

# Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*)

Peter A. Biro, Ashley E. Morton, John R. Post, and Eric A. Parkinson

**Abstract:** In this study we identify the size-dependent risk of winter starvation mortality as a strong selective pressure on age-0 rainbow trout (*Oncorhynchus mykiss*) that could promote the risk-taking behaviour and allocation of energy to lipids previously observed in young trout cohorts. Age-0 trout subjected to simulated winter starvation conditions gradually depleted lipid reserves to a critical minimum lipid content below which death occurred. Small fish with lower lipid content exhausted lipid reserves earlier, and experienced high mortality rates sooner, than larger fish with greater lipid content. Consequently, winter starvation endurance was dependent upon size-dependent lipid reserves and winter duration. To validate the laboratory findings in the field, we stocked several size classes of hatchery-raised trout with known lipid content at the start of winter into two experimental lakes, and estimated survival and lipid depletion at winter's end. Larger age-0 trout had greater initial lipid reserves than smaller trout. Individuals depleted most of their lipid reserves over the winter, and experienced mortality that ranged from just under 60% for the largest individuals to just over 90% of the smallest individuals. Many survivors had lipid contents near, but none were below, the minimum lipid content determined in the laboratory.

**Résumé :** Nous identifions dans cette étude le risque de mourir de faim pendant l'hiver, un facteur relié à la taille, chez des truites arc-en-ciel (*Oncorhynchus mykiss*) d'âge 0, comme une importante pression sélective qui pourrait favoriser des comportements de prises de risques, de même que l'allocation de l'énergie vers les lipides, ce qui a été observé antérieurement chez des cohortes de jeunes truites. Des truites d'âge 0 soumises à des conditions simulées de manque de nourriture en hiver perdent graduellement leurs réserves de lipides jusqu'à un contenu minimal critique de lipides, sous lequel la mort s'ensuit. Les petits poissons avec de faibles contenus de lipides épuisent leurs réserves de lipides et subissent de forts taux de mortalité plus tôt que les poissons plus grands avec un contenu de lipides plus élevé. En conséquence, la tolérance au manque de nourriture en hiver est reliée aux réserves de lipides qui sont fonction de la taille, ainsi qu'à la durée de l'hiver. Afin de valider ces résultats de laboratoire en nature, nous avons commencé au début de l'hiver des truites de pisciculture de différentes tailles ayant un contenu de lipides connu dans deux lacs expérimentaux et estimé à la fin de l'hiver la survie et la perte de lipides. Les truites d'âge 0 plus grandes ont des réserves initiales de lipides plus importantes que les truites plus petites. Les individus ont épuisé l'essentiel de leurs réserves de lipides au cours de l'hiver et subi une mortalité qui a varié de juste sous 60 % chez les plus grands à juste au-dessus de 90 % chez les plus petits. Plusieurs des survivants avaient des contenus en lipides près du minimum déterminé en laboratoire, mais aucun n'avait de valeur inférieure.

[Traduit par la Rédaction]

## Introduction

Identifying constraints on prey fish behaviour is critical to our mechanistic understanding of factors affecting growth and recruitment of prey fish populations. In temperate environments, fish experience short growing seasons that precede periods of winter resource scarcity, which imposes a time constraint on acquiring energy reserves sufficient to survive winter (Schultz and Conover 1999; Post and Parkinson 2001). This time constraint can affect the trade-off between growth and mortality rates in prey, resulting in an

increase in risk-taking behaviour by individuals striving to accumulate an energy insurance for the winter (Rowe and Ludwig 1991; McNamara and Houston 1994; Johansson and Rowe 1999).

If over-winter mortality is inversely proportional to accumulated energy reserves, then the risk of winter starvation operating within the constraint of a short growing season has the potential to be a strong selective pressure that could promote risk-taking behaviour (e.g., Schindler 1999; Biro et al. 2003b, 2003c) and allocation of energy to lipids in prey fish populations (Schultz and Conover 1999; Post and Parkinson

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**P.A. Biro,<sup>1</sup> A.E. Morton, and J.R. Post.** Division of Ecology, Department of Biological Sciences, University of Calgary, 2500 University Drive Northwest, Calgary, AB T2N 1N4, Canada.

**E.A. Parkinson.** Fisheries Research and Development Section, British Columbia Ministry of Fisheries, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada.

<sup>1</sup>Corresponding author (e-mail: [pbiro@ucalgary.ca](mailto:pbiro@ucalgary.ca)).

2001). Over-winter mortality due to depletion of lipid stores is often cited as a major source of mortality for young fish populations (e.g., Bernard and Fox 1997; Hurst and Conover 1998; Pratt and Fox 2002). Studies generally show that smaller individuals experience higher mortality than larger ones. However, size-dependent depletion of energy reserves and high over-winter mortality of young fish are not universal, and may vary with the species considered, fish density, predator density, and food abundance (e.g., Garvey et al. 1998; Gotceitas et al. 1999; see review in Pratt and Fox 2002). In fact, most of the literature dealing with this issue is focused on Perciformes (e.g., Oliver et al. 1979; Toney and Coble 1980; Post and Evans 1989) and comparatively little has been done with Salmoniformes (but see Gardiner and Geddes 1980; Simpkins et al. 2003). The most frequently invoked mechanism for size-dependent over-winter mortality is size-dependent starvation endurance. Larger fish of the same species not only tend to have greater lipid reserves than smaller individuals at the start of winter (reserves  $\propto$  mass<sup>b</sup>), but also have lower specific metabolic rates than smaller individuals (metabolism  $\propto$  mass<sup>-b</sup>; Shuter and Post 1990; Schultz and Conover 1999; Post and Parkinson 2001). Therefore, smaller individuals should experience higher winter mortality due to a combination of lower energy reserves and higher metabolism, provided they are starving.

Whole-lake experiments have shown that the presence of predatory rainbow trout (*Oncorhynchus mykiss*) elicits refuging behaviour and dramatically reduces growth and survival of age-0 rainbow trout relative to age-0 trout in lakes without them (Landry et al. 1999; Biro et al. 2003b). The high mortality rates of age-0 trout, which occur despite the use of refuges, are thought to result from density (Biro et al. 2003a) and food-dependent risk-taking (Biro et al. 2003b, 2003c) brought on by the need to accumulate lipid stores prior to winter (Post et al. 1999; Post and Parkinson 2001; Biro et al. 2003c). Indeed, larger age-0 trout have relatively more lipid than smaller individuals by autumn, suggesting that strong size- and lipid-dependent over-winter mortality selects for energy allocation to lipids (Post and Parkinson 2001). However, high over-winter mortality and a link to depleted lipid stores remain to be demonstrated for this species in order to identify risk of winter starvation as a major selective pressure on young fish. If there is such a link, this would support the hypothesis that predation and over-winter mortality are the two major selective forces that shape the behaviour and life history of young fish and affect their growth and survival.

In this study we show in laboratory and whole-lake experiments that over-winter mortality of young trout is high, size-dependent, and linked to the depletion of lipid reserves to a critical minimum whole-body lipid concentration. First, we quantify the size-dependent depletion of lipid reserves and mortality of age-0 rainbow trout subjected to simulated winter conditions in an outdoor greenhouse, in the absence of predators. This establishes a link between mortality and lipid depletion, and identifies lipid concentration at death. Next we validate the laboratory results by quantifying the size-dependent over-winter mortality and depletion of lipid reserves of age-0 trout stocked into two natural lakes containing fish predators. We then compare the observed size-

dependent loss of lipids in these two lakes with lipid loss predicted from metabolic allometry, assuming starvation.

## Methods

### Laboratory experiment

We simulated winter conditions in four 450-L aquaria held in an outdoor greenhouse, where we implemented a factorial experiment using two size classes of age-0 rainbow trout and two feeding regimes. Water temperature was maintained at  $3 \pm 1$  °C for the duration of the 100-day experiment. We obtained trout from a local hatchery, raised them under two temperature and feeding regimes to produce two size classes, and clipped the adipose fin of the larger trout to identify them. We stocked each tank with 200 large trout (mean fork length (FL) = 40 mm, mean mass = 0.75 g, standard error (SE) = 0.04 g,  $n = 35$ ) and 165 small trout (mean FL = 31 mm, mean mass = 0.41 g, SE = 0.019 g,  $n = 35$ ). There were no differences among replicate tanks in the initial lengths of large or small trout ( $F < 1$  and  $P > 0.05$  in each case). At this time, a random subsample of 30 large and 30 small fish were frozen for subsequent lipid analysis. Each tank and common filter boxes were equipped with airstones to allow adequate circulation and oxygen. Each tank had a Styrofoam™ cover on the water surface that simulated ice cover but still allowed fish to experience a natural winter photoperiod. Two tanks were randomly allocated to the feeding treatment, in which fish were fed ad libitum daily, and remaining replicate fish cohorts went unfed for the duration of the experiment. Any dead fish were collected each day and frozen for lipid analysis. In addition, we sampled seven large and seven small trout from each replicate every 30 days to monitor changes in lipid concentration of survivors over time.

To obtain sufficient material for lipid analysis, trout were pooled into groups of seven. Thus, lipid concentration of dead fish was represented by a seven-fish aggregate sample collected over 1–7 days, where the midpoint date of the period over which individuals were collected was taken as the date of death for each aggregate sample. Fish were thawed, weighed, measured for length, and dried at 50 °C for 96 h. The dry samples were reweighed and then ground with mortar and pestle. Lipids were extracted using the methanol and chloroform procedure of Folch et al. (1957) and adopted by Post and Parkinson (2001). This method involves measuring 0.3–0.5 g (measured to within  $\pm 0.0001$  g) of finely ground sample into a test tube, adding 16 mL of a 50:50 methanol:chloroform solution, and then heating the mixture to a boil in a water bath set at 61 °C. The mixture is then cooled to room temperature and chloroform added to bring the volume up to 25 mL. This solution is then filtered through a No. 1 Whatman filter paper into a separatory funnel. Next, 10 mL of 0.9% saline solution is added to the funnel to remove impurities from the mixture. This leaves pure lipid in the methanol–chloroform layer that settles to the bottom of the funnel. This bottom layer is drained into a preweighed beaker and evaporated to dryness on a hot plate at 70 °C (Post and Parkinson 2001). The mass of the remaining lipid in the beaker represents the mass of lipid contained in the dry fish sample. We calculated mean lipid-depletion rates for small

and large trout from the difference between individual lipid concentrations at death and the mean lipid concentration for the cohort at the start of the experiment.

### Field experiment

Our two lakes are located within 20 m of one another in south-central British Columbia, Canada (49°50'N–49°56'N, 120°33'W–120°34'W). The lakes lack naturally reproducing fish populations, are 2.0 and 3.2 ha, and are relatively shallow (maximum depths 9–14 m). The littoral habitat in the lakes consists of aquatic macrophytes (primarily *Chara* sp. and *Myriophyllum* sp.) located in small patches, open sediment, gravel, rocks, woody debris, and fallen trees. The lakes are best described as mesotrophic, based upon phosphorus concentrations (Biro et al. 2003c).

Rainbow trout for the field experiment were raised from eggs collected from wild populations in Tunkwa Lake at the Fraser Valley Trout Hatchery (B.C. Fisheries Branch). The trout were sorted into four non-overlapping size classes based on fork length (and uniquely marked with fin clips) that ranged from 50 to 105 mm (for the four size classes: mean FL = 61, 69, 78, and 89 mm; standard deviation (SD) = 0.3, 0.2, 0.2, and 0.5 mm;  $n = 50$  for each). Lakes K1 and K2 each received 400 of each size class on 17 November 2000 after the fish were chilled from 12 °C down to 3 °C during the 4-h transit from the hatchery. Two centimetres of ice were present on the lakes on the day of stocking, indicating they had frozen over recently. After stocking into shallow water, the fish were clearly visible beneath the ice; they appeared to be in good condition, and no losses of fish were observed.

Spring gillnet sampling was used to estimate survival of age-0 trout over the winter. Gillnetting took place over a 5-day period in lakes K1 and K2, beginning on 23 April 2001, when the ice cover had receded at least 10 m from the shoreline; all ice had melted before the last day of netting. Sinking experimental gillnets were set for 5 consecutive nights in all habitats, and comprised graded mesh sizes ranging from 13 to 50 mm (stretched mesh). Gillnet densities were standardized among lakes on an area basis and ranged from 400 to 500 m<sup>2</sup>·ha<sup>-1</sup>·night<sup>-1</sup>, following the identical netting effort and protocol used previously in these lakes (Post et al. 1999; Biro et al. 2003b). Mortality and growth over the winter were estimated by adjusting the total 5-night catch of age-0 trout of a given length by an established size-specific vulnerability function determined from previous mark-recapture experiments in the lakes (for details see Biro et al. 2003b). The gillnets caught trout >45 mm FL, which is well within the minimum size of the stocked trout. We did not use mesh sizes larger than 50 mm, to prevent killing adult trout from the recreational fishery. Consequently, we do not have a direct estimate of potential predator density during the winter. However, lakes K1 and K2 are stocked with yearling trout every spring at 250 and 180 trout·ha<sup>-1</sup>, respectively.

Several hundred captured trout from each lake were frozen for lipid analysis. Random samples, stratified by length, of the frozen age-0 trout were selected from each lake for regression analyses. The stratified samples included the full range of body sizes of fish captured in our gillnets. To infer the extent of over-winter lipid loss in the field experiment,

we compared the observed allometry of lipid loss to the loss of lipids that is predicted from metabolic allometry assuming starving fish. We used the metabolic slope coefficient for rainbow trout (Rand et al. 1993), and our estimates of mean lipid loss rates for our two sizes of trout used in the laboratory experiment, to fit the intercept of the relationship by least-squares. We applied this predicted mass-dependent rate of lipid loss to the autumn lipid concentrations of the hatchery fish, and the observed winter duration, to obtain a predicted loss of lipids over the winter. In addition, we used the observed allometry of autumn lipid concentration, and mean lipid concentration at death, to calculate the proportion of lipids that could be lost before death.

### Statistical analysis

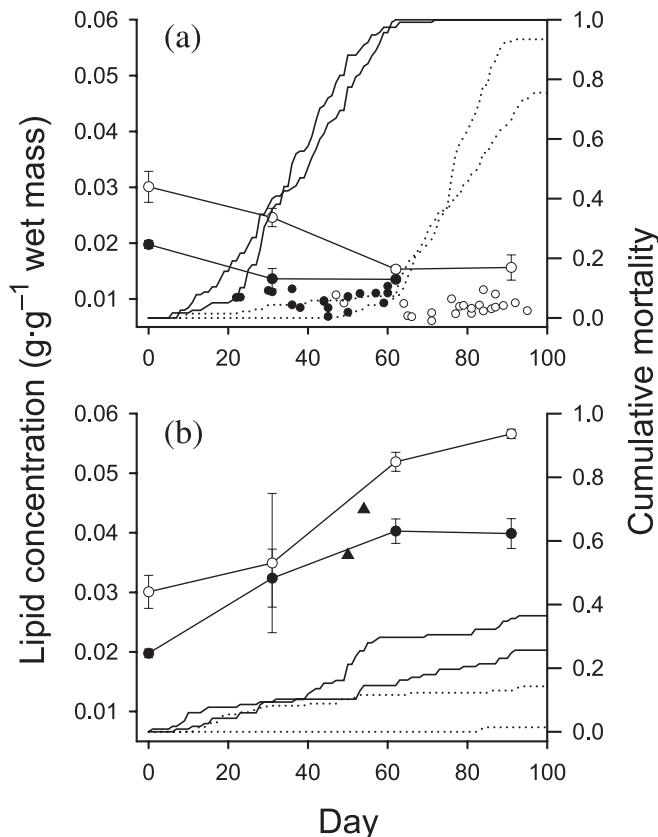
We used maximum-likelihood techniques, rather than less powerful traditional parametric statistics, on mortality data from the two field experiments because each datum (replicate) represents the response in a single lake. Significance in this analysis was determined using Type III likelihood-ratio tests that are asymptotically  $\chi^2$  distributed with  $df = 1$  (Burnham and Anderson 1998). Thus, a likelihood-ratio test with  $P < 0.05$  indicates a statistical model that is significantly more likely than a null model in which the response is constant (i.e., slope estimate not different from zero). All remaining analyses used standard linear regression or analysis of covariance. Age-0 trout mortality data were analyzed assuming normally distributed errors on arcsine square-root transformed data. We arcsine square root transformed all dependent variables that were expressed as a proportion to satisfy normality assumptions, and log<sub>10</sub>-transformed fish length and mass to satisfy linearity assumptions. To ensure statistical independence in the regression analyses of lipid allometries, we used length rather than mass as the predictor variable.

## Results

### Laboratory experiment

Starved age-0 trout lost substantial lipids and experienced high mortality (Fig. 1a). Lipid concentrations at the time of death were similar for small and large trout that were starved (0.0099 and 0.0081 g lipid·g<sup>-1</sup> wet mass, respectively, suggesting a minimum lipid threshold for survival (Fig. 1a). Lipid concentration at death was related to trout size ( $F_{[1,38]} = 9.3$ ,  $P < 0.005$ ) but not to time of death or to the interaction between time and fish size ( $P > 0.5$  in both cases; Fig. 1a). Smaller trout began dying earlier than larger trout, the timing of their death being related to the decline in lipid content of survivors to near the average lipid content at death (Fig. 1a). High mortality rates began later for larger trout because they had relatively more lipid at the outset (Fig. 1a). Clearly, high mortality rates for trout with low lipid stores (at approximately 30 days for small trout and 80 days for large trout) resulted in upwardly biasing lipid estimates of survivors at 60 days for smaller trout and 90 days for larger trout (Fig. 1a). Small starved trout had all died before the end of the experiment, owing to their lower lipid content at the start of winter, and slightly higher minimum lipid threshold (Fig. 1a). In contrast to our expectations, the estimated

**Fig. 1.** Lipid concentrations and cumulative mortality of two size classes of age-0 rainbow trout (*Oncorhynchus mykiss*) experiencing winter conditions in a outdoor greenhouse under starvation (a) and fed to satiation (b). Mean lipid concentrations ( $\pm$  standard error) of survivors over time and mortality trajectories for each replicate tank are shown. Individual data points represent lipid concentration of age-0 trout at the time of their death. Solid lines and symbols represent the small size class; dotted lines and open symbols represent the large size class. The solid triangles in b represent lipid concentration at death for fed fish that had died as a result of fungal infection.



mean rate of lipid depletion was lower for small trout than for large trout (0.000 244 6 and 0.000 296 2 g lipid·g wet mass<sup>-1</sup>·day<sup>-1</sup>, respectively).

By contrast, lipid concentration increased over time when trout were fed ad libitum, and their mortality was low (Fig. 1b). Mortality was attributed to fungal infections in all cases, and the lipid concentration of dead fish was similar to that of survivors. As expected, larger fed trout accumulated more lipid than smaller fed trout (Fig. 1b).

### Field experiment

Over-winter mortality of age-0 trout was high and size-dependent (Fig. 2a). Smaller age-0 trout experienced greater over-winter mortality than did larger trout ( $R^2 = 0.86$ ; likelihood-ratio test:  $\chi^2 = 15.8$ ,  $P < 0.0001$ ). Trout from lake K2 experienced lower mortality than fish from lake K1 (lake effect:  $\chi^2 = 4.9$ ,  $P < 0.03$ ;  $R^2 = 0.92$ , for the combined size and lake effects; Fig. 2a), but there was no interaction effect between fish size and lake origin ( $P > 0.1$ ). The mean mass of survivors was 5.99 g in lake K1 and 7.26 g in lake K2.

These differences in mean mass must to some extent reflect over-winter growth rates given that maximum fish lengths were greater in spring than in autumn (Figs. 2b and 2c), and the mean mass of these cohorts was only 4.4 g in autumn ( $n = 400$ ). Total over-winter mortality of age-0 trout cohorts in lakes K1 and K2 was 73% and 67%, respectively.

At the start of winter, larger age-0 trout had greater lipid mass than did smaller trout ( $F_{[1,28]} = 531$ ,  $P < 0.0001$ ,  $R^2 = 0.95$ ; Fig. 2b). By spring, age-0 trout had significantly depleted lipid stores (season effect:  $F_{[1,84]} = 215$ ,  $P < 0.0001$ ), but there was no interaction between season and fork length ( $F_{[1,84]} = 0.09$ ,  $P > 0.7$ ). Parallel lipid allometries on log-log-transformed data indicate that larger fish actually lost more lipid than did smaller fish, contrary to our expectations (Fig. 2b). Spring samples of age-0 trout from lake K2 had significantly greater lipid mass at a given length than fish from lake K1 (lake effect:  $F_{[1,54]} = 16$ ,  $P < 0.0002$ ) but the slopes did not differ (interaction effect:  $F_{[1,54]} = 0.01$ ,  $P > 0.9$ ). There was greater between-individual variation in lipid stores at a given length for fish captured in the spring than at the start of winter (K1:  $R^2 = 0.87$ ; K2:  $R^2 = 0.79$ ,  $P < 0.0001$  in each case).

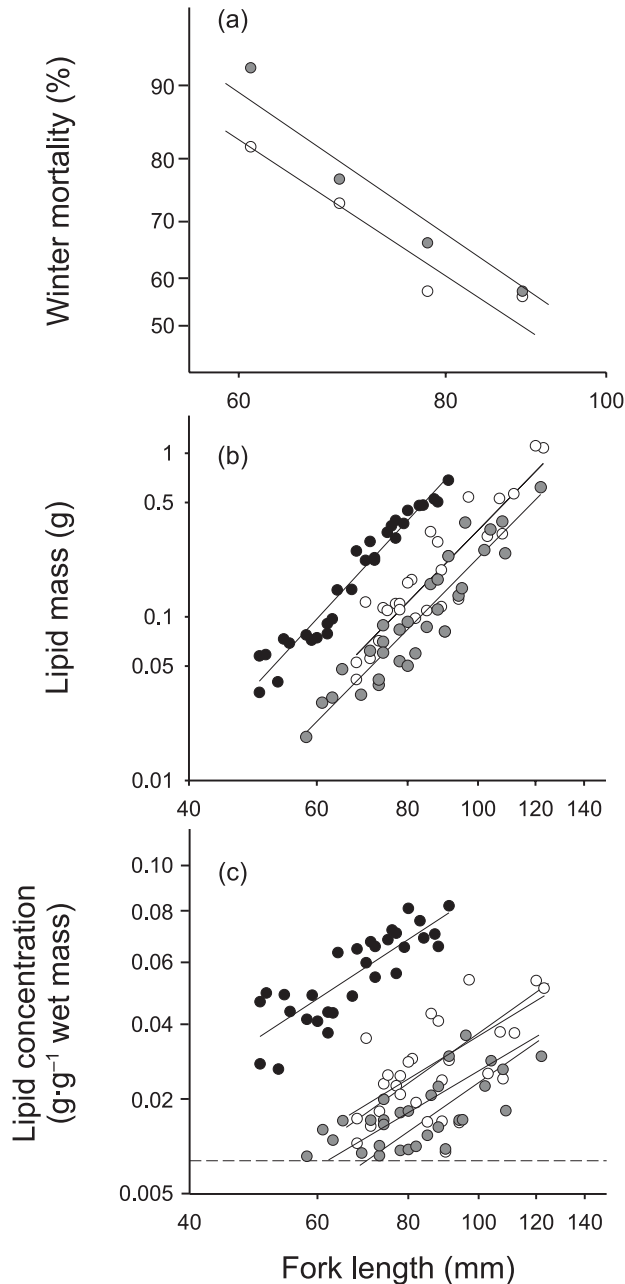
At the start of winter, larger age-0 trout had higher lipid concentrations than did smaller trout ( $F_{[1,28]} = 68$ ,  $P < 0.0001$ ,  $R^2 = 0.71$ ; Fig. 2c). Age-0 trout had significantly lower lipid concentrations at the end of the winter (season effect:  $F_{[1,84]} = 252$ ,  $P < 0.0001$ ) but there was no interaction between season and fork length ( $F_{[1,84]} = 1.52$ ,  $P > 0.2$ ). Again, parallel relationships on this nonlinear scale indicate that larger fish lost a greater proportion of accumulated lipids than did smaller fish. Spring samples of age-0 trout from lake K2 had higher lipid concentrations, at a given length, than fish from lake K1 (lake effect:  $F_{[1,54]} = 16$ ,  $P < 0.0002$ ), but the slopes did not differ (interaction effect:  $F_{[1,54]} = 0.7$ ,  $P > 0.4$ ). As before, there was greater inter-individual variation in lipid concentration for fish captured in the spring than for those captured at the start of winter (K1:  $R^2 = 0.43$ ; K2:  $R^2 = 0.33$ ,  $P < 0.002$  in each case).

Although larger fish lost absolutely more lipids than smaller fish over winter (Fig. 2b), the proportion of lipids lost over the course of the winter was between 60% and 80% of their lipid stores, which is near the maximum (approximately 85%) that they could afford to lose before starving to death (Fig. 3). In addition, there was little difference in lipid consumption between small and large fish (Fig. 3). This observation contrasts dramatically with the rapid nonlinear decline in the proportion of lipids lost that is predicted by size-dependent metabolic rates for starving fish (Fig. 3). Consequently, the metabolic allometry prediction correctly predicted lipid loss of the smallest fish but greatly underestimated lipid loss for the larger fish (Fig. 3). Survivors of all sizes lost much of their available lipids (that in excess of the minimum lipid concentration), bringing many individuals near death by starvation (Figs. 2c and 3).

### Discussion

Our study sought to determine if over-winter mortality of age-0 trout is high and inversely proportional to accumulated body reserves, as expected for starving individuals. If so, then over-winter mortality is a strong selective force that

**Fig. 2.** (a) Mortality of age-0 rainbow trout as a function of mean autumn fork length in two replicate lakes. (b) Allometry of lipid mass for age-0 trout in autumn and spring in two replicate lakes. (c) Allometry of lipid concentration in autumn (solid symbols) and spring in two replicate lakes. Open and shaded symbols represent estimates in lakes K2 and K1, respectively. The broken line represents the mean lipid concentration at death for starving trout determined in the laboratory experiment.



could promote density- and food-dependent risk-taking behaviour (Biro et al. 2003a, 2003b, 2003c) and allocation of energy to lipids observed for age-0 trout cohorts (Post and Parkinson 2001; this study). Together, the results of the laboratory and whole-lake experiments indicate that the risk of winter starvation is high and size-dependent, and is related to the loss of lipids to a threshold minimum lipid concentration. Therefore, the risk of winter starvation is a strong se-

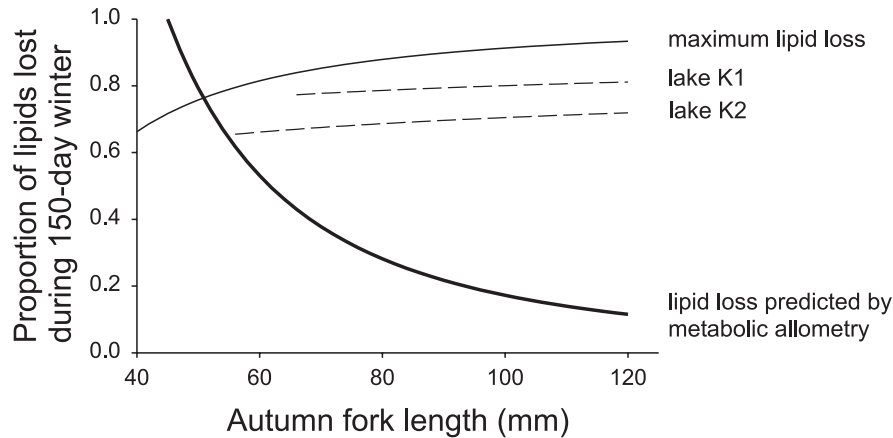
lective pressure that can promote risk-taking and lipid accumulation in populations of age-0 trout. In a more general sense, identifying predation (Biro et al. 2003b, 2003c) and over-winter starvation risks (this study) as major selective pressures for young fish may explain, at least in part, the high mortality rates typically observed for young fish populations during the growing season.

In the laboratory, age-0 trout gradually depleted lipid reserves over time and died when they reached a critical minimum lipid concentration of just less than 1% of total wet mass. The onset of high mortality rates coincided with the depletion of lipid reserves to levels near this critical lipid concentration, at which the first fish to die were those with the lowest lipid reserves at the outset. Larger trout, with more lipid reserves, began to die 40 days later than the small size class of trout with less lipid reserves. In the field, a similar pattern emerged in which smaller fish with fewer lipid reserves experienced greater mortality than larger trout with more lipid. The observation that no individuals were observed with less than 1% lipid in the spring suggests that this critical minimum lipid concentration needed for survival is robust, and the actual value does apply to fish of a much larger size range than that used in the laboratory experiment. In other studies, evidence has also been found for minimum lipid content thresholds for survival in fishes (see Post and Parkinson 2001), but to our knowledge our study is the first to convincingly demonstrate a minimum lipid threshold, and high over-winter mortality, in controlled laboratory and field experiments for a salmonid species. However, Simpkins et al. (2003) also found evidence for a threshold minimum lipid content for rainbow trout in the laboratory. Unfortunately, direct comparison is difficult, as they report the critical concentration of 3.2 as a percentage of dry mass (Simpkins et al. 2003), and wet mass to dry mass ratios are not constant among individuals or groups.

The smallest individuals in our field experiment appeared to lose lipids at a rate that was consistent with standard metabolic allometry of inactive, starving fish. Our predictions indicated that fish less than 50 mm FL would die over the course of the observed 150-day winter. Indeed, we observed only one individual less than 60 mm FL by spring in the lakes. To our surprise, however, larger individuals consumed more of their lipid reserves than predicted by standard metabolic allometry. Greater lipid consumption suggests higher energy costs for larger fish, perhaps because of increased activity rates associated with competitive and (or) predatory interactions with other trout. It is difficult to assess whether fish died directly from starvation per se, or were eaten by larger trout as they neared starvation in poor condition. However, it is possible that potential cannibals may increase starvation mortality of young trout by increasing activity levels through predator avoidance. This would result in smaller individuals being eaten or starving to a greater extent than larger ones, and could explain the 60% mortality observed for the largest size class (90 mm FL) that are not likely to be consumed by adult rainbow trout (Johnsson 1993; Biro et al. 2003b).

Mortality of age-0 trout declined dramatically with increases in body size, with trout of 60 mm FL or less experiencing at least 90% mortality and those of 90 mm FL only 60%. Clearly, this indicates that winter food shortage and

**Fig. 3.** Proportion of lipid stores lost over the 150-day winter in relation to fish mass at the onset of winter. The thin solid line represents the proportion of lipid reserves that could be consumed before death by starvation, based on a minimum lipid content of 0.9% determined in the laboratory. The thick solid line represents the loss of lipids predicted from metabolic allometry, assuming inactivity and starvation. The broken lines represent the observed loss of lipids over the winter in our two experimental populations calculated by subtracting autumn and spring lipid allometries in Fig. 2, in which the length of each broken line represents the range of body sizes of fish surviving to the end of the winter.



starvation may be a major mechanism of population regulation for age-0 trout, which amplifies the strong density- and food-dependent mortality they experience during the growing season. For instance, growing-season mortality of age-0 trout ranges from as little as 55% to as much as 98%, and is related to density- and food-dependent individual risk-taking behaviour (Post et al. 1999; Biro et al. 2003a, 2003c). Mean autumn mass, and therefore mean lipid content, of age-0 trout also declines with increases in density and (or) reductions in food abundance (Post and Parkinson 2001; Biro et al. 2003a, 2003c) that would, in turn, reduce over-winter survival. If, for instance, age-0 trout experienced 90% mortality during the growing season, and reached a population mean body size that would result in 70% over-winter mortality (as in our field experiment), then only 3% of the cohort would remain alive as age-1 individuals the following growing season. Such a pattern of fish mortality may be common for age-0 fishes in temperate waters. For instance, recruitment of age-1 striped bass (*Morone saxatilis*) was better predicted by the winter duration than by indices of age-0 abundance in one field study (Hurst and Conover 1998). The magnitude of over-winter mortality that we observed in the field is likely an underestimate of the mortality of trout reared naturally in lakes, owing to greater lipid content in our hatchery-reared trout. Indeed, the hatchery-raised fish had a lipid allometry with the same slope as that observed for fish reared naturally in lakes, but with a significantly higher intercept, indicating greater lipid stores at a given length (hatchery fish:  $\log_{10}$  lipid mass =  $-4.76 + 4.81(\text{FL})$ , 95% confidence interval (CI) =  $-5.1$  to  $-4.4$  and  $4.4$ – $5.3$  for the intercept and slope, respectively; naturally reared fish:  $\log_{10}$  lipid mass =  $-5.2 + 5.1(\text{FL})$ ; Post and Parkinson 2001).

Starvation of young trout was clearly not the result of an inability to feed or metabolize food at low temperatures. Age-0 trout increased lipid reserves during winter when they were provided with food ad libitum in the greenhouse experiment, and there was evidence that fish also did grow to a small extent in the field. Small and large trout nearly doubled their lipid concentration over the 100-day winter when

fed, indicating that young rainbow trout can perform quite well at these low temperatures when provided with abundant prey. Therefore, the combined results from both experiments indicate that winter prey is rare in lakes, given that the nonfeeding treatment in the greenhouse provided food conditions much closer to those experienced by fish in the field than did the ad libitum treatment in the greenhouse. We speculate that the reason why the age-0 trout were able to grow to a small extent in the field, yet consume most of their lipid reserves, was a greater availability of food early in the winter, which allowed some growth, followed by food shortage during the remainder of the winter. Future studies might examine lipid depletion and mortality of a greater size range of young trout to develop a functional relationship for size- and lipid-dependent probability of mortality. In addition, examining the costs and benefits to young fish adopting different energy-allocation strategies, and the short- and long-term mortality consequences of those strategies, would shed light on the patterns of behaviour and energy allocation we see in young fish populations.

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## References

- Bernard, G., Fox, M.G. 1997. Effects of body size and population density on overwinter survival of age-0 pumpkinseeds. *N. Am. J. Fish. Manag.* **17**: 581–590.

- Biro, P.A., Post, J.R., and Parkinson, E.A. 2003a. Density-dependent mortality is mediated by foraging activity for prey fish in whole-lake experiments. *J. Anim. Ecol.* **72**: 546–555.
- Biro, P.A., Post, J.R., and Parkinson, E.A. 2003b. Population consequences of a predator-induced habitat shift by trout in whole-lake experiments. *Ecology*, **84**: 691–700.
- Biro, P.A., Post, J.R., and Parkinson, E.A. 2003c. From individuals to populations: risk-taking by prey fish mediates mortality in whole-system experiments. *Ecology*, **84**: 2419–2431
- Burnham, K.P., and Anderson, D.R. 1998. Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York.
- Folch, J., Lees, M., and Stanley, G.H.S. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **266**: 497–509.
- Gardiner, W.R., and Geddes, P. 1980. The influence of body composition on the survival of juvenile salmon. *Hydrobiologia*, **69**: 67–72.
- Garvey, J.E., Wright, R.A., and Stein, R.A. 1998. Overwinter growth and survival of age-0 largemouth bass (*Micropterus salmoides*): revisiting the role of body size. *Can. J. Fish. Aquat. Sci.* **55**: 2414–2424.
- Gotceitas, V., Methven, D.A., Fraser, S., and Brown, J.A. 1999. Effects of body size and food ration on over-winter survival and growth of age-0 Atlantic cod, *Gadus morhua*. *Environ. Biol. Fishes*, **54**: 413–4220.
- Hurst, T.P., and Conover, D.O. 1998. Winter mortality of young-of-the-year Hudson River striped bass (*Morone saxatilis*): size-dependent patterns and effects on recruitment. *Can. J. Fish. Aquat. Sci.* **55**: 1122–1130.
- Johansson, F., and Rowe, L. 1999. Life history and behavioral responses to time constraints in a damselfly. *Ecology*, **80**: 1242–1252.
- Johnsson, J. 1993. Big and brave: size selection affects foraging under risk of predation in juvenile rainbow trout, *Oncorhynchus mykiss*. *Anim. Behav.* **45**: 1219–1225.
- Landry, F., Post, J.R., and Parkinson, E.A. 1999. Spatial ontogeny of lentic age-0 rainbow trout, *Oncorhynchus mykiss*: whole lake manipulations of population size structure. *Can. J. Fish. Aquat. Sci.* **56**: 1916–1928.
- McNamara, J.M., and Houston, A.I. 1994. The effect of a change in foraging options on intake rate and predation rate. *Am. Nat.* **144**: 978–1000.
- Oliver, J.D., Holeton, G.F., and Chua, K.E. 1979. Overwinter mortality of fingerling smallmouth bass in relation to size, relative energy stores, and environmental temperature. *Trans. Am. Fish. Soc.* **108**: 130–136.
- Post, J.R., and Evans, D.O. 1989. Size-dependent overwinter mortality of young-of-the-year yellow perch (*Perca flavescens*): laboratory, in situ enclosure and field experiments. *Can. J. Fish. Aquat. Sci.* **46**: 1958–1968.
- Post, J.R., and Parkinson, E.A. 2001. Energy allocation strategy in young fish: allometry and survival. *Ecology*, **82**: 1040–1051.
- Post, J.R., Parkinson, E.A., and Johnston, N.T. 1999. Density-dependent processes in structured fish populations: assessment of interaction strengths in whole-lake experiments. *Ecol. Monogr.* **69**: 155–175.
- Pratt, T.C., and Fox, M.G. 2002. Influence of predation risk on the overwinter mortality and energetic relationships of young-of-year walleyes. *Trans. Am. Fish. Soc.* **131**: 885–898.
- Rand, P.S., Stewart, D.J., Seelbach, P.W., Jones, M.L., and Wedge, L.R. 1993. Modelling steelhead population energetics in Lakes Michigan and Ontario. *Trans. Am. Fish. Soc.* **122**: 977–1001.
- Rowe, L., and Ludwig, D. 1991. Size and timing of metamorphosis in complex life cycles: time constraints and variation. *Ecology*, **72**: 413–427.
- Schindler, D.E. 1999. Migration strategies of young fishes under temporal constraints: the effect of size-dependent overwinter mortality. *Can. J. Fish. Aquat. Sci.* **56**: 61–70.
- Schultz, E.T., and Conover, D.O. 1999. The allometry of energy reserve depletion: test of a mechanism for size-dependent winter mortality. *Oecologia (Berl.)*, **119**: 474–483.
- Shuter, B.J., and Post, J.R. 1990. Climate, population viability, and the zoogeography of temperate fishes. *Trans. Am. Fish. Soc.* **119**: 314–336.
- Simpkins, D.G., Hubert, W.A., Martinez del Rio, C., and Rule, D.C. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. *Trans. Am. Fish. Soc.* **132**: 576–589.
- Toneys, M.L., and Coble, D.W. 1980. Mortality, hematocrit, osmolality, electrolyte regulation, and fat depletion of young-of-the-year freshwater fishes under simulated winter conditions. *Can. J. Fish. Aquat. Sci.* **37**: 225–232.