to exact a survival cost (Hutchings, 1994). However, to test this hypothesis, one requires data on both survival and lipid utilization for reproductive and non-reproductive individuals. To our knowledge, these data are not available for any population of fish.

The primary purpose of our study was to quantify the effects of body size, sex, and reproduction on changes in lipid content during winter in a natural, unexploited population. To do so, the lipid composition of gonadal and non-gonadal (hereafter, somatic) tissue was measured for brook trout sampled in autumn, within days of spawning, and immediately after winter.

MATERIALS AND METHODS

Brook trout were sampled randomly by electrofisher from a 50-m long section of Watern Cove River, a comparatively small (mean width and depth of 4.80 and 0.17 m, respectively), 6-km long river on Cape Race, south-eastern Newfoundland, Canada, on 2 October 1995 and 11 April 1996. On average, female trout in this population reproduce initially at 3.2 years of age, at a length of 12.7 cm, and produce 65 4.2 mm-diameter eggs (Hutchings, 1993, 1996). Anaesthetized fish were frozen at -4°C shortly after capture, a temperature at which they were maintained until lipid extraction.

In the laboratory, fork length was measured to the nearest mm and weights to the nearest 0.0001 g. Sex and reproductive state were determined by visual inspection of the gonads. Reproductive and non-reproductive females were identified by the presence of large (>3 mm diameter), orange eggs, or by small (<1 mm diameter), white primary oocytes, respectively. Reproductive males were identified by the presence of large, white gonads; gonads of non-reproductive males were distinguished by thin, narrow, clear bands of tissue.

Lipids were quantified separately for somatic tissue and gonads, following the chloroform/methanol extraction method described by Folch et al. (1957). Lipid content of somatic tissue was estimated as the mean of duplicate aliquots of homogenized tissue samples. Gonadal lipid content, measured for ripe gonads only, and the somatic lipid content of some small fish (<5 g) were measured from single extractions per sample because of small tissue volume.

Our estimates of lipid reductions between October and April may under-represent the lipid reductions experienced by brook trout in Watern Cove River. Firstly, we cannot discount the possibility that some growth, associated with an increase in lipids, occurred during winter. Secondly, the overwinter mortality of individuals that experienced particularly high losses of lipids between October and April may have been greater than the mortality of trout that lost comparatively little lipid.

RESULTS

ASSOCIATION BETWEEN BODY SIZE AND LIPID CONTENT

The autumn and late-winter collections yielded similar sample sizes of reproductive and non-reproductive trout ($n=76$ and 74 for October and April, respectively; Fig. 1). Among reproductive individuals, the average length of males (11.7 ± 0.3 cm s.e., $n=28$) and females (11.6 ± 0.3 cm, $n=19$) did not differ

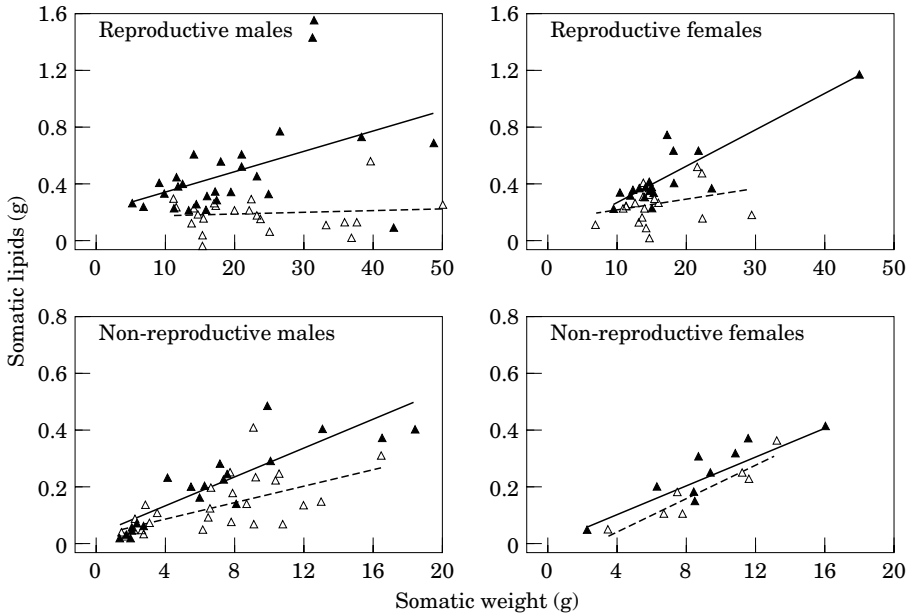


FIG. 1. Bivariate associations between somatic lipids and somatic body weight (i.e. excluding gonads), and associated linear regressions, for reproductive and non-reproductive brook trout from Watern Cove River, Newfoundland, in October 1995 (\blacktriangle) and April 1996 (\triangle).

significantly in autumn ($F_{1,47}=0.01$, $P=0.92$), although males (12.8 ± 0.4 cm, $n=22$) were marginally longer than females (11.7 ± 0.2 cm, $n=19$) in late winter ($F_{1,41}=5.88$, $P=0.02$). Among non-reproductive individuals, there were no significant differences in average length of males (7.9 ± 0.4 cm, $n=20$) and females (9.1 ± 0.5 cm, $n=9$) in autumn ($F_{1,29}=2.43$, $P=0.13$) or in late winter (males: 8.7 ± 0.4 cm, $n=26$; females: 8.9 ± 0.7 cm, $n=7$; $F_{1,32}=0.06$, $P=0.82$).

Among reproductive individuals, the absolute amount of lipids in the soma was positively associated with somatic body weight in autumn but not in late winter (Fig. 1, Table I). Among non-reproductive individuals sampled in October and in April, somatic lipids increased significantly with somatic weight (Fig. 1, Table I).

CORRELATES OF SEASONAL CHANGES IN LIPID CONTENT

Somatic lipids declined during winter, irrespective of sex or reproductive state. Among reproductive males, somatic lipids declined from an average of 0.48 ± 0.06 g s.e. in early autumn to 0.19 ± 0.03 g in early April ($F_{1,48}=15.33$, $P<0.001$); among reproductive females, somatic lipids declined from 0.44 ± 0.05 g in autumn to 0.27 ± 0.03 g in late winter ($F_{1,36}=9.22$, $P=0.004$). Data for non-reproductive males and females were pooled, given their lack of differences in body size (noted previously) and lipid content in October ($F_{1,27}=1.06$, $P=0.31$) and in April ($F_{1,31}=1.50$, $P=0.23$). Between October and April, somatic lipids of non-reproductive fish declined on average from 0.21 ± 0.02 g in early October to 0.15 ± 0.02 g in early April.

TABLE I. Model parameters for linear regressions between somatic lipids (g) and somatic body weight (g) for brook trout sampled from Watern Cove River, Newfoundland, in October, 1995, and April, 1996

Sex	Reproductive state	Month	Slope	Intercept	<i>r</i>	<i>P</i>
Male	Reproductive	October	0.014	0.21	0.41	0.017
		April	0.001	0.17	0.11	0.685
	Non-reproductive	October	0.025	0.03	0.86	<0.001
		April	0.014	0.03	0.68	<0.001
Female	Reproductive	October	0.025	0.02	0.85	<0.001
		April	0.007	0.15	0.14	0.269
	Non-reproductive	October	0.028	0	0.89	<0.001
		April	0.029	-0.07	0.92	0.002

Significant seasonal declines in the slopes of linear regressions relating somatic lipids to somatic weight (as determined by ANCOVA) suggest that, independent of sex, larger reproductive individuals lost proportionately more somatic lipids between early October and early April than smaller individuals (Table I, Fig. 1). (The significant seasonal decline in regression slope for reproductive females remains when the datum for the largest female is excluded from the analysis.) The regressions between somatic lipids and somatic weight for non-reproductive males and females did not differ between seasons.

Overwinter reductions in lipid were highest for reproductive males and lowest for non-reproductive trout. For reproductive males, average total lipid content in late winter, expressed as a percentage of total fresh body weight (1.22%), represented a significant 58% reduction ($F_{1,48}=39.90$, $P<0.0001$) when compared with total lipid content immediately prior to spawning (2.89%) (Fig. 2). From an average of 3.19% of total fresh body weight in October, total lipid content of reproductive females declined 42% ($F_{1,36}=69.73$, $P<0.0001$) to an average 1.84% in late winter. By comparison, total lipid content of non-reproductive fish declined only 24% during winter from an average 2.75% in autumn to 2.08% in late winter.

Comparing sexes, neither somatic ($F_{1,45}=0.07$, $P=0.80$) nor total (i.e., gonads and soma) lipid composition ($F_{1,45}=1.22$, $P=0.28$), as percentages of fresh body weight, differed between reproductive males and females in autumn. However, total lipid composition of females in late winter was significantly greater than that of males ($F_{1,40}=12.25$, $P=0.001$). The lipid composition of ripe gonadal tissue also differed between sexes in autumn, testes having a lower lipid content (6.1% of total fresh gonad weight) than ovaries (9.6%) ($F_{1,36}=9.02$, $P=0.005$). On average, in autumn, gonadal lipids comprised 8 and 19%, as percentages of total fresh body weight, of the total lipid content of reproductive males and females, respectively.

Overwinter loss of lipids differed between reproductive and non-reproductive trout (Fig. 2). Comparing non-reproductive individuals to reproductive males, total lipid content did not differ in autumn ($F_{1,54}=0.31$, $P=0.58$) but non-reproductive fish lost significantly less lipids during winter ($F_{1,41}=16.92$,

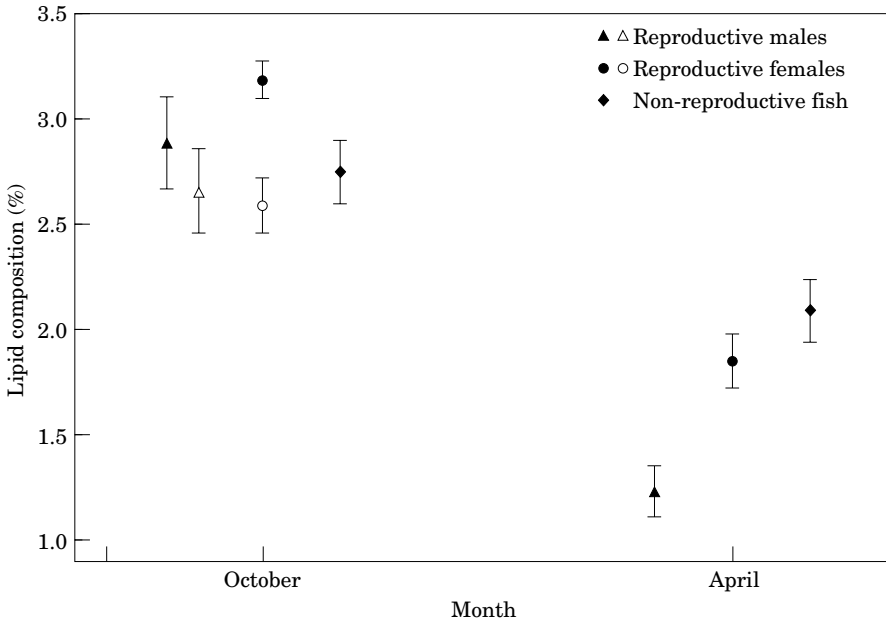


FIG. 2. Somatic (open symbols) and total (closed symbols) lipid content, as percentages of fresh body weight, of reproductive male, reproductive female, and non-reproductive brook trout from Watern Cove River, Newfoundland, in October 1995 and April 1996. Data are means and error bars represent one standard error.

$P < 0.0001$). By contrast, reproductive females possessed significantly more lipids, relative to body weight, than non-reproductive fish in autumn ($F_{1,47} = 4.94$, $P = 0.03$), although total lipid content did not differ between post-reproductive females and non-reproductive trout in late winter ($F_{1,52} = 1.23$, $P = 0.27$). Given the lack of difference between somatic lipids of reproductive females and total lipids of non-reproductive fish in autumn ($F_{1,46} = 0.53$, $P = 0.47$), the higher total lipid content of reproductive females in October can be attributed to the lipids contained in the ovaries.

ASSOCIATION BETWEEN LIPID REDUCTION AND OVERWINTER SURVIVAL

Earlier mark-recapture estimates of overwinter survival, based on autumn collections of more than 700 fish, revealed significant differences among post-reproductive males, post-reproductive females, and non-reproductive trout in Watern Cove River (Hutchings, 1994). A survival cost of reproduction in this population was suggested by an almost twofold reduction in average survival of post-reproductive trout relative to that of non-reproductive trout. Overwinter survival probabilities of 0.27 ± 0.04 s.e., 0.36 ± 0.04 , and 0.58 ± 0.04 for post-reproductive males, post-reproductive females, and non-reproductive trout, respectively, are negatively associated with the average overwinter reduction of lipids documented here for the same categories of fish (Fig. 3).

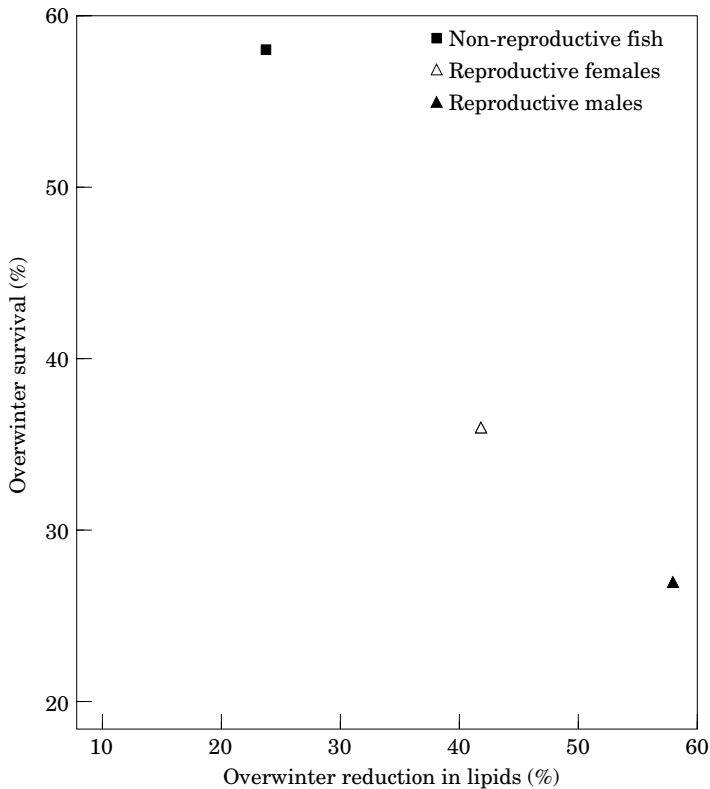


FIG. 3. Negative association between overwinter (October through April) survival and overwinter reduction in total lipids for reproductive and non-reproductive brook trout in Watern Cove River, Newfoundland.

DISCUSSION

Winter can be an energetically stressful period for north-temperate fishes (Cunjak, 1988), as reflected by substantial overwinter declines in lipid content, e.g. brook trout (Cunjak & Power, 1986; present study), Arctic charr *S. alpinus* L., (Dutil, 1986; Jørgensen *et al.*, 1997), Atlantic salmon *Salmo salar* L. (Jonsson *et al.*, 1991), North Sea plaice *Pleuronectes platessa* L. (Rijnsdorp & Ibelings, 1989), roach *Rutilus rutilus* L., and perch *Perca fluviatilis* L. (Griffiths & Kirkwood, 1995). Energy losses that cannot be attributed to the physiological processes and behaviour associated with reproduction, as estimated from overwinter reductions experienced by non-reproductive individuals, can be significant, e.g. a 24% decrease in lipids for brook trout (present study), and 9% and 30% reductions in energy (lipids and protein combined) for North Sea plaice (Rijnsdorp & Ibelings, 1989) and Arctic charr (Dutil, 1986), respectively.

The present study suggests that body size, reproduction, and sex can influence the depletion in lipids experienced by brook trout during winter. For example, we found that larger reproductive individuals lost proportionately more lipids, relative to body size, during winter than smaller reproductive trout, although such an effect of body size was not evident among non-reproductive individuals.

This evidence of increased lipid losses with increasing body size might reflect an age-specific increase in reproductive effort (e.g. gonads as a percentage of body size increase with age in some brook trout populations; Hutchings, 1993).

There is also evidence to suggest that the energetic demands of reproduction can be considerable, particularly if one compares the energetic losses of reproductive individuals with those of non-reproductive individuals during the same time interval. The present study identified an average overwinter loss of lipids in reproductive brook trout (males and females combined), as a percentage of total body weight, more than double that of non-reproductive individuals (Fig. 2). Dutil (1986) noted that post-reproductive Arctic charr in a Canadian population contained 35–46% less energy than non-reproductive charr in spring. In addition, during their winter spawning period (December to April), the energy loss of reproductive North Sea plaice can be three to five times that of non-reproductive plaice (Rijnsdorp & Ibelings, 1989).

The increased loss of energy experienced by reproductive individuals may be responsible for one or more reproductive costs. One such cost would be a reduction in future fecundity effected by a post-reproductive decrease in growth rate. For Watern Cove River brook trout, reproduction is estimated to reduce growth rate by two-thirds (Hutchings, 1993). Perhaps of greater significance is the negative correlation between overwinter survival and overwinter reductions in lipids (Fig. 3). Such an association is consistent with the hypothesis that the energetic demands of reproduction are responsible for increased mortality among reproductive individuals (Gadgil & Bossert, 1970; Bell 1980; Wootton, 1990). Given an absence of other external sources of mortality experienced by this population (e.g. interspecific predation and competition, angling; Hutchings, 1993, 1994), the loss of lipids associated with reproduction may contribute significantly to the survival cost of reproduction experienced by trout in Watern Cove River. A similar link between lipid content and mortality is suggested by the observation that overwinter mortality of Lough Neagh (Northern Ireland) roach appears to be inversely related to fat content (Griffiths & Kirkwood, 1995). However, the negative association between overwinter lipid reductions and overwinter mortality documented here need not reflect a causal relationship. Males, for example, may have lower survival for reasons that are not linked to the depletion of lipids, e.g. the physical consequences of mate competition evident in some salmonids (Hutchings & Myers, 1987).

If the energetic demands of reproduction contribute significantly to post-reproductive mortality, sexual differences in post-reproductive energy losses may produce differences in mortality between sexes. The degree to which energetic losses concomitant with reproduction differ between sexes varies among species. In a northern Norwegian population of Arctic charr, post-reproductive females lost ~80% of their body lipids during winter compared with a 50–55% reduction in post-reproductive males (Jørgensen *et al.*, 1997). Among North Sea plaice, females also experienced a greater energy deficit than males, losing 44% of their energy during spawning as compared with only 27% for males (Rijnsdorp & Ibelings, 1989). Alternatively, some studies suggest that males may incur greater energetic costs. Although total energy loss among Norwegian post-reproductive Atlantic salmon was reported to be similar between the sexes (~52%), Jonsson *et al.* (1991) found the non-gonadal lipid reduction among males (84%) to be

considerably greater than that of females (53%). Such a male bias in post-reproductive lipid reduction was also evident in Watern Cove River brook trout (Fig. 2).

Thus, differential loss of lipids between sexes may produce sex-specific differences in post-reproductive survival. Evidence of such a link is suggested by the overwinter survival probabilities and lipid reductions reported here for male and female brook trout. Although this appears to be the only study for which survival and lipid data are available by sex, there is some evidence to suggest that male-biased sex ratios in Québec (Canada) populations of yellow perch, *Perca flavescens* Mitchell, can be attributed to sexual differences in mortality resulting from sexual differences in lipid content (Newsome & Leduc, 1975).

Reproduction demands energetic capital from mature individuals. The amount undoubtedly varies among species and populations, and within populations over time. The degree to which within-population variation is a function of environmental heterogeneity and adaptive phenotypic plasticity bears examination. Our research on brook trout suggests that the energetic requirements of reproduction exact significant costs to fitness, particularly in the form of reduced post-reproductive survival. The hypothesis proposed herein that sexual differences in the energetic constraints of reproduction may effect sexual differences in post-reproductive survival merits further study.

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