Ecology of Freshwater Fish 2004: 13: 185–196
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ECOLOGY OF
FRESHWATER FISH

Energy use in spawning Atlantic salmon

Hendry AP, Beall E. Energy use in spawning Atlantic salmon. Ecology of Freshwater Fish 2004: 13: 185–196. © Blackwell Munksgaard, 2004

Abstract – We studied some of the factors that might influence energy use in spawning Atlantic salmon (*Salmo salar*). Single females were placed into an experimental channel with either one or three males, after which spawning was monitored continuously. Male status was confirmed using genetic parentage analysis. Daily fat loss was monitored with the Torry Fish Fatmeter and validated through biochemical analyses. Several comparisons were in the expected direction but not statistically significant and therefore require further study: daily fat loss appeared higher for dominant males relative to subordinate males and in the three-male treatment relative to the one-male treatment. Most of the variation among individuals remained unexplained, suggesting that several as yet unknown factors strongly influence fat loss in spawning salmon. A large and significant effect was that daily fat loss was higher for females than for males, a difference that might contribute to the shorter spawning duration typical of females.

A. P. Hendry, E. Beall

UMR ECOBIOP, Écologie Comportementale et Biologie des Populations de Poissons, Station INRA d'Hydrobiologie de Saint-Pée sur Nivelle, 64310 Saint-Pée sur Nivelle, France

Key words: breeding; cost of reproduction; competition; lipid; aggression; courtship

Andrew P. Hendry, Redpath Museum and Department of Biology, McGill University, 859 Sherbrooke St. W., Montréal, Québec, Canada H3A 2K6; tel.: +1 514 398 4086/ext. 00880; fax: +1 514 398 3185; e-mail: andrew.hendry@mcgill.ca

Accepted for publication November 17, 2003

Un resumen en español se incluye detrás del texto principal de este artículo.

Introduction

Energy constraints shape many aspects of life history and behavior (Sibly & Calow 1986) and are often most evident during breeding, when energy expenditure is high and energy intake is low. Such constraints seem particularly important for salmonids because they do not feed during the spawning period (Jones 1959; Kadri et al. 1995) and instead use stored energy to fuel reproduction (i.e., capital breeding). Major energy expenditures include migration, development of gonads and secondary sexual traits, construction of nests, courtship, and intrasexual competition (Semenchenko 1986, 1987; Jonsson et al. 1991, 1997; Fleming 1996, 1998; Hinch & Rand 1998; Hendry & Berg 1999; Hendry et al. 1999, 2000, 2001; Healey et al. 2003). These expenditures: (i) lead to reductions in energy stores that average 12.5–78.8% (review: Hendry & Berg 1999); (ii) accelerate senescence in semelparous species (Hendry & Berg 1999; Hendry et al. in press); and (iii) decrease the likelihood of repeat spawning in iteroparous species (Jonsson et al. 1997).

We investigated several factors that might influence energy use in spawning Atlantic salmon (*Salmo salar*). We placed single females with either one or three males (all of wild origin) into an experimental stream channel. We then used continuous videotape recordings to quantify their behavior, and the noninvasive Torry Fish Fatmeter (Kent 1990) to estimate their fat loss. Behavioral observations were used to assign male status, and these assignments were later confirmed through genetic parentage analysis of eggs that were excavated from nests. The reliability of the fatmeter was examined through biochemical analyses of individual fish at the end of the experiment. In the following, we use 'energy' when referring to some unknown combination of fat and protein and 'fat' when referring specifically to lipids. In spawning Atlantic salmon, the two should be closely related because most (but not all) of the metabolizable energy comes from fat stores (Jonsson et al. 1991, 1997).

Specific objectives

Our first objective was to compare fat loss (daily loss of percentage fat) between dominant and subordinate males in the three-male treatment. Courtship in spawning salmonids can be quite vigorous (Tautz & Groot 1975; Fleming et al. 1996; de Gaudemar & Beall 1999), and dominant males perform most of the

courtship acts (Petersson & Järvi 1997; Berejikian et al. 2001; Healey et al. 2003). Aggressive behavior is also common (Foote 1990; Fleming et al. 1996; Quinn et al. 1996; Quinn 1999), particularly by dominant males (Petersson & Järvi 1997; Healey & Prince 1998; Healey et al. 2003). Intense courtship and high aggression by dominant males should elevate their activity levels (Healey et al. 2003) and cause increased energy expenditure and fat loss relative to subordinate males. However, the effects of male status might also be influenced by sex ratio, spawner density, and the absolute and relative size of males (Semenchenko 1986, 1987).

Our second objective was to compare male fat loss between the one-male (no competition) and three-male (competition) treatments. Males that do not experience competition will not expend energy on male–male aggression. In contrast, males that do experience competition often show high rates of aggression (Foote 1990; Fleming et al. 1996; Quinn et al. 1996; Petersson & Järvi 1997; Healey & Prince 1998; Quinn 1999; Healey et al. 2003) and should therefore have increased energy expenditure and fat loss. In support of this prediction, male reproductive life span is negatively correlated with the intensity of competition (van den Berghe & Gross 1986; Hendry et al. 2001). Alternatively, an increase in male–male competition might actually reduce energy expenditure if the corresponding decrease in the availability of females reduces male movement and rates of courtship, or if less competitive males cease fighting for females (Semenchenko 1986, 1987).

Our third objective was to compare female fat loss between the one-male and three-male treatments. Female–female competition was absent from our experiment but male–male competition might influence female energy use. For example, fighting among males may hinder nest construction by females, damage partially constructed nests, or increase egg retention. Moreover, because increased male courtship leads to increased female activity (de Gaudemar & Beall 1999; de Gaudemar et al. 2000a), the effects of competition among males on rates of courtship might indirectly influence female energy use. Direct tests for such effects are lacking but circumstantial evidence is suggestive. For example, qualitative observations of spawning Atlantic salmon indicate that fighting among males often disturbs nesting females (Fleming 1998; A. Hendry & E. Beall, personal observation). Perhaps owing to such disturbance, females spawn a smaller proportion of their eggs as the male bias in sex ratio increases past 3 : 1 (Chebanov 1986; Semenchenko 1986).

Our fourth objective was to compare fat loss between males and females. In 11 of 12 studies, populations, the proportional energy cost of reproduction was higher for females than for males (review: Hendry & Berg 1999). The exception was the study by Jonsson et al. (1997) who proposed that energy loss was similar between the sexes. In addition, differences in energy use between males and females may depend on their relative size. For example, Jonsson & Jonsson (2003) found that small females have less proportional energy loss than small males, whereas large females have similar or perhaps greater proportional energy loss than large males. Unfortunately, estimates of energy loss in the above studies included costs of migration, gonad development, and secondary sexual development, whereas we are specifically interested in the cost of spawning behavior. Only a few studies have quantified energy losses during spawning itself, and these have found that energy (and fat) loss was greater for males than for females in three populations (Gilhousen 1980; p. 37; Hendry et al. 1999) but similar for males and females in two populations (Gilhousen 1980; p. 37; Williams et al. 1986; p. 45). Comparable data are not available for iteroparous salmonids.

Individual-based analyses

Achieving the above objectives requires precise estimates of energy (or fat) loss for individual fish. Only two studies have adopted this approach, both for sockeye salmon (*Oncorhynchus nerka*). In the first, Semenchenko (1986, 1987) found that male energy loss (estimated as the pre- versus postspawning difference in metabolic rate and body mass) depended on interactions among body size, sex ratio, and status (status was estimated based on the pre- versus postspawning difference in testes mass). In the second study, Healey et al. (2003) used electromyogram telemetry (EMG) to measure muscle activity in wild fish. A series of published empirical relationships were then used to estimate tail-beat frequencies from EMG pulse intervals, swimming speeds from tail-beat frequencies, oxygen consumption from swimming speeds, and finally energy loss from oxygen consumption. Healey et al. (2003) conclude that: (i) aggression and digging are energetically costly; (ii) subordinate males use less energy than dominant males; (iii) dominant males and nest-building females use energy at similar rates; and (iv) females have greater total energy loss than males.

Our study complements previous work in being the first to: (i) measure fat loss in individual salmonids; (ii) estimate energy loss during spawning for an iteroparous salmonid; and (iii) experimentally examine the influence of male–male competition on energy loss in an iteroparous salmonid. Our source populations were very small (about 100 adults) and therefore our sample sizes were as well (four replicates of the one-male

treatment, three of the three-male treatment). For this reason, our conclusions are tentative, and the greatest value of our study is to influence future work where more fish are available. We therefore focused our efforts on intensive and complementary analyses that would demonstrate the overall utility of our approach.

Materials and methods

Study site and experimental fish

Our study was conducted during December 1997 and January 1998 in an experimental channel beside the Lapitxuri Stream, a tributary to the Nivelle River in south-western France (for a map, see de Gaudemar et al. 2000b). The experimental channel was divided into 13 sections, each 10 m long and 2.8 m wide, with a 0.6-m layer of gravel (1–8 cm diameter). Water flow was maintained at approximately 150 $l \cdot s^{-1}$, which generated water velocities of 0.20–0.35 $m \cdot s^{-1}$ and water depths of 0.2–0.4 m, consistent with spawning sites in the Nivelle River (Beall & de Gaudemar 1999). This experimental channel has been the site of many studies on the spawning behavior of Atlantic salmon (e.g., Beall & Marty 1987; Beall 1994; Morán et al. 1996; Thomaz et al. 1997; de Gaudemar & Beall 1998, 1999; de Gaudemar et al. 2000a,b; Garcia-Vazquez et al. 2001).

Atlantic salmon adults were captured during their upstream migration (October–November) in fish ladder traps at the Uxondoa and Olha dams on the Nivelle River. The population of Atlantic salmon in the Nivelle River is very small (111 individuals in 1997), and so we took only five males and nine females for our experiment. An additional eight males and four females were obtained from the Bidassoa River, Spain, which also has a small population (101 individuals in 1997). The fish from these two rivers were mixed for our experiments because the rivers are less than 10 km apart, fish often stray between them, and the sample sizes were not sufficient to include river as a factor in our analyses. Consistent with the apparent similarity between these two populations, we could not detect any qualitative effects of river origin on behavior or energy use. At capture, each fish was anesthetized (0.5 $mg \cdot l^{-1}$ of phenoxy-2-ethanol), measured for length, weighed, and marked with an individual tattoo under the skin (injected with a panjet and alcian blue dye). Fish were then transferred to the Lapitxuri channel, where they were held in fiber glass tanks without feeding (wild salmon do not feed during this period).

Study design and implementation

The experiment had two treatments, each involving mature males and recently ovulated females. In the one-male treatment (four replicates), one male and one female were placed together in a channel section, with no other salmon present. In the three-male treatment (three replicates), three mature males and one mature female were placed together in a channel section, again with no other salmon present. When the first female from a replicate had finished spawning, she was removed and killed (with an overdose of anesthetic) and a new female was added. The males in each replicate thus spawned sequentially with two different females (except in replicate #4 for the one-male treatment, where a second female was not available). Two females were used in succession because they completed spawning in 2–7 days and we needed males to spawn for longer, thus increasing our ability to detect temporal trends in fat stores. Basic information about each replicate is provided in Table 1.

When starting a replicate, each fish was anesthetized and tagged through its dorsal musculature with a small, flat tag. These tags varied in shape (round, square, triangle), which allowed the identification of individuals on videotape recordings. We could not detect any effect of these tags on the fish. The fat content of the muscle tissue of each fish was estimated using a Torry Fish Fatmeter (Model 692-STD). (Note that we are estimating fat in the muscle tissue only and therefore do not consider the large amount of fat lost in the form of spawned eggs.) The fatmeter uses a low-powered microwave sensor (2 mW output at 2000 MHz) to measure water content, from which fat content is estimated based on fat/water relationships in fish muscle tissue (Kent 1990). For the fatmeter measurements, each fish was laid flat on its side and wiped clean of mucus (Douirin et al. 1998). Readings were then taken three times at each of three locations above the lateral line: (i) midway between the posterior edge of the operculum and the anterior insertion of the dorsal fin; (ii) centered below the posterior insertion of the dorsal fin; and (iii) centered below the adipose fin. The fish recovered quickly from these procedures and no fish died during the experiment.

Spawning fish were videotaped continuously using two cameras: an Ikegami Model ICD-42E Type F/L and a Panasonic Model CF12.5A-SND-P (with a Newvicon tube sensitive to visible and infrared light). At night, 300 W infrared projectors (filtered to exclude wavelengths below 830 nm) were used for illumination. Atlantic salmon often spawn at night (almost exclusively in this experiment), and infrared light is a reliable way to videotape their behavior without disturbance (de Gaudemar & Beall 1999; de Gaudemar et al. 2000a,b). At least once each day, the positions of all nests were mapped. Every 1–3 days, all fish within a replicate were captured, anesthetized, and subjected to fatmeter measurements (as above).

Table 1. Information on the fish used in each replicate.

Treatment/replicate #	Mass (g)	Initial fat (%)	Duration (days)	% of time	GSI	Ovipositions
One-male/#1						
1st female	2320	1.21	1.96		3.8 (110)	4 (3)
2nd female	1810	0.91	2.84		3.2 (252)	5 (3)
Male	2105	0.64	4.80		2.8	9 (6)
One-male/#2						
1st female	2390	1.13	2.94		2.3 (5)	§
2nd female	2480	1.08	6.00		1.9 (6)	8 (6)
Male	2370	0.94	8.94		3.8	§
One-male/#3						
1st female	2430	0.96	5.99		2.4 (10)	5 (2)
2nd female	2565	0.69	4.00		1.9 (3)	3 (2)
Male	2715	0.62	12.99		2.2	8 (4)
One-male/#4						
1st female	2240	0.84	5.98		8.9 (0)†	3 (2)
Male	1940	1.12	5.98		2.1	3 (2)
Three-male/#5						
1st female	2370	0.70	2.87		1.4 (0)	7 (7)
2nd female	2675	1.28	3.10		2.0 (32)	12 (10)
Alpha male	2755	1.82	5.97	100	3.0	19 (17)
Beta male	2695	1.92	5.97	76.0	4.8	–
Gamma male	1845	1.51	5.97	76.0	4.2	–
Three-male/#6						
1st female	2150	1.04	3.98		6.1 (0)†	8 (4)
2nd female	1890	0.79	4.04		6.2 (596)‡	4 (3)
Alpha male	2865	1.02	8.02	100	2.2	12 (7)
Beta male	2480	1.00	8.02	81.0	7.1	–
Gamma male	2005	1.01	8.02	81.0	4.0	–
Three-male/#7						
1st female	2300	1.03	6.96		2.3 (7)	3 (3)
2nd female	2340	0.98	5.01		1.7 (7)	9 (7)
Alpha male	2115	0.72	11.97	100	2.8	12 (10)
Beta male	1855	0.96	11.97	50.6	5.0	–
Gamma male	2695	0.99	11.97	50.6	3.8	–

'Mass' is body mass at the start of the experiment. 'Initial fat' is the average Torry Fish Fatmeter reading at the start of the experiment. 'Duration' is the total time each fish was in a replicate. '% of time' is the percentage of the total time that each male was of the indicated status (alpha, beta, gamma). 'GSI' is the proportion of body mass composed of gonadal tissue at the end of the experiment (number of free eggs remaining in the body cavity in parentheses). 'Ovipositions' is the total number of oviposition events for each fish (the number of videotaped ovipositions sufficiently clear for analysis is in parentheses).
†These females had many over-ripe eggs bound to the skein.
‡This female had many loose ripe eggs and probably had not finished spawning.
§We were unable to obtain any useable recordings for the first female in this replicate.

When the second female in a replicate had completed spawning, all fish in that replicate were removed, killed, and subjected to fatmeter measurements (as above). Each male was 'stripped' by hand to determine the amount of free milt and then dissected to determine the remaining testes mass. Each female was dissected to determine the total mass of gonadal tissue, as well as the number of eggs (normal and 'overripe') remaining in its body cavity. A piece of dorsal muscle tissue (about 10 cc, excluding the skin) was dissected from each of the three locations used for fatmeter measurements. These muscle samples and the remaining somatic tissue (including the viscera) were frozen at −20 °C.

Behavioral analysis

Videotape recordings (a total of 804 h) were scanned for oviposition events ('ovipositions'). At each oviposition in the three-male treatment, dominant (alpha) males were defined as those closest to the female, chasing away intruding males, and releasing sperm before the other males. All other males were considered subordinate, including beta males (next closest to the female) and gamma males (farthest from the female). Spawning behavior was quantified over 10-min intervals at four different times around each oviposition: 120–110 min before, 60–50 min before, 10–0 min before, and 0–10 min after. These times correspond to different phases of spawning behavior for Nivelle Atlantic salmon in the Lapitxuri channel (de Gaudemar & Beall 1999; de Gaudemar et al. 2000a).

The female spawning behaviors we quantified included 'digging' (rapid tail movements against the gravel while the female was turned on her side), 'probing' (arched back, with the anal fin extended into the nest gravel), and 'covering' (similar to digging, but of shorter duration and upstream of the nest after

oviposition). Male courtship behaviors included 'quivering' (high frequency undulations beside the female) and 'crossing-over' (back and forth movements across the back of the female). The above categories corresponded to those used by Tautz & Groot (1975) and have been adopted by other authors (e.g., de Gaudemar & Beall 1999; Berejikian et al. 2000; de Gaudemar et al. 2000a). Male aggressive behaviors included 'threat displays' (short-duration movement toward another male), 'chasing' (acceleration toward another male without contact), 'ramming' (head-to-body contact with another male), 'biting', 'lateral display' (parallel orientation to another male, with fins extended), 'T-display' (perpendicular orientation to another male directly downstream), and 'back-pedalling' (crossing in front of another male, often while drifting downstream). These categories have been used to varying degrees by other authors (e.g., Jones 1959; Schroder 1981; Foote 1990; Beall 1994; Healey & Prince 1998; Petersson et al. 1999; Quinn 1999; Berejikian et al. 2000; Healey et al. 2003).

The frequencies of each behavior (number per 10 min) were calculated for each fish/oviposition/interval combination and then summed within each general behavior category (female spawning, male courtship, male aggression). These summed frequencies were then averaged across all ovipositions for each fish/interval combination (the two females in each replicate were pooled). Two-factor repeated-measures anova was then used to compare the frequency of female spawning behaviors across intervals (repeated) and treatments (fixed). Similar analyses were performed for male courtship, once using dominant males and once summing across all males in a replicate. Single-factor repeated-measures anovas were used to compare male aggression among the intervals in the three-male treatment, once using dominant males and once summing across all males in a replicate. Subordinate males performed too few behaviors for separate analyses.

Genetic parentage analysis

The behavioral observations revealed that a single male was dominant at all oviposition events in each three-male replicate (Table 1). The reproductive success of these males was examined using genetic parentage analysis. Approximately, 1 month after spawning, the water level in the channel was lowered and one redd was excavated for each female in each three-male replicate (i.e., two redds per replicate). Excavations entailed digging through the gravel until an egg pocket was discovered and then removing the exposed eggs with a hand-held glass tube attached to a rubber bulb. Recovered eggs were preserved in 95% ethanol. The excavated eggs and samples of adipose fin tissue from the adults were genotyped using the procedures described by Martinez et al. (2000) at nine loci (five minisatellites: pSsa-A45/1, pSsa-A4A5/2, pSsa-A60, pStr-A22/1, pStr-A5; four microsatellites: SSOSL417, SS3, SS4, SS6). Paternity assignment was based on direct comparison of the molecular weight of progeny alleles with alleles of the mother and putative fathers. All progeny could be unambiguously assigned to a father. Owing to the lack of variation in paternity (see below), only a subset of the eggs excavated from each redd were genotyped ($N = 30$–140).

Estimating fat loss and verifying the fatmeter estimates

The total change in percentage fat was estimated for each fish as the difference between its average starting and ending fatmeter readings. This difference was divided by the number of elapsed days (Table 1) to estimate daily fat loss (% fat per day). One-way repeated-measures anova (here analogous to a paired-sample *t*-test) was then used to compare daily fat loss between dominant (alpha) males and subordinate (beta and gamma averaged within replicates) males in the three-male treatment. Two-way repeated-measures anova was then used to compare daily fat loss between males and females (repeated) and the one-male and three-male treatments (fixed). This last anova was performed two times, once using alpha males and once using the average of beta and gamma males within each replicate (the average value for the females in a replicate was used in both cases).

The Torry Fish Fatmeter has already proven useful for estimating the fat content of fish tissue (Kent 1990; Kadri et al. 1995; Douirin et al. 1998) but has not been used for spawning fish. We tested its reliability in this context by comparing percentage fat readings to fat content as measured through standard biochemical analyses (AOAC 1990). Each muscle sample and the entire remaining somatic tissue of each fish were thawed and homogenized separately in a blender. One subsample of each homogenate was dried at 105 °C for 24 h to determine the proportion of dry matter. Two other subsamples were freeze dried and their fat extracted using the Soxhlet method with petroleum ether as the solvent. The proportions of fat per unit of dry mass and per unit of wet mass were then calculated for each sample. Simple linear regressions within each sex were then used to compare percentage fat estimates from the fatmeter and the biochemical analyses. First, the average fatmeter estimate for each location on each fish was compared to the biochemical estimate of fat content at that location (three data points per fish). Second, the average fatmeter estimate for each fish was compared to the average biochemical estimate of the muscle samples from each fish (one

data point per fish). Third, the average fatmeter estimate for each fish was compared to the biochemical estimate of fat content in the entire remaining somatic tissue (one data point per fish).

Results

Dominance and behavior

A single male was dominant at all recorded ovipositions within each replicate (Table 1). Genetic parentage analysis confirmed that each behaviorally dominant male fathered all of the analyzed eggs within his replicate ($N = 80$–201 eggs per replicate, see also Table 5 in Martinez et al. 2000). Postspawning gonadal-somatic index (GSI) ((mass of strippable milt + gonad mass)/total body mass) was always lower for dominant males than for subordinate males (Table 1) and was similar between the one-male and three-male treatments (using dominant males for latter).

Female spawning behaviors matched those previously observed in the Lapitxuri channel (de Gaudemar & Beall 1999; de Gaudemar et al. 2000a). In general, digging was highest 120 and 60 min before oviposition, probing was highest immediately before oviposition, and covering was highest immediately after oviposition. The combined frequency of all female spawning behaviors differed among intervals ($F = 47.26$ and $P < 0.001$) and was lowest 120 min before oviposition, increased to a peak immediately before oviposition, and decreased immediately after oviposition (Fig. 1A). However, the combined frequency of these behaviors did not differ significantly between the one-male and three-male treatments ($F = 2.820$ and $P = 0.154$; interaction: $F = 0.28$ and $P = 0.839$; Fig. 1A).

In the one-male treatment, quivering by males increased steadily until oviposition and then decreased sharply (see also de Gaudemar & Beall 1999). Crossing-over was low throughout. In the three-male treatment, nearly all of the courtship behaviors were performed by the dominant male (Fig. 1B). In comparison to the one-male treatment, quivering by the dominant male was slightly higher and followed the same temporal trend, but crossing-over was about six times more frequent. This difference in crossing-over is consistent with Schroder's (1981; p. 158) conjecture that 'crossing-over movements allow a male to continuously inspect both sides of a female and thus presumably detect and evict rivals'. The frequency of all courtship behaviors, excluding subordinate males, differed significantly among intervals ($F = 6.73$ and $P = 0.004$; lowest immediately after oviposition, Fig. 1B) but not between treatments ($F = 0.536$ and $P = 0.497$; interaction: $F = 2.042$ and $P = 0.151$). Similar results were obtained for the total frequency of courtship behaviors by all males in a replicate (interval: $F = 2.874$ and $P = 0.071$; treatment: $F = 2.822$ and $P = 0.154$; interaction: $F = 0.900$ and $P = 0.464$).

The total frequency of male aggressive behaviors varied dramatically among replicates (highest in #7) but only slightly among time intervals (dominant males: $F = 3.625$ and $P = 0.084$; all males: $F = 3.432$ and $P = 0.093$; Fig. 1C). In each replicate, nearly all of the aggressive acts were performed by dominant males. This may occur in part because the cameras only recorded aggressive acts near the female's redd, where dominant males were present more often than subordinate males. However, because at least two males were present each time an aggressive act was recorded, the subordinate males clearly received more aggression from the dominant male than vice versa.

Fat stores

Daily fat loss was qualitatively higher for dominant than for subordinate males (estimated marginal means: -0.020 vs. -0.013; Fig. 2) but the difference was not significant ($F = 0.927$ and $P = 0.437$), perhaps owing to the small number of replicates ($N = 3$) and the correspondingly low statistical power (0.092). Using dominant males to represent the three-male treatment, daily fat loss was: (i) higher for females than for males (-0.055 vs. -0.014; $F = 10.720$ and $P = 0.022$); (ii) qualitatively higher, but not significantly so, in the three-male than in the one-male treatment (-0.040 vs. -0.029; $F = 0.252$ and $P = 0.637$); and (iii) not influenced by an interaction between sex and treatment ($F = 0.004$ and $P = 0.951$; Fig. 2). Similar results were obtained when subordinate males were used to represent the three-male treatment: sex ($F = 10.918$ and $P = 0.021$), treatment ($F = 0.139$ and $P = 0.724$), and interaction ($F = 0.042$ and $P = 0.846$; Fig. 2). Statistical power to detect a significant treatment effect was low in both cases (using dominant males, power = 0.070; using subordinate males, power = 0.061). Results were the same if sexes were compared within treatments or if treatments were compared within sexes. Daily fat loss was positively, but weakly, correlated with initial fatmeter readings for females ($r^2 = 0.304$; $P = 0.051$) but not for males ($r^2 = 0.152$; $P = 0.187$).

Fatmeter readings at the end of the experiment were highly correlated with biochemical estimates of fat content. First, correlations were strongly positive for all fatmeter locations considered as separate data points: by dry mass (males: $r^2 = 0.672$ and $P < 0.001$; females: $r^2 = 0.301$ and $P < 0.001$; Fig. 3A) and by wet mass (males: $r^2 = 0.725$ and $P < 0.001$; females: $r^2 = 0.307$ and $P < 0.001$; Fig. 3B). Second, correla-

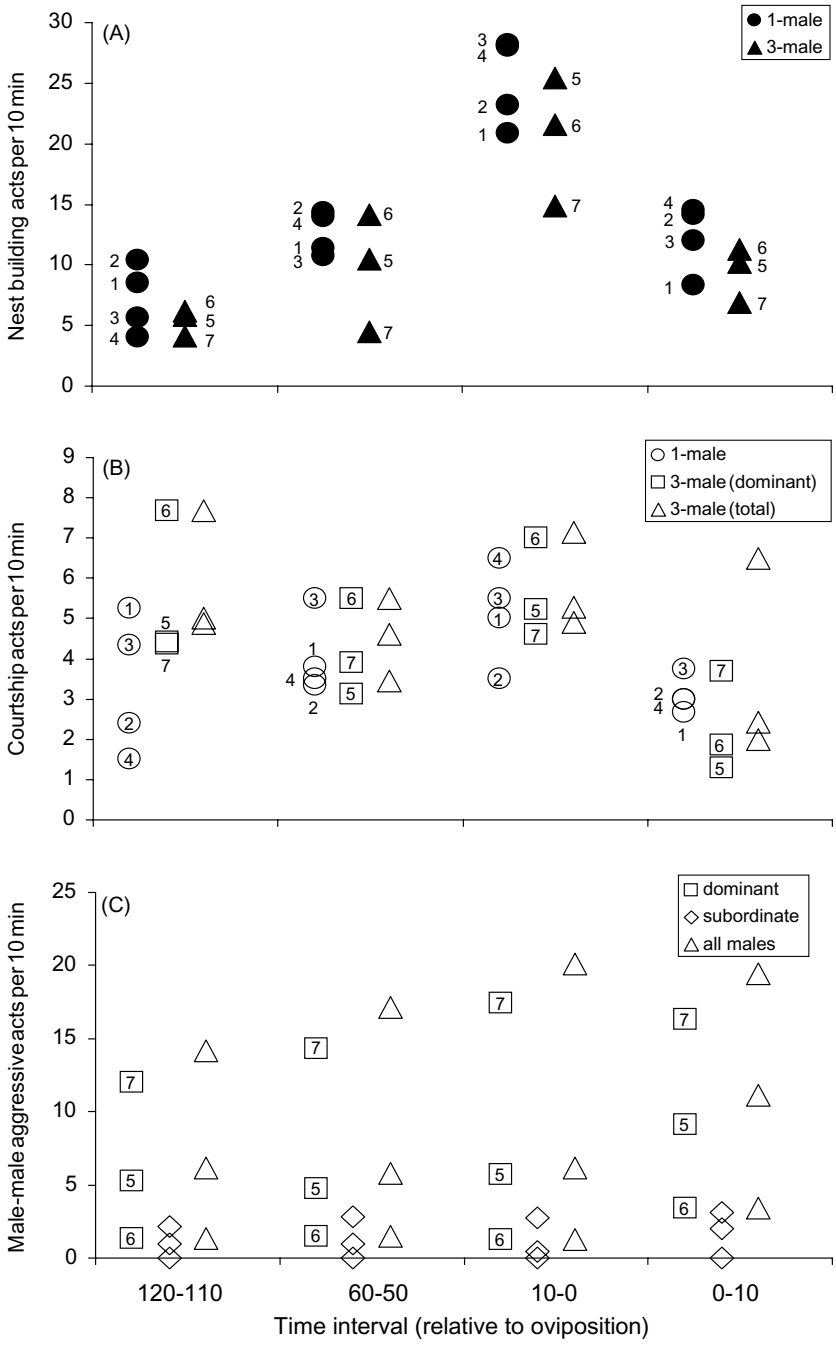

Fig. 1. Results of behavioral analysis. All graphs show the frequency of behaviors at the four different time intervals (numbers indicate specific replicates). Panel (A) shows the total frequency of nest building acts by females (averaged within each replicate). Panel (B) shows the total frequency of courtship acts by males in the one-male treatment, and by alpha (dominant) and all males (total) in the three-male treatment. Specific replicates for all males can be inferred by comparison to alpha males. Panel (C) shows the total frequency of all aggressive acts by alpha (dominant), beta and gamma (subordinate), and all males in the three-male treatment. Specific replicates are not labeled for subordinate males because aggression was very low. Specific replicates for all males can be inferred by comparison to dominant males.

tions were strongly positive for the average fatmeter estimate per fish versus the average biochemical estimate for the muscle samples at those locations: by dry mass (males: $r^2 = 0.718$ and $P < 0.001$; females: $r^2 = 0.775$ and $P < 0.001$) and by wet mass (males: $r^2 = 0.847$ and $P < 0.001$; females: $r^2 = 0.787$ and $P < 0.001$). Third, correlations were strongly positive for average fatmeter estimates versus the fat content in the entire remaining somatic tissue: by dry mass (males: $r^2 = 0.862$ and $P < 0.001$; females: $r^2 = 0.455$ and $P = 0.011$; Fig. 4A) and by wet mass (males: $r^2 = 0.872$ and $P < 0.001$; females: $r^2 = 0.486$ and $P = 0.008$; Fig. 4B).

Discussion

We compared daily rates of fat loss for dominant versus subordinate males, males in one-male versus three-male treatments, females in one-male versus three-male treatments, and males versus females. The first three comparisons yielded qualitative differences in the predicted direction that were not statistically significant. We therefore consider potential explanations for the general lack of statistical significance before examining each comparison in detail. One possibility is that the qualitative differences were real but the statistical tests were not powerful enough to

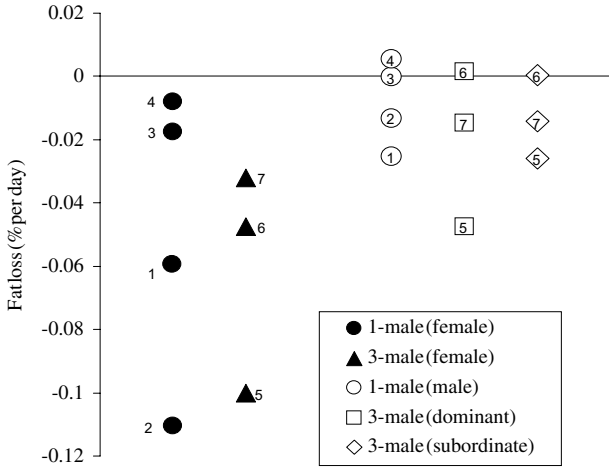

Fig. 2. The change in percentage fat per day for females and males in each replicate. Points for females are the average of the two females in that replicate (except for replicate #4, which had only one female).

provide confirmation. This is certainly plausible because the sample sizes were small (three or four replicates per treatment) and the statistical power was correspondingly low (<10%). Unfortunately, larger sample sizes were not possible because we used all available fish. Another possibility is that the qualitative differences were not real and the statistical tests accurately reflected this fact. Future work in systems that have more available fish should be able to discriminate between these possibilities. In the following, we discuss the qualitative differences but with the proviso that our sample sizes were too small for definitive interpretations of nonsignificant results. Our main purpose is therefore to generate hypotheses and illustrate methods for future experiments.

The greatest unknown in our experiment was the Torry Fish Fatmeter, simply because it had not previously been used to monitor spawning fish. The fatmeter readings at the end of the experiment were

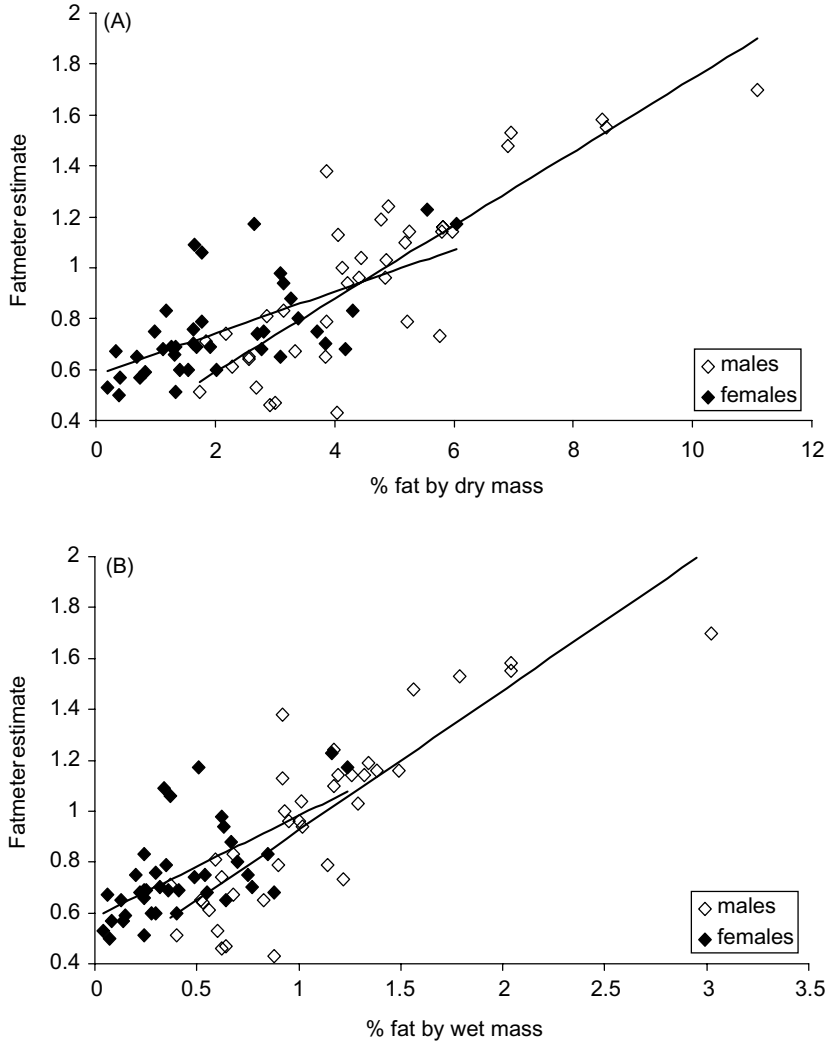

Fig. 3. Relationships between Torry Fish Fatmeter estimates of fat in the muscle tissue at specific locations on each fish and biochemical estimates of fat for those locations. Biochemical estimates are for fat by dry mass (A) and by wet mass (B).

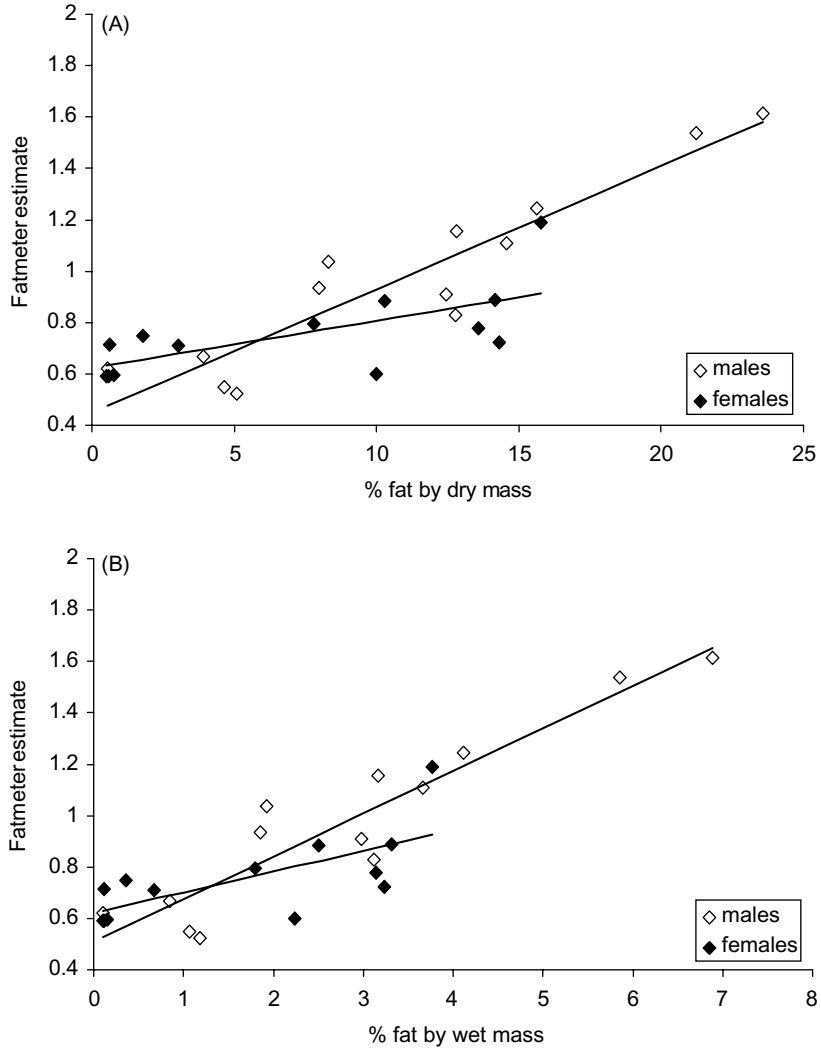

Fig. 4. Relationships between Torry Fish Fatmeter estimates of fat in the muscle tissue of each fish (average of the three locations) and biochemical estimates of fat content for the entire body of each fish. Biochemical estimates are for fat by dry mass (A) and wet mass (B).

highly correlated with standard biochemical estimates of fat content (Figs 3 and 4) but some caution is nonetheless warranted. The fatmeter directly measures water content and then uses published water/fat relationships to estimate fat content (Kent 1990). Unfortunately, the published relationships were not developed for spawning fish, where fat content is very low (Jonsson et al. 1991, 1997) and some muscle protein is metabolized (Hendry & Berg 1999; Jonsson & Jonsson 2003). Also, some fat used during spawning could come from the viscera, which the fatmeter ignores. Fortunately, viscera fat stores are low by the time Atlantic salmon start spawning (Jonsson et al. 1997). Additional work validating the fatmeter for use on spawning fish would be helpful because our results suggest it may provide a useful tool.

Fat loss in relation to status, competition, and sex

Comparing dominant and subordinate males, the former performed nearly all of the courtship acts (Fig. 1B) and were more aggressive (Fig. 1C). These higher activity levels should cause higher energy expenditure by dominant males than by subordinate males (Healey et al. 2003). This seemed to be the case, at least qualitatively, but the trend was because of a single replicate (#5, Fig. 2) and was not statistically significant. The dominant male with the highest levels of aggression (#7) was the only dominant male that was not also the largest in its replicate (Table 1), suggesting that high levels of aggression can compensate for small size. However, this male did not have the greatest fat loss (Fig. 2). The dominant male with the greatest fat loss was instead the male that started with the most fat (#5, Table 1). The two males with which he was competing also had high initial fat stores (Table 1), suggesting that the difference in energy use among males within this replicate was indeed because of their status (i.e., dominant vs. subordinate).

These results suggest a new hypothesis. Perhaps fat loss is similar for dominant and subordinate males when both start with low fat stores, but is higher for

dominant males when both start with high fat stores. Maintaining dominance when fat stores are low may require energy provided by protein catabolism, which could substantially compromise postspawning survival. The depletion of protein in iteroparous Atlantic salmon (Jonsson et al. 1991, 1997) appears lower than that in semelparous Pacific salmon (Hendry & Berg 1999), but within-population variation could still be substantial. We suggest that males with higher initial fat stores may have a fitness advantage because they can maintain dominance for longer or achieve higher postspawning survival.

Comparing males between the one-male and three-male treatments, aggression was absent from the former but common in the latter (Fig. 1C) and courtship rates were similar (Fig. 1B). Dominant males in the three-male treatment were thus more active than males in the one-male treatment, which should cause greater energy loss in the former (Healey et al. 2003). This seemed to be the case, at least qualitatively, but again the trend was because of a single replicate (#5, Fig. 2) and was not statistically significant. As above, the dominant male that used the most fat was not the most aggressive male but was instead the male that started with the highest fat stores. In general, fat loss during spawning appears low for most males, regardless of whether they are alone, dominant, or subordinate (Fig. 2). High fat loss can occur but only for a dominant male that starts with high fat stores. This again raises the question of whether males with low rates of fat loss also have low rates of total energy loss, or whether they subsidize metabolism through the depletion of muscle protein (as suggested above). Future work would benefit from measuring the loss of both fat and protein, perhaps by combining fatmeter readings with biochemical analyses of muscle 'cores' taken from individual fish (e.g., Hendry et al. 2001).

Comparing females between the one-male and three-male treatments, the latter did not take longer to spawn, construct more nests, or retain more eggs (Table 1). Evidence that male–male competition disturbs females was thus lacking, presumably because dominant males were rarely challenged by subordinate males and so females were rarely disturbed. Females did have higher fat loss in the three-male treatment, at least qualitatively, but the trend was not statistically significant and fat loss was highly variable within treatments (Fig. 2). This high variability suggests that factors other than male–male competition play an important role in female fat loss, even when female–female competition is absent. One such factor might be body size (Jonsson et al. 1997; Jonsson & Jonsson 2003), but the average size of females in a replicate was not correlated with their average fat loss (compare Fig. 2 with Table 1). Another factor might be the intensity of nest building, but this did not vary appreciably or consistently among replicates (Fig. 1A). Yet another factor might be the overall level of courtship by males, but a consistent pattern was not evident here either (compare Fig. 2 with Fig. 1B). Finally, fat loss in females did not correlate well with initial fat stores (compare Fig. 2 with Table 1). Thus, although male–male competition may influence fat loss in females, other as yet unknown factors generate substantially more of the variation.

Comparing males and females, daily fat loss was much higher for the latter (Fig. 2). This difference could not be attributed to: (i) variation in initial fat stores because they were not higher for females (Table 1); (ii) variation in spawning duration because females that were intermediate in this regard actually lost the most fat (Table 1); or (iii) the loss of eggs because fatmeter readings were above the lateral line. Thus, daily fat loss during spawning appears higher for females than males, at least under our experimental conditions. This result is particularly striking because our experiment excluded female–female competition, which might further increase fat loss. Higher rates of fat loss for females than males was consistent with a previous individual-based study (Healey et al. 2003) but not with group-based studies (Gilhousen 1980; Williams et al 1986; Hendry et al. 1999). More work needs to be performed before we can resolve the discrepancy between these two approaches.

Higher daily fat loss for females than males suggests that females may have: (i) a shorter spawning duration, (ii) lower pre- or postspawning energy costs, or (iii) lower postspawning survival. Lower prespawning energy costs seem unlikely because females must produce eggs, which require much more energy than does sperm (Jonsson et al. 1997; Hendry et al. 2000). We also know that females do not have lower postspawning survival in the wild (Fleming 1996) or in our experimental channel: over the last 15 years, mortality was similar for males (66 of 276 fish, 23.9%) and females (75 of 251 fish, 29.9%). Thus, females may have lower postspawning energy costs or shorter spawning durations. The former is unknown but the later appears true in general (Fleming 1996, 1998; Fleming et al. 1996) and in our experimental channel (Table 1; E. Beall, unpublished data). It thus seems plausible that the shorter spawning duration of Atlantic salmon females, relative to males, has evolved in part because it reduces energy costs and improves the chances of repeat breeding, both of which should increase reproductive success. It is also possible, of course, that the arrow of causality flies in the other direction and that shorter spawning durations in females evolved for some other reason, which then allowed increased rates of energy expenditure during spawning.

Resumen

1. Estudiamos varios factores que pueden influir el uso de la energía en reproductores de *Salmo salar*. Hembras individuales fueron colocadas en un canal experimental con uno o tres machos. Posteriormente, la puesta fue monitorizada de forma continua con grabación de video (por la noche con luz infrarroja) Estimas comportamentales del status masculino (dominante o subordinado) fueron confirmadas a través de análisis de parentesco genético. Las perdidas diarias de lípidos fueron monitorizadas con un medidor de lípidos no-invasivo Torry Fish que fue validado a través de análisis bio-químicos.
2. Los resultados de varias comparaciones estuvieron en la dirección esperada pero no fueron estadísticamente significativos y por ello, requieren de mas estudios: la pérdida diaria de lípidos pareció mayor en los machos dominantes respecto de los subordinados y tanto en machos como en hembras en los tratamientos de 3 machos respecto del tratamiento con un macho.
3. La falta de significación estadística en estas comparaciones puede deberse a las siguientes causas: A. El poder estadístico fue bajo; B. La pérdida de lípidos en los machos fue alta solo cuando un individuo fue dominante y comenzó con almacenaje alto de lípidos; C. La pérdida de lípidos en las hembras fue muy variable en los tratamientos. La mayor parte de la variación entre individuos permaneció no explicada lo que sugiere que varios factores, todavía no identificados, influyen poderosamente sobre pérdida de lípidos en reproductores de *S. salar*. Un efecto grande y significativo fue que la pérdida de lípidos diaria fue mayor en las hembras que en los machos, una diferencia que puede contribuir a la menor duración de puesta típica de las hembras.

Acknowledgements

Atlantic salmon were provided by the Nivelle Fisherman's Association and the Diputacion Foral de Navarra (M. Lamuela). B. de Gaudemar helped design and carry out the experiment and interpret the behavioral observations. Experiments in the Lapitxuri channel were conducted with the help of J.-C. Vignes, M. Parade, F. Lange, and especially S. Glise. The Torrey Fish Fatmeter was loaned by B. Fauconneau. Behavioral analyses of the videotape recordings were performed by A. Rojao. The biochemical analyses were performed by C. Darget in the Fish Nutrition laboratory at Saint-Pée sur Nivelle. The genetic parentage analysis was performed by P. Martinez in the laboratory of E. Garcia-Vazquez. S. Kaushik provided helpful comments. A. Hendry was supported by funding from the Department of Hydrobiologie, INRA.

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