

Impact of macroinvertebrate diet on growth and fatty acid profiles of restocked 0+ Atlantic salmon (*Salmo salar*) parr from a large European river (the Allier)

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Abstract: The influence of macroinvertebrate diet on growth and fatty acid profiles of Atlantic salmon (*Salmo salar*) parr released at the fry stage in three different riffles was studied in a large temperate river (Allier, France). Comparisons were made between sites and between restocked parr and hatchery-reared counterparts fed with a traditional fish diet. Significant differences were observed along the longitudinal gradient of the river and between restocked and hatchery-reared parr. Growth performance and nutritional status were higher in the hatchery and in downstream riffles and low in the most upstream site. These differences appeared to be related to different types of diet and consequently to variations in polar and neutral lipid intakes. The most favorable site for optimum growth appeared to be the intermediary riffle, with values close to those achieved in the hatchery. Simuliids and baetids, preferentially consumed in downstream sites, constitute an interesting type of food, showing quite different fatty acid composition from vegetable oils. This could be of interest for composing a new diet formula for young salmon intended for river restocking, imitating these macroinvertebrate fatty acid profiles.

Résumé : L'influence de la consommation de proies naturelles sur la croissance et les profils d'acides gras de tacons de saumon atlantique (*Salmo salar*) de repeuplement déversés dans différents radiers a été étudiée sur un grand cours d'eau européen : l'Allier, France. Des comparaisons ont été effectuées entre trois sites de déversement étagés de l'amont vers l'aval, ainsi qu'entre les tacons relâchés et leurs homologues maintenus en salmoniculture et nourris d'aliments artificiels, formulés à partir de farines de poissons. Les meilleures performances de croissance et de statut nutritionnel ont été observées en salmoniculture ainsi que dans les radiers aval, tandis que les plus faibles ont été enregistrées sur le radier situé le plus en amont. Ces observations semblent être liées aux différences de régime alimentaire, et par conséquent, à des teneurs variables en lipides neutres et polaires. Le radier le plus favorable à la croissance des tacons semble être le site intermédiaire qui présente des performances proches de celles observées en salmoniculture. En outre, les simuliidés et les baetidés, proies préférentiellement consommées dans les sites aval, semblent constituer une nourriture intéressante pour les juvéniles en raison de leurs profils particuliers d'acides gras. Ces diptères pourraient ainsi permettre de formuler de nouveaux aliments artificiels destinés aux tacons de repeuplement.

Introduction

Salmonid farming in France traditionally uses diets based on marine fish meal and oil because farmed species are commonly carnivorous. This is also the case in rearing young Atlantic salmon (*Salmo salar*), even though juveniles and parr are known to be freshwater residents and in their natural environment would encounter a diet consisting mainly of freshwater invertebrates (Thonney and Gibson 1989). However, according to several studies (Ghioni et al. 1996; Sushchik et al. 2003; Torres-Ruiz et al. 2007), the fatty acid (FA) compositions of such insect larvae are quite different from those of the fish oils used in farm diets. Invertebrates showed higher levels of 18:2n-6 and 18:3n-3,

intermediate levels of 20:5n-3 and 20:4n-6, and lower levels, if any, of 22:6n-3 than found in fish-oil based diets. Lipids from freshwater invertebrates consumed by wild salmon parr presented FA profiles closer to those of vegetable oil than of fish oil diets (Bell et al. 1997). However, despite obvious economic and environmental arguments, vegetable oils are still little used in the production of salmon for river restocking: in general, salmonid aquaculture prefers the traditional diet based on marine fish oil.

Studies on anadromous salmonids have shown that the FA composition of wild parr and smolts differs from that of farmed counterparts (Bergström 1989; Ahlgren et al. 1994). Farmed salmon fed with fish oil based diets were particu-

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larly rich in $n-3$ highly unsaturated FA (HUFA) and had a high $n-3$ to $n-6$ polyunsaturated FA (PUFA) ratio (Bell et al. 2002), whereas wild parr and farmed parr fed with vegetable oils showed more C_{18} PUFA, less $n-3$ HUFA, and a lower $n-3$ to $n-6$ PUFA ratio (Bell et al. 1998, 2003b). However, the most important fact is that these differences in diet may affect parr–smolt transformation (Bell et al. 1997; Bendiksen et al. 2003), a primordial stage in the salmonid life cycle. Fish oil based diets can inhibit the desaturase activity in parr and thus affect smoltification, whereas vegetable oil based diets seem to prevent this inhibition (Tocher et al. 2000). They also seem to enhance the ability of the fish to osmoregulate and thus to adapt to salinity changes (Sargent et al. 1999). Dietary FA must also provide enough energy to ensure sufficient parr growth, whether in the form of monounsaturated FA (MUFA) (mainly 18:1 $n-9$, 22:1 $n-9$) or other easily catabolized compounds (18:3 $n-3$) (Henderson 1996, Bell et al. 2003b). Studies on salmonids undergoing parr–smolt transformation also showed that 18:1 $n-9$, 22:1 $n-9$, 18:3 $n-3$, and 18:2 $n-6$ were used differently depending on their contribution to different dietary formulae (Tocher et al. 2000; Bendiksen et al. 2003). They can be used as selective substrates either for β -oxidation and (or), in the case of both 18:3 $n-3$ and 18:2 $n-6$, for bioconversion into HUFA when dietary 22:6 $n-3$ and 20:4 $n-6$ are insufficient (Henderson 1996; Bell et al. 1997). It therefore seems wasteful to continue to use marine fish oil diets to raise young salmon for river restocking.

In the wild, just a few insect orders (Diptera, Ephemeroptera, Plecoptera, and Trichoptera) are consumed by parr due to special habitat (riffle), morphological, and energetic constraints (Wankowski and Thorpe 1979; Keeley and Grant 1997). Moreover, the diversity and composition of available prey, which have been shown to vary along the upstream–downstream gradient in a large European river (Descroix et al. 2009), could affect parr lipid metabolism. Kaushik et al. (2006) observed that differences in prey diversity between the upper and lower reaches of the River Nivelle could explain the upstream–downstream differences in FA composition recorded in parr. This means that freshwater invertebrates are more diverse in their FA composition than is generally thought, a fact that recent studies tend to confirm (Sushchik et al. 2007; Torres-Ruiz et al. 2007). Mainly MUFA and PUFA content differed among macroinvertebrate taxa. In addition, Trichoptera (Hydropsychidae) and Ephemeroptera (Ephemerillidae) exhibited high percentages of $n-3$ FA (e.g., eicosapentaenoic acid: 10%–23%) and low percentages of $n-6$ FA, while Diptera (Chironomidae) and Oligochaeta generally had low percentages of 18:2 $n-6$ and 18:3 $n-3$ (Ghioni et al. 1996; Torres-Ruiz et al. 2007). Young salmon will therefore encounter different dietary FA intakes from one site to another, which could affect growth, overwintering, and subsequent smoltification in the wild (Nordgarden et al. 2002).

In this context, the objective of the present study was to investigate the dietary origin of the main FA accumulated by restocked *S. salar* parr during their high-growth period, which will be involved in overwintering and further migration processes. The influence of natural food (aquatic macroinvertebrates) on salmon growth and the FA composition of whole-body neutral lipids (NL) and polar lipids (PL) was

analyzed. Emphasis was placed on 16:0 (known to be incorporated into PL), 16:1 $n-7$ and 18:1 $n-9$ (used as oxidation substrate for energy; Bell et al. 1997), and C_{18} PUFA, 20:4 $n-6$, 20:5 $n-3$, and 22:6 $n-3$ (regarded as essential dietary FA). The study was undertaken in three restocking riffles located along the upstream–downstream gradient in the River Allier (France), but comparisons were also performed between hatchery-reared parr fed with a traditional diet based on marine fish oil and the restocked parr from the same broodstock (Chanteuges salmon hatchery). Our aim was to evaluate the importance of natural food, where FA composition varies over time and between sites, compared with hatchery feed, where composition is constant.

Materials and methods

Study sites

The study was undertaken from June to October 2006 in the River Allier, which belongs to the upstream river network of the Loire basin (France) (Fig. 1). The Loire–Allier axis is famous for its wild salmon population, the last in Europe able to make long-distance migration in freshwater (900 km). The wild population is therefore reinforced by restocking the river with 0+ parr (in May) and 1+ smolts (in April) from the Chanteuges salmon hatchery (Haute-Loire, France; see Fig. 1).

Our sampling sites were located in Haut-Allier, the upper part of the River Allier, historically known as a natural breeding area for *S. salar*. There, the river is a fourth- to fifth-order stream (Strahler 1957) with a watershed of 2900 km². Three sites were selected according to their upstream–downstream distribution along the river’s longitudinal gradient. Site 1 offers middle-rhithron habitats belonging to the trout zone (Huet 1959). Site 2 is located in the grayling zone with lower-rhithron stream characteristics. Site 3 is typical of the upper-potamon fluvial areas located in the barbel zone. Sampling was only performed in riffles where no natural spawning had been observed during the previous autumn (2005). A detailed description of riffle habitats in each site can be found in Descroix et al. (2009).

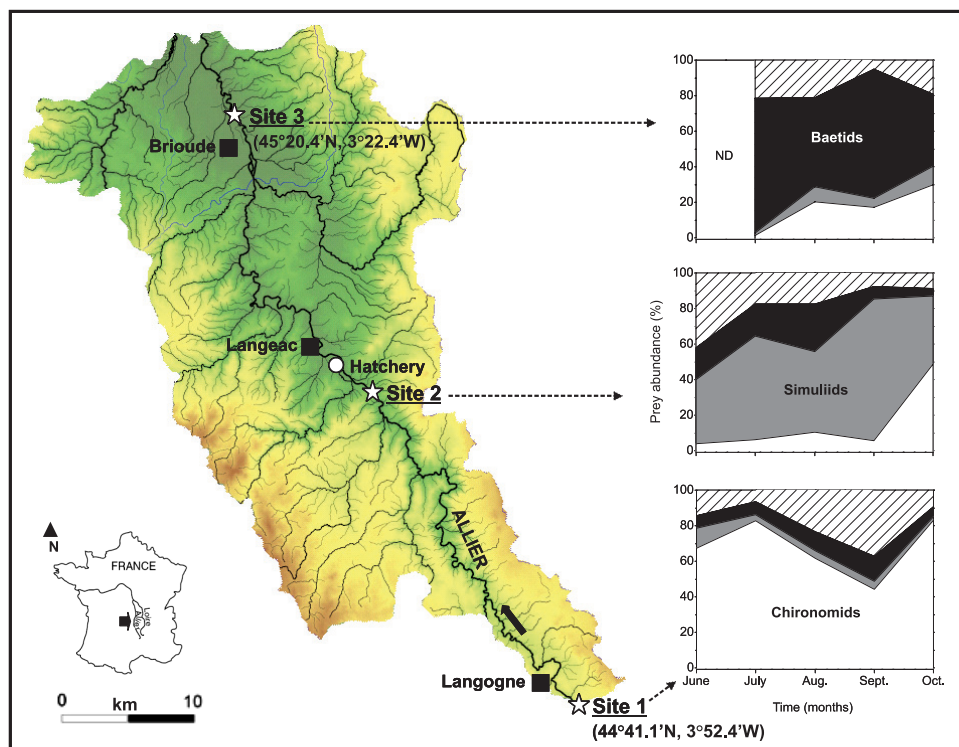
Fish rearing and field sampling

Fish samples

All parr analyzed in this study originated from artificial spawning of wild *S. salar* caught each autumn in the River Allier. In the Chanteuges salmon hatchery (see Fig. 1) and after yolk sac resorption, juveniles were reared in circular 400 L fibreglass tanks with 18 000 fish per tank. Constant water flow (pumped from the River Allier) and a current speed of 8–10 cm·s⁻¹ in the periphery and close to 0 cm·s⁻¹ in the center were maintained in all tanks under natural photoperiod. Fish were fed to apparent satiation eight times daily with commercial pelleted food (Nutra HP 0.3, Skretting, France). This diet contains 57% crude protein, 17% lipids, 8.5% carbohydrates, 10% ash, 0.5% cellulose, 1.3% phosphorus, and 19.9 MJ·kg⁻¹ digestible energy.

In May, an initial sample of these juvenile farm salmon (15 fish, total length ranging from 3 to 4 cm) was collected prior to the beginning of the experiment. Remaining juveniles were then restocked in the river, except for one batch,

Fig. 1. Prey relative abundance in the stomachs of Atlantic salmon parr sampled in three riffles from the upper River Allier. Prey items: hatched, others; black, baetids; grey, simuliids; white, Chironomids; ND, no data. Sampling riffles (stars) are located on the map as site 1, site 2, and Site 3. The circle indicates the location of the Chanteuges salmon hatchery. (Modified from Descroix et al. 2009.)



which was isolated from the rest and randomly divided between three 1000 L tanks containing 1250 fish each, i.e., hatchery-reared. Their diet consisted of fish oil based pellets (Nutra HP 1.0, Skretting, France) containing 53% crude protein, 19% lipids, 10.5% carbohydrates, 10% ash, 0.5% cellulose, 1.3% phosphorus, and 20.1 MJ·kg⁻¹ digestible energy (more details of the FA composition of this artificial diet are given in Table 1). Parr were fed six times a day until the end of the experiment (October). A mean of seven reared salmon were collected each month from June to October. All fish were anaesthetized and immediately frozen in liquid nitrogen prior to lyophilization, dry weight determination, and lipid analysis.

All other batches of juveniles were released in the river in late May 2006. In the three selected riffles, release densities were around 10–15 fry·m⁻². After 3 weeks of acclimatization, sampling started and was conducted monthly during the same period as for hatchery-reared parr (June–October). At each site and date, six to nineteen 0+ parr were captured by backpack electrofishing. Stomach contents were extracted by pulsed gastric lavage and diet materials were preserved in 4% formaldehyde. A total of 130 restocked parr were thus collected, of which 69 were immediately frozen in liquid nitrogen pending lyophilization, dry weight determination, and lipid analysis.

Stomach content analysis and macroinvertebrate samples

Restocked salmon food items were identified and enumerated under a binocular microscope. Macroinvertebrate prey were identified to the genus level for Diptera, Ephemeroptera, and Trichoptera and to the family level for other taxa.

Each item's contribution to stomach fullness was described as percentage prey abundance (%A_i) (Amundsen et al. 1996):

$$%A_i = (\Sigma S_i / \Sigma S_t) \times 100$$

where S_i is the stomach content (in this case, number) for prey i and S_t is the total stomach content for all prey categories in the fish. Although prey proportions varied between sites, baetid nymphs and chironomid and simuliid larvae predominated over the survey as a whole (Descroix et al. 2009), and these three families were therefore specifically analyzed for FA composition. They were collected from a minimum of four locations within each riffle using a Surber benthic sampler fitted with a 250 μm mesh net. All sampled macroinvertebrates were kept overnight in a tank filled with river water at 4 °C to achieve gut clearance. The next morning, all baetid, chironomid, and simuliid specimens were carefully hand-sorted and counted. Specimens of each family were pooled in preweighed vials to obtain appropriate biomass for lipid analysis. When possible, three replicated samples were formed for each taxon, freeze-dried, weighed (Mettler AE163 electrobalance, sensitivity 0.001 mg), and stored at -40 °C prior to lipid extraction.

Lipid analysis

Lipids were analyzed in whole salmon parr, natural food (insect larvae), and artificial pellets. Total lipids were extracted with chloroform–methanol according to the method of Folch et al. (1957). For each sample, NL were separated from PL (mainly phospholipids) in prepacked solid-phase silica columns (SPE Strata NH₂). Columns were precondi-

Table 1. Fatty acid composition (weight % of total fatty acids) in total lipids extracted from commercial pellets and freshwater invertebrates.

| Fatty acid | Nutra pellets | Baetids | Simuliids | Chironomids | <i>P</i> |
|-----------------------|---------------|-------------|--------------|--------------|----------|
| 14:0 | 6.19±0.10a | 2.55±0.32b | 1.32±0.10d | 3.99±0.21c | <0.001 |
| 16:0 | 17.73±0.20a | 25.43±2.42b | 20.83±0.48ab | 17.44±0.36a | <0.01 |
| 18:0 | 3.48±0.00a | 10.67±2.25b | 7.41±0.41ab | 11.20±0.83b | <0.001 |
| Total SFA | 30.13±0.30a | 41.84±3.50b | 36.71±0.84ab | 39.91±1.06b | <0.01 |
| 14:1 | 0.06±0.00a | 0.04±0.02a | 4.53±0.33b | 0.58±0.08a | <0.001 |
| 16:1 _{n-7} | 6.47±0.10a | 10.36±1.33b | 9.88±0.34ab | 7.30±0.48ab | <0.05 |
| 18:1 _{n-9} | 9.71±0.40a | 4.14±0.48b | 11.99±0.46d | 6.28±0.33c | <0.001 |
| 18:1 _{n-7} | 2.53±0.10a | 5.37±0.63b | 3.86±0.24b | 2.43±0.22a | <0.001 |
| 20:1 _{n-9} | 2.91±0.40a | 0.06±0.02b | 0.15±0.04b | 0.14±0.05b | <0.001 |
| 22:1 _{n-9} | 3.14±0.10a | | | 0.03±0.01b | <0.001 |
| 22:1 _{n-7} | 0.09±0.00a | | | | <0.001 |
| Total MUFA | 25.67±1.30a | 22.12±1.68b | 31.35±0.82c | 20.33±0.59b | <0.001 |
| 16:2 _{n-4} | 1.00±0.10a | 10.84±4.57b | 0.38±0.07a | 1.65±0.24a | <0.01 |
| 16:3 _{n-4} | 1.29±0.10a | 0.22±0.06b | | 0.53±0.06c | <0.001 |
| 16:4 _{n-3} | 0.17±0.01a | 0.13±0.04a | 0.12±0.03a | 0.86±0.11b | <0.001 |
| 18:2 _{n-6} | 8.47±0.10a | 1.96±0.25b | 5.18±0.27d | 7.09±0.28c | <0.001 |
| 20:2 _{n-6} | 0.17±0.01ab | 0.10±0.09 b | 0.08±0.03b | 0.55±0.17a | <0.05 |
| 20:4 _{n-6} | 0.82±0.01a | 0.55±0.15a | 2.27±0.319b | 0.77±0.09a | <0.001 |
| Total <i>n-6</i> PUFA | 10.16±0.10a | 2.91±0.38b | 8.01±0.45c | 9.07±0.39ac | <0.001 |
| 18:3 _{n-3} | 1.33±0.00a | 7.74±1.25b | 9.48±0.71b | 17.12±0.84c | <0.001 |
| 18:4 _{n-3} | 2.24±0.10a | 0.76±0.13b | 0.92±0.06b | 0.89±0.10b | <0.001 |
| 20:4 _{n-3} | 0.62±0.00a | 0.11±0.04b | 0.10±0.02b | 0.52±0.12a | <0.001 |
| 20:5 _{n-3} | 12.27±0.60a | 7.49±0.99b | 10.60±0.40a | 6.33±0.48b | <0.001 |
| 22:5 _{n-3} | 1.43±0.10a | | | | <0.001 |
| 22:6 _{n-3} | 10.30±0.60a | | 0.06±0.02b | | <0.001 |
| Total <i>n-3</i> PUFA | 29.13±0.20a | 17.17±1.83b | 21.31±0.84bc | 25.90±1.11ac | <0.001 |
| Total PUFA | 43.73±0.50a | 32.42±3.88b | 31.14±1.08b | 38.74±0.95ab | <0.01 |
| <i>n-3/n-6</i> | 2.87±0.10 | 11.78±5.05 | 2.85±0.17 | 2.98±0.24 | 0.08 |
| Unidentified | 0.48±0.01 | 3.64±2.39 | 0.79±0.22 | 1.04±0.25 | 0.24 |

Note: Values are mean ± SE. Sites and months are pooled and results of ANOVA are shown. Different letters indicate significant differences between each type of food. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

tioned with hexane and total lipid extract dissolved in chloroform was then added. The NL fraction was eluted with chloroform–2-propanol (2:1 *v/v*) and PL were eluted with diethylether–acetic acid (100:2 *v/v*) followed by methanol. After solvent evaporation, lipid extracts were methylated with BF₃ (14% *w/v* in methanol) to convert FA into their corresponding methyl esters (FAME). Samples were heated at 100 °C for 45 min, resuspended in hexane, and concentrated under N₂. FAME were separated and analyzed on a Chrompack CP9001 gas chromatograph fitted with a Supelco Omegawax column and an FID detector (split injection; injector temperature 260 °C, carrier gas helium, oven rise from 140 to 245 °C at 3 °C·min⁻¹). Individual FAME were identified by comparing retention times with those obtained from Supelco and our own laboratory standards and quantified using 13:0 as internal standard added just before FA derivatization. Peaks registering less than 0.1% of total area were considered nondetected. GC/MS was used to confirm identification of some FA. FAME were injected in a GC6850 Agilent gas chromatograph fitted with a 5975B Agilent mass spectrometer. Spectra separated with a DB wax column were identified using our own spectrum library and the American Oil Chemists' Society's lipid library (<http://www.lipidlibrary.co.uk>).

Data analysis

The statistical analyses were performed on the most important encountered FA: 16:0, 16:1_{n-7}, and 18:1_{n-9}, known as a specific oxidation substrate for energy especially during the seaward migration, 18:2_{n-6}, which is converted into 20:4_{n-6}, and 18:3_{n-3}, which is elongated and desaturated to 20:5_{n-3} and then to 22:6_{n-3} (Tocher et al. 2000; Bell et al. 2003a; Bendiksen et al. 2003). FA 20:4_{n-6} and 20:5_{n-3} are involved in eicosanoid synthesis and therefore in adaptation to salinity changes (Sargent et al. 1999).

Means and standard errors were calculated to characterize FA concentrations (milligrams per gram dry weight) in farmed and restocked parr, commercial pellets, and natural prey. Site/hatchery, date, and invertebrate taxon effects were analyzed by analysis of variance (ANOVA) followed where appropriate by Tukey's post hoc test to determine significant differences. All data were checked for normality and variance homogeneity before analysis and log transformed when necessary. Discriminant function analysis was performed to differentiate parr groups that tended to have similar FA concentrations according to growth period location (hatchery, site 1, site 2, or site 3). One discriminant function analysis was performed for FA in NL and another for FA in PL. All statistical analyses used XLStat-Pro soft-

ware (Addinsoft, Paris, France). Differences were considered significant at $P < 0.05$.

Results

Feeding, growth, and total FA content in farmed and restocked parr

Feeding

Over the entire survey, 25 prey taxa, mostly aquatic insects (mean = 99%), were identified in parr stomachs. In the three riffles, the predominant prey were Diptera larvae ($A_i = 72\%$ on average) and Ephemeroptera nymphs ($A_i = 22\%$) (Fig. 1). More specifically, chironomid larvae (Orthoclaadiinae, Chironominae, and Tanypodinae) provided major contributions to salmon diet in site 1 ($A_i = 71\%$), simuliid larvae (*Simulium* and *Prosimulium*) in site 2 ($A_i = 58\%$), and baetid nymphs (*Baetis rhodanii*, *Acentrella sinaica*, and *Procloeon bifidum*) in site 3 ($A_i = 60\%$). Others prey items, such as Coleoptera (*Elmis*), Gastropoda (*Ancylus fluviatilis*), Oligochaeta, Plecoptera (*Capnia* spp. and Leuctridae), and Trichoptera (*Psychomyia pusilla*, *Hydropsyche* spp., and *Rhyacophila* sensu stricto), were also consumed but generally constituted no more than an average of 14% of the diet and were therefore regarded as secondary or occasional prey (Fig. 1).

Growth

Significant variation in mean parr dry weight (Fig. 2) was found between restocking riffles and hatchery (ANOVA and post hoc Tukey's tests: $F_{[3, 172]} = 16.60$, $P < 0.01$). At the end of the survey, the maximum dry weights were reached in sites 2 (2.12 ± 0.24 g) and 3 (1.83 ± 0.32 g), with lower values in the most upstream site, site 1 (0.64 ± 0.06 g). In the hatchery, the maximum dry weight of reared fish was greater than for restocked fish, reaching 4.31 ± 1.09 g in October ($F_{[3, 31]} = 6.89$, $P < 0.01$). Furthermore, it is of interest to note that growth in the intermediate riffle (site 2) was the closest to that observed in the hatchery, although individual dry weight increased more regularly in the latter. Significant monthly variations were also recorded in each restocking riffle and hatchery tank ($F_{[5, 172]} = 18.36$, $P < 0.01$): growth rates were low during the first 2 months after restocking (June and July) and increased over time as of July (Fig. 2).

Total FA content

During the 2 months following restocking, NLFA storage (milligrams per gram dry weight) was higher in hatchery-reared parr than in restocked fish in any riffle (ANOVA and post hoc Tukey's tests: $F_{[3, 12]} = 20.48$, $P < 0.01$) (Fig. 2). On the other hand, at the end of the study, no significant differences in NLFA amounts were observed between restocking riffles and hatchery salmon ($F_{[3, 13]} = 2.46$, $P = 0.12$). Significant monthly variations were observed in fish from each restocking riffle and from the hatchery ($F_{[5, 75]} = 23.96$, $P < 0.01$). After a marked decrease between May and June, these FA increased and stabilized around 28 mg.g dry weight⁻¹ in all sites except in site 3 (16 mg.g dry weight⁻¹) (Fig. 2).

PLFA relative concentrations decreased from May to August in the hatchery and in each restocking riffle ($F_{[5, 74]} =$

42.55, $P < 0.01$). At the end of the survey, PLFA contents in farmed fish were the closest to those observed in site 2, i.e., slightly lower than in fish growing in sites 1 and 3 (Fig. 2).

FA analysis of natural food versus commercial pellets

The FA composition of total lipids extracted from each type of food (prey and commercial pellets) is shown in Table 1. Saturated FA represented 30% of total FA in Nutra pellets and more than 36% in invertebrate prey. FA 16:0 was the predominant saturated FA, ranging between 17% and 25%, with quite similar proportions in Nutra pellets and chironomid and simuliid larvae (around 17%–21%). FA 18:0 was also present but at smaller values and was more abundant in insect larvae (7%–11%).

MUFA generally represented 20%–32% of total FA in all diets. FA 16:1n–7 was the most abundant MUFA in both baetids and chironomids, while 18:1n–9 predominated in both hatchery diet and simuliids. Significant differences were also recorded with 20:1n–9 and 22:1n–9, which were present in pellets (2%–3%) but were found in low or negligible concentrations in insect larvae (Table 1).

PUFA were more abundant in the hatchery (44% of total FA) compared with wild diets (31%–39%). FA 18:2n–6 was detected in low proportions in baetids (<2%) and in larger proportions in pellets (8%), chironomids (7%), and simuliids (5%). FA 18:3n–3 was found in greater percentages in invertebrates (7%–17%), especially chironomids, than in hatchery diet (<2%). In contrast, 18:4n–3 was observed in negligible proportions in larvae and represented just 2% of the total FA in pellets. Negligible proportions of 20:4n–6 were also found in all diets, except in simuliids (2%). On the other hand, 20:5n–3 was present in abundance, particularly in hatchery diet and simuliids (11%–12%). In baetids and chironomids, this component represented 6%–7% of total FA. The most remarkable differences were observed for 22:6n–3: Nutra pellets showed about 10 times as much 22:6n–3 as did insect larvae, which seemed to contain only small proportions or none at all. In general, n–3 PUFA were always more abundant than n–6 PUFA in all types of diet. The sums of n–3 and n–6 PUFA, however, were higher in pellets and chironomids than in other diets, although the n–3 to n–6 ratio showed no significant difference between types of food (Table 1).

NLFA and PLFA compositions of farmed and restocked parr: differences and temporal changes

Based on the eight selected FA (16:0, 16:1n–7, 18:1n–9, 18:2n–6, 18:3n–3, 20:4n–6, 20:5n–3, and 22:6n–3), discriminant function analysis differentiated groups of parr that tended to have similar FA concentrations in NL and PL (Fig. 3). In NLFA and PLFA, the first two discriminant analysis functions explained 97% and 100% of intergroup variance, respectively (NLFA: Wilks' $\lambda = 0.00$, $P < 0.01$; PLFA: Wilks' $\lambda = 0.01$, $P < 0.01$). In NLFA, 79% of the variance was observed on the first discriminant function and separated the FA concentrations of parr fed with Nutra pellets in the hatchery from those released in the upper Allier riffles. The second discriminant function showed a less pronounced difference (18% of intergroup variance), discriminating the FA composition of salmon collected in the most

Fig. 2. Mean temporal changes (May–October) in (a) dry weights and mean amounts of total fatty acids extracted from (b) neutral lipids (NLFA) and (c) polar lipids of hatchery-reared Atlantic salmon parr and restocked parr (sites 1–3). Results of two-way ANOVA and Tukey’s post hoc tests are shown with different letters indicating significant differences between months in each site. ND, no data.

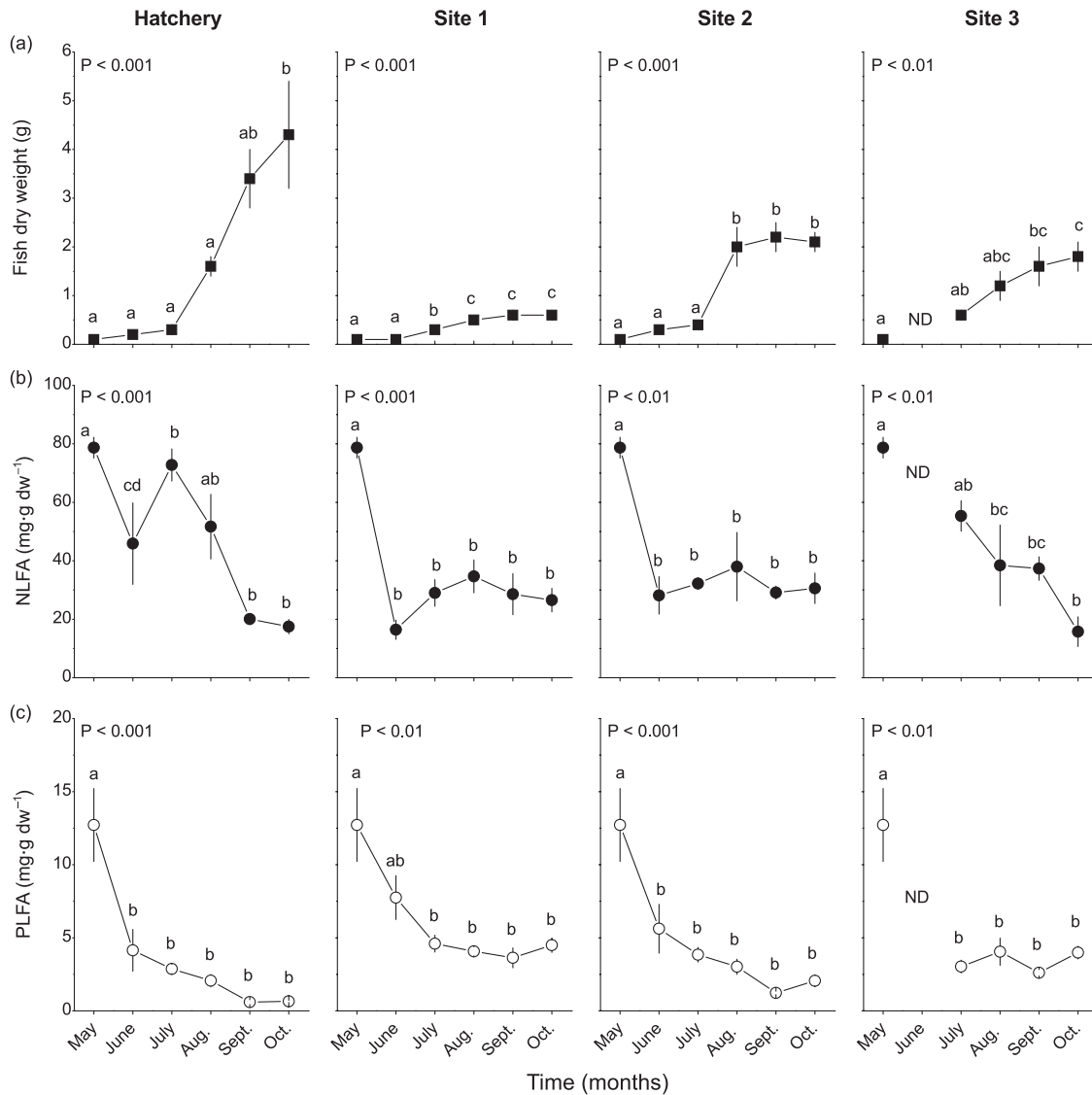
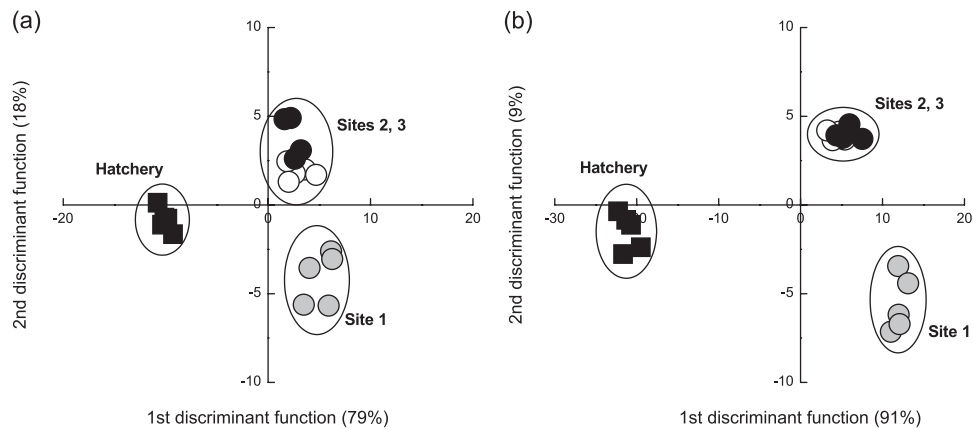


Fig. 3. First two components of the discriminant function analysis with individual fatty acids (mg·g dry weight⁻¹) extracted from Atlantic salmon (a) neutral lipids and (b) polar lipids as covariates and sample location (hatchery and sites 1–3) as grouping variable.



upstream riffle (site 1) from those sampled in the other sites (sites 2 and 3). These same significant differences were observed with PLFA, the first axis explaining 91% of intergroup variance and the second axis 9%. Thus, some NLFA and PLFA showed significantly different evolution between hatchery-reared parr and their counterparts released in riffles (Fig. 3).

At the beginning of the survey (June–July), the concentrations of 18:1*n*–9, 18:2*n*–6, 20:5*n*–3, and 22:6*n*–3 appeared to be higher in the NL of farmed than in those of restocked parr (Fig. 4). The same trend was observed with 16:0, 16:1*n*–7, and 20:4*n*–6, although not significant in these cases. On the other hand, farmed parr contained smaller amounts of 18:3*n*–3 (0.69 ± 0.11 mg·g dry weight⁻¹) in their NL in June–July than did those released in riffles (range 2.03 ± 0.80 to 6.98 ± 1.50 mg·g dry weight⁻¹). At the end of the study (in October), these values did not show significant differences between farmed and restocked parr, except for 18:1*n*–9, 18:3*n*–3, and 20:4*n*–6. FA 18:1*n*–9 was more abundant in NL of farmed (3.06 ± 0.38 mg·g dry weight⁻¹) than in those of restocked parr (range 1.22 ± 0.39 to 2.16 ± 0.36 mg·g dry weight⁻¹). Restocked parr contained significantly higher amounts of 18:3*n*–3 (range 1.39 ± 0.51 to 3.42 ± 0.85 mg·g dry weight⁻¹) and 20:4*n*–6 (range 0.21 ± 0.09 to 0.39 ± 0.06 mg·g dry weight⁻¹) than farmed parr (0.16 ± 0.03 and 0.13 ± 0.02 mg·g dry weight⁻¹, respectively) (Fig. 4).

For PLFA, no significant differences were recorded from June to July between hatchery and restocked salmon, except for 18:2*n*–6, 18:3*n*–3, and 20:5*n*–3 (Fig. 5). Restocked parr showed higher levels of these PUFA than did farmed parr. In October, on the other hand, all of the various FA were more abundant in restocked than in farmed parr. Moreover, these FA concentrations seemed to be higher in the first riffle (site 1) than in the others, except for 20:5*n*–3 (Fig. 5).

Diet effects on FA composition in farmed and restocked salmon parr

The potential contribution of the NLFA and PLFA supply of the different foods (Nutra pellets and insect larvae) eaten by salmon parr in the hatchery and natural reaches during the entire survey is shown in Fig. 6. In the hatchery, all FA were primarily provided in the form of NL in pellets, except 18:2*n*–6 which, was also provided as PL (1.49 mg·g dry weight⁻¹). For parr collected in riffles, a significant proportion of FA came from NL prey (chironomids in site 1, simuliids in site 2, and baetids in site 3) but also from PL prey, especially in sites 2 and 3. Moreover, in these sites, FA were provided as PL by more than one type of prey. NL 16:0 and NL 16:1*n*–7 were particularly abundant in the prey predominating in the stomach contents of restocked parr from sites 2 and 3. In contrast, their contribution remained lower in parr from site 1 and hatchery, especially with respect to PL input. The second MUFA, 18:1*n*–9, was strongly represented in the NL of artificial food. It was also found in large quantities in the NL and PL of simuliids and markedly in the PL of baetids ingested by parr from site 2. Concerning *n*–3 PUFA, the main difference was observed for 18:3*n*–3 and 22:6*n*–3. PUFA 18:3*n*–3 was, logically, recorded in small quantities in pellets but was found in abundance in the natural diet of restocked parr, especially in the first riffle, in the

form of either NL or PL. In contrast, 22:6*n*–3 was absent from macroinvertebrate NL and PL (Table 1). PUFA 20:5*n*–3 was provided in large quantities by the NL from Nutra pellets and chironomid larvae, whereas huge quantities of 20:5*n*–3 were detected in the PL from aetids and simuliids consumed by parr from site 2 and to a lesser extent from site 3. Finally, for *n*–6 PUFA, diet was rich in 18:2*n*–6 whatever the place of growth (hatchery or riffles), whereas only simuliids ingested by parr in site 2 provided significant amounts of 20:4*n*–6, in the form of both PL and NL (Fig. 6).

Discussion

Despite the variety of prey potentially available in the riffle habitats of the River Allier, only three prey families predominated in the stomach contents of restocked *S. salar* parr: baetid nymphs and chironomid and simuliid larvae. These macroinvertebrate prey have been reported as being a major part of salmon parr diet in numerous studies undertaken in reaches of temperate and northern streams (Thonney and Gibson 1989; Keeley and Grant 1997). However, 0+ parr feeding varied in the River Allier along the upstream–downstream gradient. Thus, chironomid larvae were the predominant prey ingested in the upstream riffle (site 1), simuliid larvae in the intermediary riffle (site 2), and baetid nymphs in the downstream riffle (site 3). In sites 1 and 2, parr fed mostly on larvae that predominated in the benthic fauna in terms of numerical abundance (chironomids or simuliids; Descroix et al. 2009). This was not the case in the last riffle (site 3) where the main prey ingested (baetids) was a significant but not predominant taxon in the benthos (Descroix et al. 2009). The importance of secondary prey as a source of FA could not be taken into account in the present study. However, apart from two site-specific sampling dates (September for site 1 and June for site 2), all secondary prey taken together never exceeded 20% of the diet.

Significant variations in parr growth performance and NLFA contents were observed between riffles and between parr reared in the hatchery and those released in the river. Such differences suggest variable growth conditions along the longitudinal gradient of the river sufficient to influence body composition. This could primarily be related to differences in the type of prey ingested as well as differential energy expenditure linked to intra- and interspecies competition (Descroix et al. 2009). It is only at the beginning of the study (June–July) that restocked parr accumulated less NLFA than hatchery-reared salmon. This could not be attributed to difficulties encountered in prey capture by inexperienced released juveniles. According to Reiriz et al. (1998), naïve restocked salmon parr acquire in 67 days, the appropriate prey selection criteria usually exhibited by their wild counterparts. From a nutritional viewpoint, it is likely that the type of prey ingested influenced the growth of restocked salmon along the longitudinal gradient in the present study. But, it is difficult to attribute these growth differences solely to variations in the FA composition of baetids, chironomids and simuliids because the digestibility of these prey and their calorific values and carbohydrate and protein contents probably differ (Gupta and Pant 1983; Batzer et al. 1993). Furthermore, many experimental studies

Fig. 4. Monthly variations in the average amounts of fatty acids extracted from neutral lipids of hatchery-reared Atlantic salmon parr and restocked parr (sites 1–3). Results of two-way ANOVA and Tukey’s post hoc tests are shown with different letters indicating significant differences between months in each site. ND, no data.

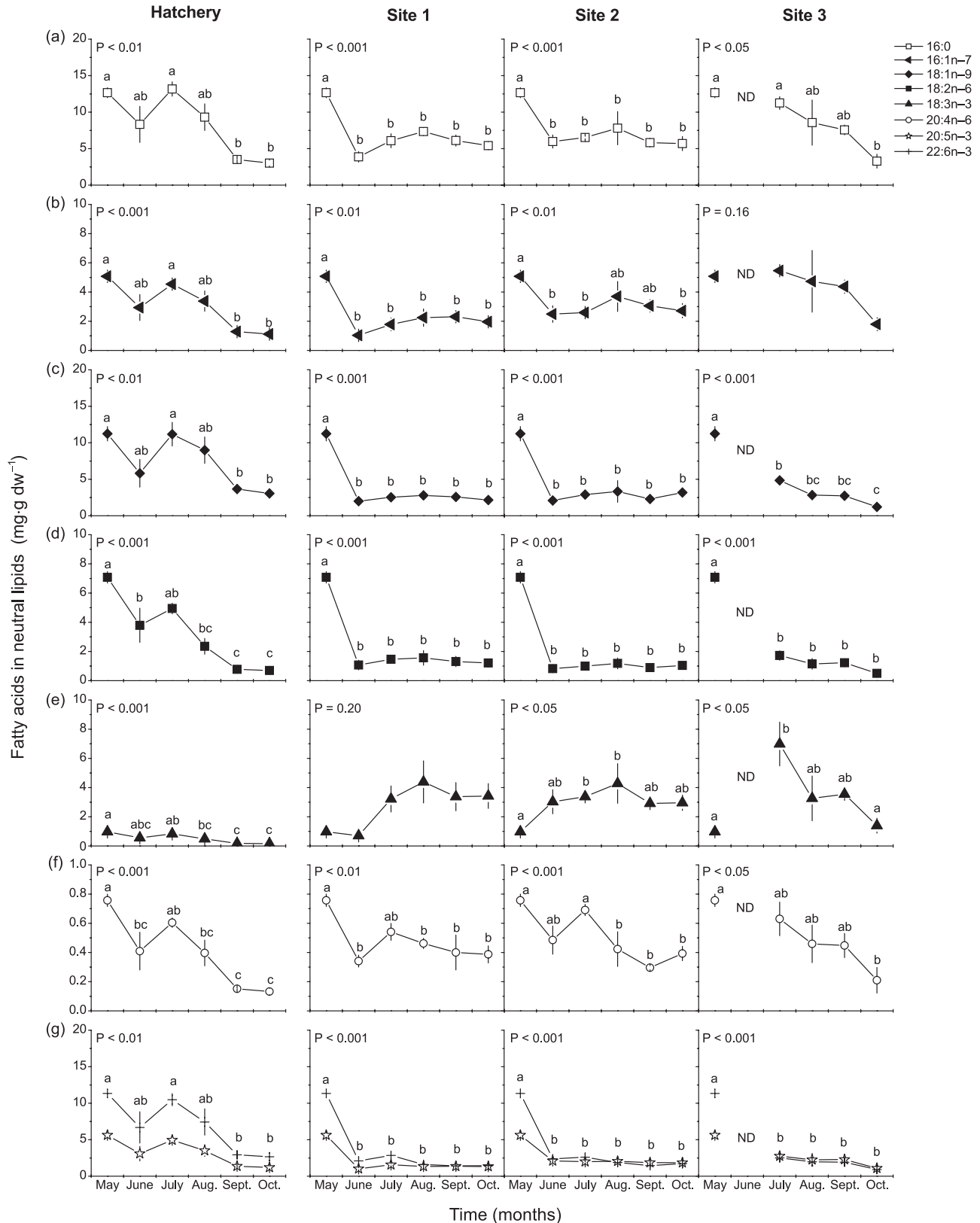


Fig. 5. Monthly variations in the average amounts of fatty acids extracted from polar lipids of hatchery-reared Atlantic salmon parr and restocked parr (sites 1–3). Results of two-way ANOVA and Tukey’s post hoc tests are shown with different letters indicating significant differences between months in each site. ND, no data.

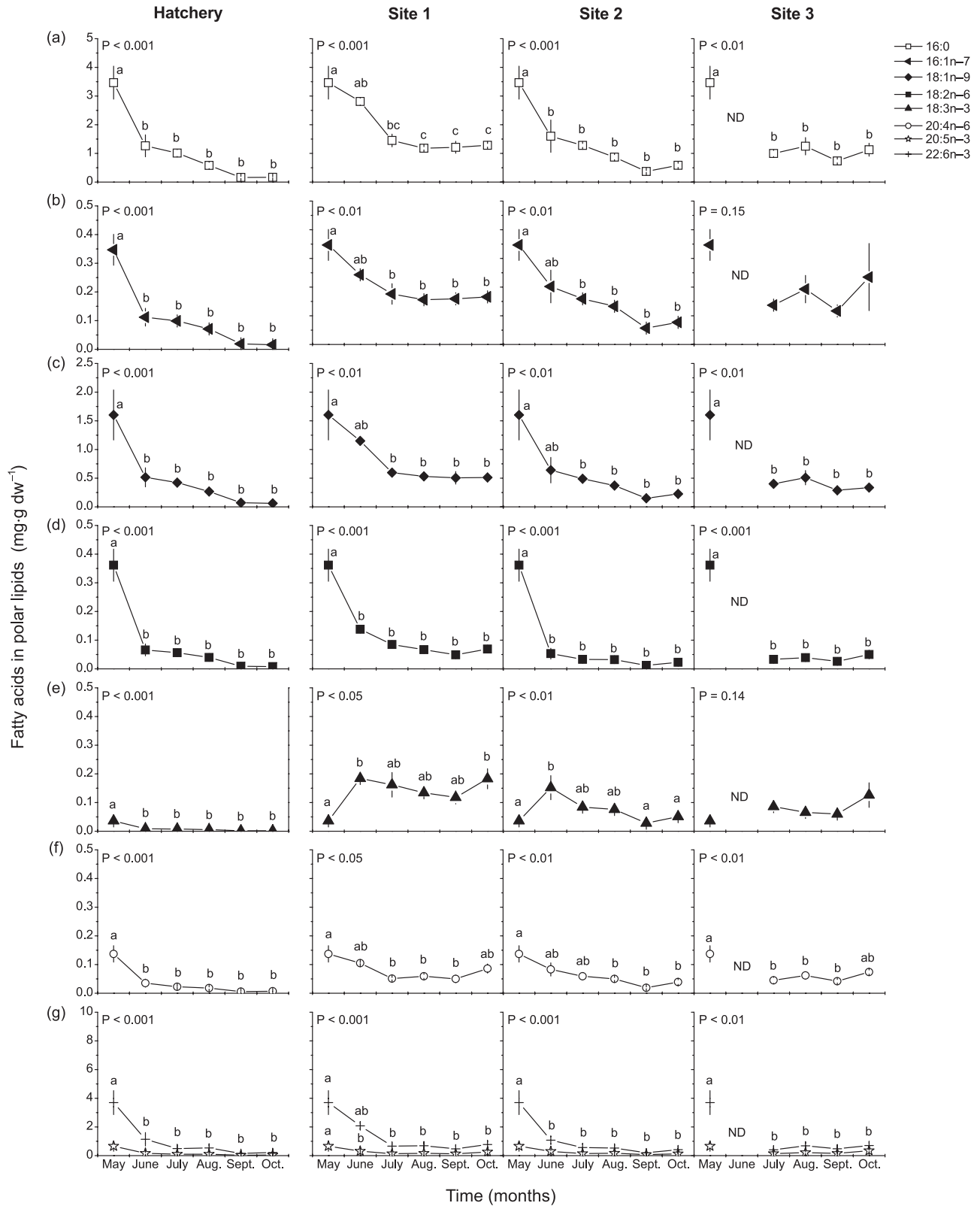
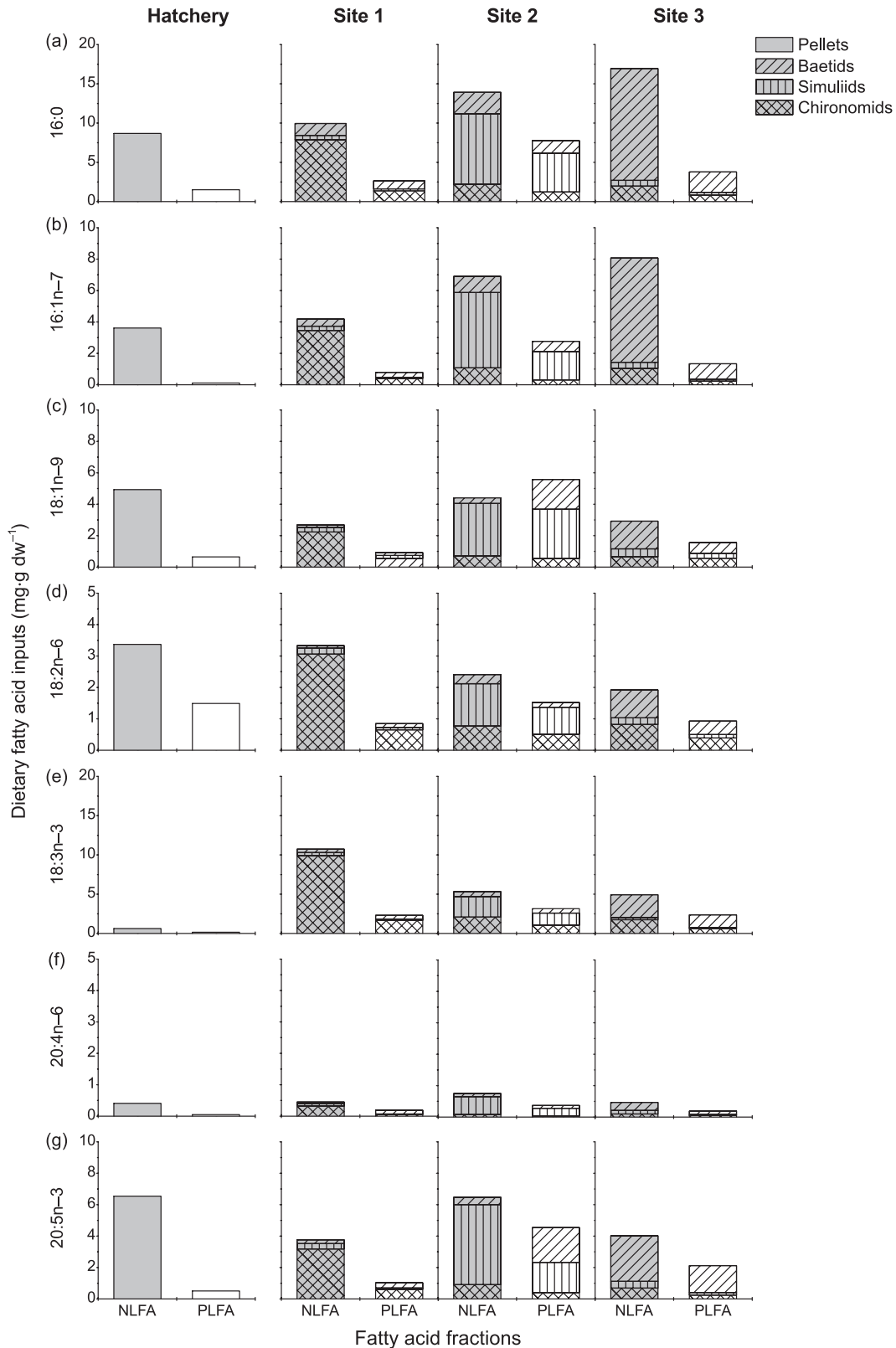


Fig. 6. Mean selected dietary fatty acid inputs from commercial pellets (left column, hatchery) and from the three predominant macroinvertebrate prey (right columns, sites 1–3). For macroinvertebrates, values were recalculated as a function of the average proportion of each prey type encountered throughout the survey in the stomach contents of parr from sites 1–3. NLFA, neutral lipid fatty acids; PLFA, polar lipid fatty acids.



have shown that when only the FA composition of artificial diets is modified (replacing fish oil by vegetable oil), the growth performances of parr or smolt remain unchanged and fish health and welfare are not affected (Robin et al. 2003; Miller et al. 2007). On the other hand, such experimental practices generally cause substantial change in the flesh FA composition of salmon parr and of several other species of anadromous salmonid (Bendiksen et al. 2003; Huang et al. 2008). Thus, one of the main reasons for conducting the present study was to assess whether different prey types would at least influence the body lipid FA composition, if not growth, of restocked salmon parr previously raised on artificial diet. In fact, the FA composition of freshwater aquatic insects turned out to be more diverse than we had thought, a fact that recent studies tend to corroborate (Sushchik et al. 2007; Torres-Ruiz et al. 2007).

For instance, MUFA and PUFA contents differed between the macroinvertebrates predominantly consumed by restocked parr. Baetid nymphs and chironomid larvae contained higher lipid levels of 16:1*n*-7 and simuliid larvae of 18:1*n*-9. These FA were reported to be the predominant monounsaturates in chironomid larvae and ephemeropteran nymphs (Bell et al. 1994; Ghioni et al. 1996). Concerning PUFA, the *n*-3 series were the principal components observed in all taxa (17%–26%), confirming previous observations by Bell et al. (1994). However, *n*-6 PUFA were detected in appreciable amounts (3%–9%), especially 18:2*n*-6. This FA exhibited the highest levels in chironomids and the lowest in baetids, which is consistent with observations by Sushchik et al. (2003). In contrast, only simuliid larvae had significant proportions of 20:4*n*-6, the major precursor of eicosanoids (Sargent et al. 1999). Another FA that may be involved in the synthesis of eicosanoids is 20:5*n*-3. This component was reported to be the most abundant *n*-3 PUFA in several taxa of freshwater insects, including Plecoptera and Ephemeroptera (Ghioni et al. 1996). This was particularly true for simuliids in this study, while 20:5*n*-3 appeared to be less abundant than 18:3*n*-3 in chironomids. Negligible amounts of 22:6*n*-3 were recorded in all analyzed prey, as previously reported (Bell et al. 1994; Goedkoop et al. 2000). It appeared from the present study that the FA composition of taxa such as simuliids and baetids was not so close to that of vegetable oil. They contained more 16:0, 18:0, and 16:1*n*-7 and less 18:1*n*-9 than rapeseed and linseed oils (Tocher et al. 2000). Regarding *n*-3 and *n*-6 PUFAs, simuliid larvae showed larger amounts of 20:4*n*-6 and 20:5*n*-3. Finally, only the FA profiles of chironomids seemed to be close to those of vegetable oil, with high percentages of 18:2*n*-6 and 18:3*n*-3 and a low percentage of 20:5*n*-3.

Discriminant function analysis distinguished restocked parr in the three riffles from the hatchery-reared parr. This clearly indicates that artificial versus river insect diet significantly affected NLFA and PLFA patterns in juvenile Atlantic salmon throughout the survey. From June to August, the levels of 16:0, 16:1*n*-7, 18:1*n*-9, and 18:2*n*-6 were lower in restocked than in hatchery-reared parr. We assume that these FA were readily mobilized to compensate for the increase in energy expenditure when young salmon were released into the riffles. FA such as 18:1*n*-9 and 18:2*n*-6 are known to be the major substrates for β -oxidation in salmonids (Hen-

derson 1996; Bell et al. 2002), while 16:0 is preferentially incorporated into PL (Tocher et al. 2008). Restocked salmon have to adapt quickly to their new environment, confronting new stimuli generally absent or limited in a hatchery environment: variable water velocity, live prey to seek and capture, etc. At the end of the survey (September–October), differences in the concentration of these FA between farmed and restocked parr tended to disappear. FA 16:1*n*-7 and 18:1*n*-9 were provided in variable but appreciable quantities, whether by natural prey or pellets. However, compared with 18:1*n*-9, 16:1*n*-7 was not detected in abundance in NLFA and PLFA, especially in restocked salmon. This specific trend seems to suggest that 16:1*n*-7 was selectively catabolized by parr living in the riffles, in preference to 18:1*n*-9. This could be a relatively new finding, since 18:1*n*-9 has often been regarded as the predominant FA used for energy cover by hatchery-reared salmon fed on various experimental diets (Bell et al. 2003*b*). FA 16:1*n*-7 is a typical diatom FA (Desvillettes and Bec 2009) that is probably more abundantly produced than 18:1*n*-9 in river ecosystems and therefore more easily transferred to higher levels in a food web (Sushchik et al. 2006).

The most pronounced differences in FA pattern linked to ingested food concerned *n*-6 and *n*-3 PUFA contents. The high level of 18:2*n*-6 in the artificial diet (8%) was linked to the incorporation of small quantities of poultry fat into Nutra pellet oil. Farmed parr had a greater amount of 18:2*n*-6 in their NL than did restocked parr, while 20:4*n*-6 was less abundant. At the end of the survey, in October, the amount of 20:4*n*-6 in the PL of hatchery-reared parr was significantly lower than that of any restocked parr. Hatchery-reared parr did not seem to biosynthesize this component, probably due to the abundant dietary supply of 20:5*n*-3 and 22:6*n*-3. Dietary HUFA (including 20:4*n*-6) are known to partially inhibit FA desaturase activity in salmonids (Tocher et al. 2006). The high level of 22:6*n*-3, logically detected in pellets and in the NLs of hatchery parr, may well have affected the conversion of 18:3*n*-3 and probably of 18:2*n*-6 into 20:4*n*-6 (Bendiksen et al. 2003). Concerning released salmon, it is interesting to note that parr from site 2, which obtained significant levels of 20:4*n*-6 from their simuliid prey, contained lower levels of this FA in their PL than did parr in either of the other sites. Our suggestion is that dietary 20:4*n*-6 may have inhibited the bioconversion of 18:2*n*-6 into 20:4*n*-6, which was not the case with the other restocked parr, owing to low levels of 20:4*n*-6 obtained from baetid or chironomid prey. Moreover, the lack of 22:6*n*-3 in prey ingested by restocked parr would in theory enable C₁₈ PUFA bioconversion activity to be maintained. However, 18:2*n*-6 could have probably been used as the principal Δ 6-desaturase endogenous conversion substrate because 18:3*n*-3 levels accumulated in the NL of restocked parr. According to Bell et al. (2003*b*), these results might suggest that 18:3*n*-3 was stored for future β -oxidation during anadromous migration or, presumably, during overwintering. Similar observations were reported in a study using deuterated 18:3*n*-3, which showed that catabolism via β -oxidation was preferred to conversion to 22:6*n*-3 in juvenile rainbow trout (*Oncorhynchus mykiss*) (Bell et al. 2001). In the present study, 22:6*n*-3 was recorded in very large quantities in Nutra pellets and not in macroinvertebrate prey. On

the other hand, the PL of restocked parr contained similar amounts of this HUFA to those observed in the PL of farmed salmon. FA 22:6*n*-3 synthesis occurs via the Sprecher pathway from 20:5*n*-3 originating from ingested prey and converted into C₂₄ PUFAs that are further retro-converted into 22:6*n*-3 (Miller et al. 2007). Because Δ6-desaturase also catalyses conversion of 18:3*n*-3 into 18:4*n*-3 and of 24:5*n*-3 into 24:6*n*-3, dietary 20:5*n*-3 is easily converted into 24:5*n*-3. Thus, 18:3*n*-3 and 24:5*n*-3 compete for Δ6-desaturase, inhibiting 18:3*n*-3 desaturation in restocked parr (Ruyter et al. 2000; Zheng et al. 2005).

The last interesting observation highlighted by the present study was that many FA, such as 16:1*n*-7, 18:1*n*-9, 18:2*n*-6, 18:3*n*-3, and 20:5*n*-3, were provided in sites 2 and 3 in the form of PL. Intact PL are required for optimal growth, development, and survival in larval and early juvenile fish of both marine and freshwater species (Coutteau et al. 1997). Indeed, Tocher et al. (2008) reported that the quantitative PL requirements of larval and juvenile salmon ranged from about 2% to 6% of the diet and apparently decreased with age. If no requirement has been observed in salmon greater than 7.5 g, this may be due to the short-term nature of the studies performed. Thus, in Atlantic salmon cultured over a 24-month growth cycle, low dietary phospholipid levels might induce growth depression and other effects over this longer period of culture (Tocher et al. 2008). In this context, it can be suggested that the high growth performances recorded from August to October at sites 2 and 3 could be partly related to the high input of these dietary PL. Indeed, salmon parr from site 1, which exhibited the lowest final growth, received the bulk of their FA in the form of NL (except 18:3*n*-3). In fish larvae and juveniles feeding on NL-rich diets, insufficient dietary PL limits lipoprotein synthesis, leading to impaired FA transport to tissue (Tocher et al. 2008). In contrast, hatchery-reared salmon had the best growth, although their diet contained mostly NLFA and few PLFA. This could indicate that there is probably some minimal level of dietary PL required by salmon parr that could be covered by Nutra pellets but not chironomid-based diet of site 1 parr. Once this minimal level of PL is met, there is probably little additional benefit for salmon. Obviously, further investigations on macroinvertebrate phospholipid structure are needed to confirm or not our suggestion and whether simuliid larvae and baetid nymphs could be of greater nutritional interest than chironomid larvae.

In conclusion, our results show that the type of prey ingested and growth performance of restocked Atlantic salmon parr varied along the longitudinal gradient of the River Allier. Site 1 can be considered as the extreme upstream limit for 0+ salmon restocking. The low growth recorded in this area could be detrimental to overwintering survival and subsequent smoltification. This can be supported by the significant percentage (12%) of parr that delay migration in the most upstream parts of the River Allier (Cuinat 1986) and by problems encountered by their future smolts to achieve complete downstream migration (P. Martin et al., unpublished data). In contrast, the most favorable site for growth was the intermediate riffle (site 2), with values close to those achieved in hatchery-reared parr. Simuliid larvae and baetid nymphs seem to be an interesting type of food. Therefore, from an ecological viewpoint, maintaining the in-

tegrity of riffle macroinvertebrate assemblages in a large river can be of great importance for salmon parr, as those insects are thought to be less tolerant to habitat and water quality degradation than chironomid larvae (Lautenschläger and Kiel 2005). Finally, simuliid larvae and baetid nymphs exhibit FA composition not so close to that of vegetable oils, as frequently postulated with freshwater insects. Because during the 2 months following restocking, NLFA storage was lower in restocked fish (in any site), it would therefore be useful to think of a new diet formula based on the FA profiles of these two families of freshwater insect. Moreover, it would be of interest to test whether such diets could be beneficial to salmon fry or parr intended for river restocking rather than commercial aquaculture and human consumption.

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