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# The effect of hatching time on the bioenergetics of northern pike (*Esox lucius*) larvae from a single egg batch during the endogenous feeding period

Awatef Trabelsi <sup>a,b</sup>, Andrzej Jaworski <sup>c</sup>, Ewa Kamler <sup>d</sup>, Jean-Noël Gardeur <sup>a</sup>, Fabrice Teletchea <sup>a</sup>, Habib Ayadi <sup>b</sup>, Pascal Fontaine <sup>a</sup>.

**a : Université de Lorraine, Unité de Recherche Animal et Fonctionnalités des Produits Animaux, USC INRA 340, 54505 Vandoeuvre-lès-Nancy, France**

**b : Laboratoire d'Ecologie Animale et de Planctonologie, Département des Sciences de la Vie, Faculté des Sciences de Sfax, BP 802, 3018 Sfax, Tunisia**

**c : Marine Scotland -Science, Marine Laboratory, PO Box 101, 375 Victoria Road, Aberdeen AB11 9DB, UK**

**d : Pond Fishery Department, Inland Fisheries Institute, Żabieniec, 05-500 Piaseczno, Poland**

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## Keywords

*Esox lucius*, chemical composition, energy content, hatching time, larvae, yolk

## Abstract

Size, caloric value and chemical composition were measured separately in the progeny of two northern pike (*Esox lucius*) females at 3-day intervals during the endogenous feeding period from hatching to final yolk resorption. Tissue, yolk and entire larvae were analysed separately in three groups of larvae that hatched at different times (between 88 and 106 degree-days post-fertilization). An integrated approach with the Gompertz model was used to compute the yolk conversion efficiency and time to maximum tissue size in early, mid and late hatched larvae. At hatching, unresorbed yolk of early hatched larvae contained more energy (39.20 J) and more protein (0.99 mg) compared to the yolk of larvae that hatched later (38.13 J and 0.92 mg protein for late hatched larvae,  $p < 0.05$ ). In contrast, a significant reduction in tissue weight (-0.7 mg DW) and protein content (-0.5 mg) was found in early hatched larvae compared to those which hatched later ( $p < 0.05$ ). Between days 9 and 12 post-hatching (108 and 144 degree-days post-hatching), close to the final yolk resorption, late hatched larvae stopped growing and their tissue began to be resorbed. This tissue resorption time was delayed in early hatched larvae which presented at the end of the experiment a greater tissue weight than late hatched ones. Yolk conversion efficiency in term of energy from hatching to complete yolk resorption stage was significantly higher for early and mid hatched larvae (51 %) compared to late hatched ones (44 %) ( $p = 0.004$ ). Furthermore, the time to maximum tissue size was found to be negatively related to hatching time which implies that early hatched larvae take longer time to switch from one developmental stage to the next. The maximum tissue dry weight and energy content were found to be reached at approximately the same age post-fertilization for both early hatched and late hatched larvae, suggesting that the principal steps in a fish's lifespan are better correlated with time of fertilization than hatching time.

## 1. Introduction

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A fish embryo develops within an egg where it is protected by an egg capsule (chorion) and a perivitelline fluid from biotic and abiotic components of the environment (Finn 2007). At hatching, an increase in locomotor and metabolic activities in newly hatched larvae is observed (Kamler and Kato 1983; Kamler 1992; Kamler et al. 1995). According to Yamagami (1988) and Fuiman and Werner (2002), the hatching event

constitutes a key ontogenetic step during the early life of fish. However, other authors like Peñáz (1983) and Balon (1984) do not consider it as an important developmental event because it is not correlated with important morphological changes and it depends on environmental factors, particularly temperature and dissolved oxygen levels. Thus, a premature or a delayed hatching can occur under different conditions, and

larvae hatch at different sizes and ontogenetic advancement (precocial vs. altricial species; Balon 1981, 2001, 2002). This was observed even among larvae from the same batch of eggs (Geffen 2002; Laurel et al. 2008; Trabelsi et al. 2013). A protracted hatching period (defined as the time span over which a batch of eggs hatches) induces larval size heterogeneity (Baras et al. 2003; Laurel et al. 2008; Trabelsi et al. 2013) that is perceived negatively par many authors. It is considered to be a key to cannibalism and growth depensation as reviewed in Baras et al. (2000), Baras and d'Almeida (2001) and Kestemont et al. (2003). Thus, studying the heterogeneity of initial size is particularly important for gaining an understanding of the population dynamics of juvenile fish and their subsequent recruitment to the adult stock of a fish population (DeAngelis et al. 1993).

Some studies focusing on the size heterogeneity induced by the hatching time both within and between egg batches were based on morphometric and growth analysis (Methven and Brown 1991; Geffen 2002; Kestemont et al. 2003; Porter and Bailey 2007; Ivanova and Svirskaya 2009; Steinfeldt et al. 2011; Trabelsi et al. 2013). An improved growth rate was usually observed in larvae that hatched at the beginning of the hatching period (Methven and Brown 1991; Geffen 2002; Kestemont et al. 2003; Porter and Bailey 2007; Trabelsi et al. 2013) and is likely to be a consequence of enhanced yolk conversion efficiency by early hatched larvae (Trabelsi et al. 2013). However, the growth rate reported by Trabelsi et al. (2013) was based on morphometric measurements which may depend on body shape and tissue hydration and can be biased by the process of shrinkage (Dawirs 1981; Ferron and Leggett

1994; Kamler 2008). Thus, to more understand the effects of hatching time on larval growth, the evaluation of the biochemical composition of larval tissue and yolk in relation to hatching time and growth should provide additional insights into the emergence of size heterogeneity at larval stage.

In the present study, we assessed size (dry weight), caloric value and chemical composition in two compartments of yolk-feeding larvae: tissue and yolk, as well as in entire larvae, during the endogenous feeding period from hatching (H) to final resorption of yolk sac (Re). This was motivated twofold: first, to better understand the effects of hatching time on the development of fish larvae from a biochemical and physiological perspective, and second, to verify the statement of Trabelsi et al. (2013) in which the hatching time was found to strongly affect larval morphology and growth of the northern pike (*Esox lucius*, further referred to as pike) larvae. The two matrices (yolk and tissue) were considered separately in order to follow the exchange of materials and energy between these two compartments. In fact, the morphometric study carried out by Trabelsi et al. (2013) did not show any relation between the size of the remaining yolk and the higher growth accomplished by early hatched larvae, which disagrees with several studies that stressed the importance of the quantity and quality of yolk during the endogenous feeding period in the development of fish larvae (Kennedy et al. 2007). Two approaches were used in the present study to describe temporal changes in the size of pike larvae: an analytical approach showing short-term changes, and an integrated approach which uses a mathematical model to describe these changes as a function of time.

## 2. Material and Methods

### 2.1. Fish and experimental facilities

The broodstock came from the pond Neuf-Étang, 8 ha, Domaine de Lindre, Moselle, France, where they were fed live prey (forage fish) throughout their life. Ovulation was induced by a single injection of carp pituitary extract (4 mg/kg body weight). Males received the same hormone (2 mg/kg). Two egg batches (weights: 200 and 510 g) were obtained by stripping and artificial fertilization of oocytes from two females (F1: 59 cm, 1.6 kg; F2: 53 cm, 1.1 kg) on 16 March 2009. In order to avoid any effects on the eggs and larvae related to polyandry, the oocyte batch for each female was fertilized with the milt of one male only.

Just after fertilization, each egg batch was placed in a plastic bag with water under pure oxygen, and transported to the laboratory in a polystyrene box. At the laboratory, egg batches (20 h-degrees post-fertilization) were acclimated gradually to hatchery water temperature (from 13.5 to 12.0 °C) and then incubated separately in three Zug jars (one for the eggs of the female F1 and two for the eggs of the female F2) directly connected to the hatchery water supply. During the entire incubation period in the laboratory, water temperature was set at 12.0 ± 0.5 °C, dissolved oxygen level ranged from 8 to 9 mg/l, and total ammonia and nitrite concentrations were kept below 0.02 and 0.2 mg/l, respectively. The photoperiod was fixed at 12L/12D (12 h daylight/12 h darkness), and light intensity at the water surface was 190 lx.

Hatching lasted approximately 1.5 days. The first larva appeared at 88 DD (day-degrees) and the last one at 106 DD. According to hatching time, larvae were designated as “early”(larvae hatched during the first 12 h), “mid”(larvae hatched during the second 12 h) and “late”hatched larvae (larvae hatched during the last 12 h), and they were collected at 94, 100 and 106 day-degrees post-fertilization, respectively.

The experimental steps for each combination of female and hatching time, beginning at hatching time, are shown in Fig. 1. Newly hatched larvae (600 larvae per hatching time from each female) were transferred into three 1.5-L net cages with plastic exoskeletal frame (200 larvae per cage) suspended within the hatchery tray (with a volume of 770 L). They were kept without feeding until the end of the experiment to avoid a possible effect of a feeding factor on our results. Three samples (replicates) of 30 larvae from each rearing cage at each hatching time group (HT1, HT2 and HT3) were taken at hatch (D0) and at every 3 days until 15 days post-hatching (D3, D6, D9, D12 and D15). Subsequently, they were preserved in 4 % formaldehyde for further analyses.

At the end of the experiment, a total of 3240 larvae (which corresponds to 90 larvae per hatching time per female at six observation days: D0, D3, D6, D9, D12 and D15) were sampled and washed with distilled water. Yolk sacs were separated from tissues with a needle under Olympus SZX7 zoom stereo microscope, and were pre-dried in a drying oven (Firlabo, Air Concept) during 24 h at 60 °C (Edmondson and

Winberg 1971). Measurements of yolk and tissue dry weight were made separately to the nearest 0.01 mg after desiccation to constant weight over silica gel. The yolks of the 30 larvae as well as their tissues were pooled separately in order to obtain a sufficient quantity of biological material for the elemental analysis.

## 2.2. Chemical composition

The chemical composition of dry matter was measured in triplicate samples that had been individually homogenized in an agate mortar, sieved and homogenized again. Elemental analyses (C, H, N and S, % dry matter) were performed from 2.5 mg sub-samples using a Flash Elemental Analyser 1112 (ThermoFinnigan 2003); methionine served as a reference. Oxygen content (O, % dry weight, DW) was determined by pyrolysis from another 2.5 mg subsamples. Caloric value (Cal. val., J/mg DW) was calculated from an equation provided by the analyser:

$$\begin{aligned} \text{Cal. val. (J/mg DW)} = & 0.004184[88\% \text{ C} \\ & + 344(\% \text{ H} - 0.125\% \text{ O}) \\ & + 25\% \text{ S}] \end{aligned} \quad (1)$$

Due to the limited amount of material available for ash analysis, ash fraction (% DW) was calculated from:

$$\begin{aligned} \text{Ash (\% DW)} = & 100\% - (\% \text{ C} + \% \text{ H} + \% \text{ N} \\ & + \% \text{ S} + \% \text{ O}) \end{aligned} \quad (2)$$

(Quelet 1961). "Protein fraction" (P, % DW) includes both protein-bound amino acids and free amino acids. It was calculated as recommended by Gnaiger and Bitterlich (1984) for aquatic organisms:

$$P\% = 5.78 N\% \quad (3)$$

## 2.3. Calculations and statistical analysis

During the analysis, we realized that the amount of pooled yolks and bodies of mid hatched larvae (HT2) from female 2 sampled at day 3 (30 larvae per sample) was insufficient for the elemental analysis (2.5 mg). Consequently, all data corresponding to D3 were not used for the statistical analysis.

Two alternative approaches were used to describe ontogenetic changes in size of pike larvae developed exclusively on endogenous reserves. The first was an ANOVA with repeated measures and the MIXED procedure for mixed models, using SAS 9.0. (SAS/STAT® User's Guide 1989), and applied to matter composition (ash and protein % DW; caloric value J/ mg DW), individual size (DW, mg/individ.), protein content (mg/individ.), ash content (mg/individ.) and energy content (EC, J/individ.). The structures of covariance are of type CSH (Heterogeneous Compound Symmetry), or AR (1) [Autoregressive (1)] for variables protein total (%) and caloric value (CV) of yolk, or UN (1) [Unstructured (1)] for variables protein tissue (mg) and ash total (mg) because of infinite

likelihood. Effects which were tested included: female (F, df = 1, 12), hatching time (HT, df = 2, 12), larval age (A, df = 4, 22) and the interactions F 9 HT (df = 2, 12), F 9 A (df = 4, 22), HT 9 A (df = 8, 22) and F 9 HT 9 A (df = 8, 22). For the assessment of the biochemical composition of yolk, measures were only done on samples taken at days 0, 6 and 9, because at the following sampling day (D12), the yolk was nearly resorbed (or completely exhausted) and technically, it was not possible to separate it from the larval body. Therefore, the degrees of freedom were modified for the main effects: F effect (df = 1, 11), HT effect (df = 2, 11), A effect (df = 2, 2) and for the interactions: F 9 HT (df = 2, 11), F 9 A (df = 2, 2), HT 9 A (df = 4, 2) and F 9 HT 9 A (df = 4, 2). The Bonferroni correction was applied to compare differences between the adjusted means (LS means). In the present study, the focus was on the effects of two important interactions, HT 9 A and HT 9 F, on the larval biochemical composition and energy content. The second approach consisted of a smooth model involving two sigmoid curves representing dry weight and energy content changes over time in the yolk and larval body (yolk ?tissue) (Jaworski and Kamler 2002). It was employed to integrate and describe ontogenetic changes in size in a yolk-feeding larva without taking into account transient changes. In the present study, the Gompertz model was used to describe each of the two curves:

$$S(\tau) = S_0 \exp(-\exp(-g(\tau_0 - \tau))) \quad (4)$$

where  $S(t)$  is the larval body size or yolk size at age  $t$ ,  $S_0$  is the asymptotic size,  $g$  is the instantaneous growth rate at the inflection point,  $t_0$  (Schnute 1981). In the present study, the model was fitted for each combination of hatching time (HT1, HT2 and HT3) and female (F1 and F2). Individual size was expressed either as dry weight (mg/individ.) or energy (J/individ.).

Gross efficiency of yolk transformation into body tissue (Calow 1977) was computed in a cumulative form (Klekowski and Duncan 1975), which is applicable for early fish developmental stages (Kamler 1992). The cumulative gross efficiencies (%) were computed from:

$$K_{1c} = 100P_c / C_{Yc} \quad (5)$$

where  $P_c$  is the amount of dry weight (mg/individ.) or energy (J/individ.) accumulated in the larval body tissue from hatching (time 0) to any time point before to final yolk resorption, and  $C_{Yc}$  is the cumulative amount of dry weight or cumulative energy consumed during the same time period from yolk. Thus, the cumulative gross efficiencies were presented in terms of dry weight ( $K_{1c}$  DW) or energy ( $K_{1c}$  EC).

An ANOVA was applied to the maximum tissue size (MTS),  $K_{1c}$  DW and  $K_{1c}$  EC, computed for two periods, (1) from hatching to the Time of Maximum Tissue Size (TMTS) and (2) from hatching to final yolk resorption. The timing of MTS and Re was captured from the Gompertz model. This part of the analysis was conducted using the STATBOX (Grim-mersoft) software. Hatching time (df = 2) was the only effect to be tested. Newman-Keuls and Bonfer-roni tests were used for the mean comparisons ( $p < 0.05$ ).

### 3. Results

Following the hatching, the larvae were collected at 94 (early hatched larvae), 100 (mid hatched larvae) and 106 (late hatched larvae) day-degrees post-fertilization. Embryonic mortality was low (less than 5 %) and no deformed larvae were observed. The results of ANOVA showing the effect of different factors and their interaction on the dry weight, caloric value, energy content and biochemical composition of the whole larvae and the two analysed body compartments are summarized in Tables 1 and 2.

#### 3.1. Dry weight

Yolk dry weight in endogenously feeding pike larvae decreased continuously (Fig. 2c), while tissue dry weight increased after hatching, reaching a maximum shortly before the final yolk depletion, and decreasing afterwards (Fig. 2e). All groups of larvae (early, mid and late hatched) in the present study exhibited the same pattern of changes in yolk and tissue dry weight, but in late hatched larvae, the changes occurred earlier. Dry weight of remaining yolk did not differ significantly between early, mid and late hatched larvae at all three sampling occasions (D0, D6 and D9, Fig. 2c). In contrast, tissue dry weight during days 0–9 post-hatching was higher for late hatched larvae than for mid and early hatched individuals (Fig. 2e). The tissue dry weight of late hatched larvae initially increased to an apparent maximum found in the sample taken on D9, and then decreased between D9 and D12. In the mid and early hatched larvae, the apparent maximum dry weight was found on D12, when they had a significantly higher tissue dry weight than late hatched ones. On day 15, early hatched larvae showed the highest tissue dry weight.

For the entire larva (yolk +tissue), dry weight decreased during the experiment (Fig. 2a). It appeared to be higher in early hatched larvae, but significant differences were found only in D6 and D15 samples.

#### 3.2. Caloric value and energy content

The caloric value of dry matter (CV, J/mg DW) in tissue during the whole experimental period was lower than that in yolk (25.2 vs. 27.5 J/mg DW in tissue and yolk, respectively, Table 1). The tissue and yolk caloric values were almost constant during the entire experimental period and did not change with regard to the hatching time (HT non-significant, Table 1). Similarly, for the whole larva, significant but small differences only were observed on day 9 between the groups of early, mid and late hatched larvae (25.58, 26.03 and 25.09 J mg<sup>-1</sup> DW, respectively). For all other days, no significant difference was observed between the three groups of larvae.

The interaction HT 9 A was found to be significant for energy content of the whole larva (Table 1). Its energy content (EC, J/individ.) decreased during the endogenous feeding period (Fig. 2b). It did not differ among the three groups in newly hatched larvae (D0). Later on, early hatched larvae had a slightly elevated EC compared to mid and late hatched larvae, but this difference was significant only at D6.

Yolk energy content decreased significantly with age and hatching time. No significant effect of the interaction HT 9 A was observed on the yolk EC (Fig. 2d), but the simple effect

HT was significant (Table 1). Early hatched larvae had significantly ( $p < 0.01$ ) higher yolk energy content (EC = 21.58 J/individ.) than late hatched larvae (EC = 17.87 J/individ.) (here the general mean values for all studied periods were compared).

For the tissue energy content, the interaction HT 9 A was found to be significant (Table 1) and its changes over time (Fig. 2f) followed the same pattern as that of dry weight (Fig. 2e). Energy content in tissue was initially (on D0) low and similar for the three groups of larvae. It increased from hatching to D12 for early and mid hatched larvae and then decreased. For late hatched larvae, the decrease began earlier, from day 9 post-hatching. On days 6 and 9, tissue energy was significantly higher in the late hatched individuals. These larvae showed the lowest tissue energy content on day 12, but were similar to the other larvae by day 15 post-hatching.

#### 3.3. Biochemical composition

The mean ash and protein percentages in the whole larva and the two analysed body compartment ranged between 4 and 6 % DW and 69 to 71 % DW, respectively (Table 2). There was no difference in ash percentage in tissue, yolk or the whole larva for early and late hatched larvae during the whole experimental period. Ash content (mg/individ.) decreased in yolk and increased in tissue during all the endogenous feeding periods and for all larvae.

A significant effect of the interaction F 9 HT was observed for yolk protein content (Fig. 3). The hatching time effect was more important for female 2 than for female 1. For both females, the yolk protein content of early hatched larvae (mean values for the post-hatching period) was significantly higher than that of late hatched ones (0.53, 0.49 and 0.43 mg/ individ. for HT1, HT2, and HT3, respectively,  $p < 0.05$ ).

The effect of the interaction HT 9 A on tissue protein content (Fig. 4) was the same as the effect of this interaction on dry weight (Fig. 2e), of which protein formed a major part (71 % DW, Table 2). Protein content initially increased, and then decreased with a peak at D9 in the late hatched larvae and delayed to D12 in mid and early hatched larvae. During this increase, the protein content of late hatched larvae was higher than in mid and early hatched larvae, with a significant result at hatching time and at D9. A weak tendency for reduced tissue protein content in the late hatched larvae compared to the mid and early hatched ones was found on days 12 and 15, but the result was not significant.

For the whole larva, the protein content decreased significantly with age and was significantly higher for early hatched larvae compared to mid and late hatched ones (0.93 mg for HT1; 0.90 mg for HT2 and 0.89 mg for HT3,  $p < 0.001$ ). Moreover, no significant difference was observed for the protein fraction in yolk and tissue. For the entire larva, the percentage of protein varied little with age.

### 3.4. Integrated approach with a Gompertz model

The curves obtained with the Gompertz model for early, mid and late hatched larvae are presented in Fig. 5. Estimated time at the inflection point for yolk (sOY) decreased with delayed hatching (sOY HT1 > sOY HT2 > sOY HT3). This order was observed in the progeny of females 1 and 2, both for dry weight and energy content (Table 3).

Time to reach maximum tissue size (TMTS, smax), expressed in dry weight or energy content was between 9 and 11 days for the three groups of larvae. The late hatched larvae attained maximum tissue size (MTS) earlier. Their TMTS was shorter

by 12 and 9 % for dry weight and energy content, respectively, compared to the early hatched larvae. Maximum tissue size did not differ significantly among the three groups of larvae (with a slight tendency for energy content at MTS to decrease from HT1 over HT2 to HT3). The same result (no significant difference) was found for the gross yolk conversion coefficient at MTS and Re in terms of dry weight (ranging from 77 to 79 % and from 54 to 58 %, respectively). In contrast, the hatching time effect was significant for the gross yolk conversion coefficient in terms of energy content, both at MTS and at Re ( $p < 0.05$  and  $p < 0.01$ , respectively, Fig. 6). Early and mid hatched larvae had a significantly higher K1c compared to the late hatched ones (the test power ranged between 60 and 76 %).

## 4. Discussion

### 4.1. Biochemical composition and energy content

In this study, biochemical and bioenergetic differences in both yolk and body tissues were observed between pike larvae hatched at the beginning, the middle and the end of the hatching period. These differences (+24 % yolk energy, +19 % yolk protein, -19 % tissue dry weight and -21 % tissue protein for early hatched larvae compared to late hatched ones, mean values for the whole experimental period, Tables 1, 2) were found at hatching and they remained visible on subsequent days post-hatching confirming that early hatched larvae were more precocial. Indeed, according to Geffen (2002), larvae coming from a single egg batch can hatch at different developmental stages and size at hatching is correlated with the developmental stage at which hatching occurs. Smaller size (lower tissue dry weight) of early hatched pike larvae observed at hatching can be a result of a smaller size at the embryonic stage. In fact, Green et al. (2006) observed in anemonefish *Amphiprion melanopus* that small size embryos measured for total length 2 h after fertilization develop into small larvae at the time of hatching. Given that a smaller embryo body size may facilitate movement inside the egg capsule (Ciuhandu et al. 2005; Ninness et al. 2006), it is possible that the precocious hatch of some pike larvae at a smaller size is a result of intensified metabolism and increased oxygen demand of these embryos which triggers an escape from egg capsules (Miller and Kendall 2009).

No significant differences in energy content of the entire larvae (Fig. 2b) or in tissue energy content (Fig. 2f) were found among early, mid and late hatched larvae at D0. Likewise, in herring *Clupea harengus*, a relative constancy of energy content of newly hatched larvae reared over a large range of temperature (3.5–17.0 °C) was observed (Overnell 1997). This may suggest that the exact timing of hatching is determined by the achievement of a particular energy level that meets all demands of newly hatched larvae during the first days of their life without egg capsule protection.

In the present study, low energy content was found in the yolk of late hatched larvae. This can be explained by the fact that yolk energy reserves expended to satisfy metabolic demands of the embryos, partly powered by anaerobic glycolysis, are higher inside than outside egg capsules. This energy generated in anaerobiosis (per mol) is only a few per cent of the energy released in full aerobic reaction (review in Kamler 2008).

The lower yolk protein content in late hatched larvae was expected because during the last period of the embryonic development, just before hatching, amino acids (a part of the protein fraction in this study) continue to be used both for tissue protein synthesis and as a catabolic fuel. According to Monroy et al. (1961), fish embryos develop at the expense of yolk protein degradation and the liberation of amino acids. For pike, studies about the yolk nutrients use during the embryonic period are scarce. The only work conducted by Desvillettes et al. (1997) was about the changes in lipid classes and fatty acid composition in eggs incubated at 15 °C. These authors showed a heavy lipid consumption during the embryonic stage which could be the outcome of thermal conditions higher than the usual temperature encountered in a broodstock (9–12 °C). This suggests that an alternative energy source (e.g. proteins) is commonly used at normal incubation temperature. For rainbow trout (*Oncorhynchus mykiss*) larvae, a small and insignificant difference in yolk protein content was observed between individuals whose egg capsules were removed 6 days before the hatching day (day 6) and natural hatched larvae on day 0 (Ninness et al. 2006). These authors argued that small differences in yolk protein content are difficult to resolve because of the high concentration of protein in yolk compared to the whole body. Another explanation possible for the disagreement between the present study and that of Ninness et al. (2006) is that the sequence of substrates mobilized to form new tissues is species-specific, i.e. for the rainbow trout (as for other trout species), proteins may not be the first fuel used from yolk to be converted into tissues. This finding is corroborated by Haliloğlu et al. (2003) observations about the use of saturated and monounsaturated fatty acids as energy substrate for rainbow trout embryo, alevin and fry.

In this study, we observed that the changes in tissue dry weight, tissue energy content and tissue protein content followed a pattern in relation to age and hatching time similar to that observed by Trabelsi et al. (2013) in morphometric parameters, larval total length and body area. In contrast, changes in yolk biochemical composition and energy in relation to age and hatching time differed from those observed in yolk sac morphometrics. This indicates that energetic, biochemical and morphometric approaches are complementary. The weakness of morphometric studies alone, particularly when applied to early stages of aquatic organisms, is that differences in body shape and hydration may be a source of bias (review in Kamler 2008).

## 4.2. Age effect on tissue and yolk biochemical composition

Transition from yolk to external food is a key critical period in fish early life (review in Kamler, 1992). The onset of exogenous feeding occurs when the larva reaches its maximum tissue size (MTS) (Rønnestad et al. 1995). In this study, the analytical approach did not allow us to determine the exact timing of maximum tissue size. For example, maximum dry weight of late hatched larvae might occur somewhere between day 6 and day 12 which is a wide time span (Fig. 2e). In contrast, the modelling approach (Fig. 5; Table 3) enabled us to determine when tissues theoretically reach their maximum dry weight and energy content. Such an approach can be recommended as a complement to the analytical approach in yolk-feeding fish that develop rapidly. For dry weight, this timing occurred at 11.0 and 9.6 days post-hatching, respectively, for early and late hatched larvae. If we take into account the difference of 1.5 days between the early hatched larvae and the late hatched ones (88 and 106 day-degrees at 12 °C), we find that maximum tissue dry weight and energy content were reached approximately at the same age post-fertilization (Fig. 7a, b). This implies that, biologically, the principal steps in a fish's lifespan are better correlated with time of fertilization than hatching time. Our findings agree with the conclusion of Peñáz (1983) who considered hatching as an event of minor importance from the developmental point of view and is not correlated with a fixed developmental stage (Hamor and Garside 1979; Heming 1982; Peñáz 1983; Eckmann 1987).

Our study showed a tendency for the time to maximum tissue size (TMTS) to decrease with the hatching time in the order:  $s_{max} HT1 > s_{max} HT2 > s_{max} HT3$  (Fig. 5; Table 3). Providing that TMTS corresponds to the time when larvae begin to swim and feed actively, our results imply that early hatched larvae take longer time to switch from one developmental stage to the next. Our findings confirm that early hatched larvae remained at the stage of "larva" for a longer period of time and reached their maximum tissue size later, which was also reported for Pacific cod *Gadus macrocephalus* by Laurel et al. (2008).

Tissue size differences between early and late hatched larvae observed from hatching to TMTS (Figs. 2e, f; 4) were mitigated at TMTS. Similar observations about initial temperature-induced morphometric differences that decreased progressively and disappeared completely when the swim bladder was inflated (just before the onset of external feeding) were reported for the common carp *Cyprinus carpio* and the grass carp *Ctenopharyngodon idella* by Korwin-Kossakowski (2008). The author called this developmental period "a compensatory growth phase" during which less advanced larvae catch up with the more advanced ones.

Close to the final yolk resorption stage (between days 9 and 12 in the present study), late hatched larvae that were initially favoured (more tissue, more protein) stopped growing and, in

the absence of an external food source, body protein-bound amino acids were mobilized. This induced a tissue resorption translated into a decrease in body size (Figs. 2e, f, 4, 5). The timing of tissue resorption seems to have been delayed for early hatched larvae and occurred after day 12. In consequence, on days 12 and 15, early hatched larvae were greater in tissue weight than late hatched ones (Fig. 2e, f).

The yolk conversion efficiency in terms of energy was significantly higher for early and mid hatched larvae compared to the late hatched ones (Fig. 6). This finding corroborates the study of Trabelsi et al. (2013) on pike larvae where the yolk conversion efficiency calculated from morphometric properties (total length/ yolk sac perimeter, Hardy and Litvak 2004) was significantly greater in earlier hatched larvae than in late hatched larvae. Increasing yolk conversion efficiency at early stages is possible if the cost of protein synthesis is reduced or if maintenance costs are reduced (Wieser et al. 1988). During the endogenous feeding period, maintenance functions cover mainly respiration (Kamler 2008). Yet, the absolute respiration rate for many fish species before exotrophy is directly related to body mass (Kamler 2008) and increases with dry weight as observed for walleye pollock larvae (Porter 2001). Given that late hatched pike larvae showed larger size and dry weight from hatching until D12, their respiration rate from hatching to TMTS should be higher. As a result, yolk energy expended to form new tissues increases and the yolk conversion efficiency decreases.

On the other hand, increased yolk conversion efficiency of early hatched larvae can be also explained by a lower protein synthesis cost. Indeed, a decline in the cost of synthesis of a unit of protein (low aerobic cost) with increasing rates of protein synthesis was reviewed by Pannevis and Houlihan (1992) and confirmed by Smith and Houlihan's (1995) observations. According to the latter authors, the oxygen consumption of the different fish cells increased with increasing rates of protein synthesis up to a limit after which it remained constant. These findings mean that fast-growing larvae expend less energy to synthesize proteins, which agrees with Trabelsi et al. (2013) results regarding the higher growth rate recorded in early hatched larvae compared to the late hatched ones during the endogenous feeding period (+8.5 % per day) and particularly during the first 5-day period after hatching (+10 % per day) compared to late hatched ones.

To conclude, the present study confirms findings of Trabelsi et al. (2013) about a precocious organization of the larval quality with the hatching time for the northern pike. The biochemical and bioenergetics studies, which were complementary to the morphometric study, showed that the earlier the hatching, the smaller the size of the newly hatched larvae, but the higher their yolk conversion efficiency. In addition to that, early hatched larvae were found to take longer time to develop into juveniles. Finally, the principal steps of larval development seem to be better correlated with time of fertilization than hatching time.

**Table 1. Female (F), hatching time (HT), age (A) and their interaction effects on dry weight (DW), caloric value (CV) and energy content (EC) of entire larva, yolk and tissue**

	Mean value	F	HT	A	HT × A	F × HT
DW (mg/individ.)						
Larva	1.29	S	NS	S	S	S
Yolk	0.76	S	S	S	S	S
Tissue	0.86	NS	S	S	S	NS
CV (J/mg DW)						
Larva	25.8	NS	NS	S	S	NS
Yolk	27.5	NS	NS	NS	NS	NS
Tissue	25.2	NS	NS	S	NS	NS
EC (J/individ.)						
Larva	33.5	NS	S	S	S	NS
Yolk	19.8	S	S	S	NS	NS
Tissue	21.9	NS	NS	S	S	NS

ANOVA, *S* significant, *NS* not significant,  $p < 0.05$

**Table 2. Female (F), hatching time (HT), age (A) and their interaction effects on the biochemical compositions of entire larva, yolk and tissue**

	Mean value	F	HT	A	HT × A	F × HT
Ash (% DW)						
Larva	5.21	NS	NS	NS	NS	NS
Yolk	4.04	NS	NS	NS	NS	NS
Tissue	5.84	NS	NS	S	S	NS
Ash (mg/individ.)						
Larva	0.06	NS	NS	S	S	NS
Yolk	0.02	NS	NS	S	NS	NS
Tissue	0.04	NS	NS	S	NS	NS
Protein (% DW)						
Larva	70.6	NS	NS	NS	NS	NS
Yolk	68.6	NS	NS	NS	NS	NS
Tissue	71.1	NS	NS	NS	NS	NS
Protein (mg/individ.)						
Larva	0.91	NS	S	S	NS	NS
Yolk	0.48	S	S	S	NS	S
Tissue	0.61	NS	S	S	S	NS

ANOVA, *S* significant, *NS* not significant,  $p < 0.05$

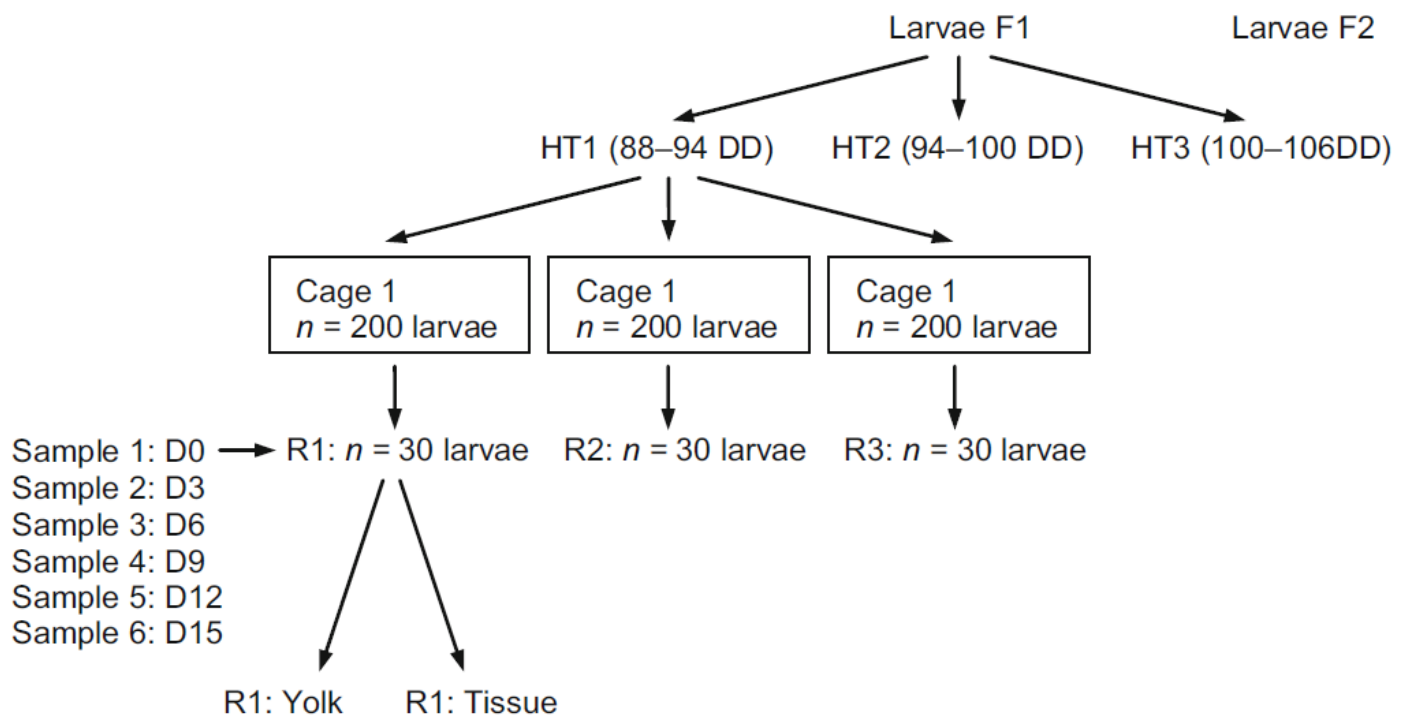


**Table 3. Parameters of Gompertz model describing changes in total larval and yolk dry weight and energy content (see the text), maximum tissue size (MTS), time at MTS (TMTS,  $s_{max}$ ) and cumulative yolk utilization efficiency ( $K_{1c}$ ), at MTS and at full yolk resorption ( $Re$ )**

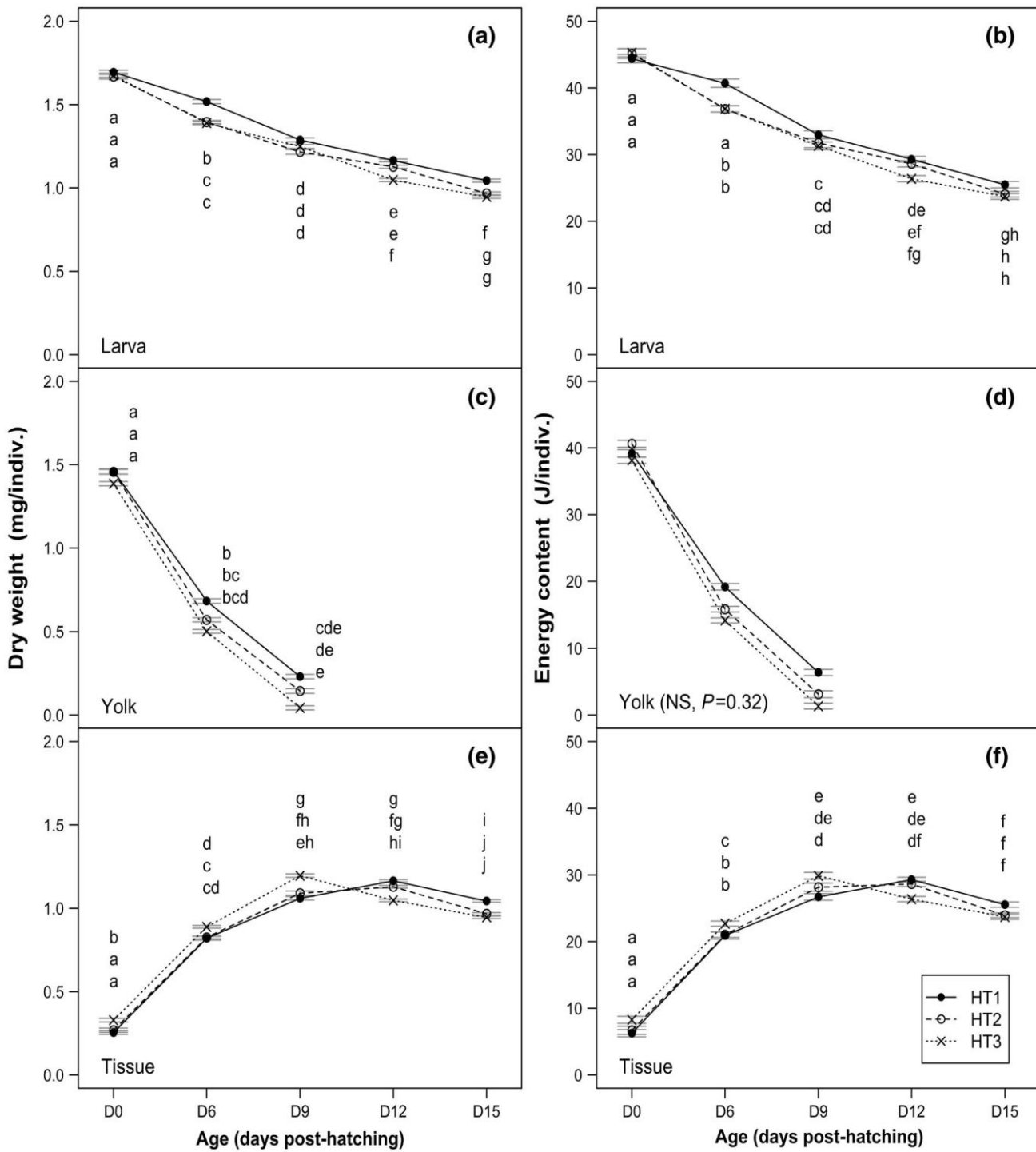
Female	Hatching time	$S_0$	$g_L$	$\tau_{0L}^a$	$g_Y$	$\tau_{0Y}^a$	MTS	$\tau_{max}^a$	$K_{1c}$ (%)	
									At MTS	At $Re^b$
Dry mass (mg/individ.)										
F1	HT1	1.84	0.123	18.8	0.237	6.0	1.18	10.9	77.9	57.0
	HT2	1.80	0.124	18.8	0.238	5.8	1.17	10.7	78.6	57.7
	HT3	1.85	0.121	17.7	0.227	5.1	1.16	10.0	76.9	52.6
F2	HT1	1.84	0.126	18.7	0.235	6.3	1.17	11.1	77.1	55.5
	HT2	1.77	0.119	18.5	0.240	5.0	1.17	9.9	79.5	59.1
	HT3	1.81	0.133	17.1	0.252	4.6	1.20	9.2	79.5	57.3
Energy content (J/individ.)										
F1	HT1	48.8	0.135	17.4	0.242	6.2	29.9	10.7	75.4	51.5
	HT2	48.3	0.129	17.5	0.234	5.9	29.7	10.6	75.6	51.4
	HT3	50.8	0.125	16.4	0.222	5.2	29.6	10.0	73.4	45.3
F2	HT1	48.7	0.138	17.6	0.243	6.6	29.9	11.0	75.2	51.3
	HT2	48.5	0.120	17.2	0.231	5.0	29.9	9.8	76.3	52.2
	HT3	52.2	0.116	16.3	0.212	4.7	29.7	9.7	72.7	43.6

a Days post-hatching

b Full yolk resorption assumed to be 0.1 % of the initial yolk size



**Figure 1. Experimental pattern. F =female (1 and2), HT =hatching time (1-3), D =sampling day (0, 3, 6, 9, 12 and 15), R =replicate (1-3). The detailed experimental pattern and sample size are only shown for one female (F1), one hatching time (HT1), one cage (R1) and one sampling day (D0), as it was the same elsewhere**



**Figure 2. Effect of the interaction between hatching time and age on larval (yolk + tissue), yolk and tissue dry weight (a, c, e) and energy content (b, d, f) (mean  $\pm$  SE) in early (HT1), mid (HT2) and late (HT3) hatched northern pike larvae. The different letters in each subplot denote significant results ( $p < 0.05$ )**

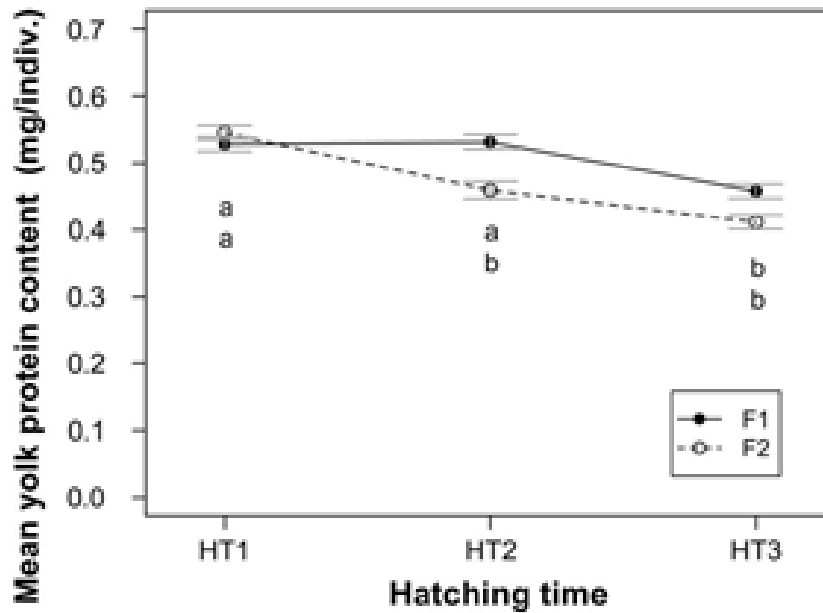


Figure 3. Effect of the interaction between female and hatching time on the yolk protein content (mean  $\pm$  SE) in northern pike larvae. F1, F2 =female; HT1–HT3 =hatching time. The different letters denote significant results ( $p < 0.05$ )

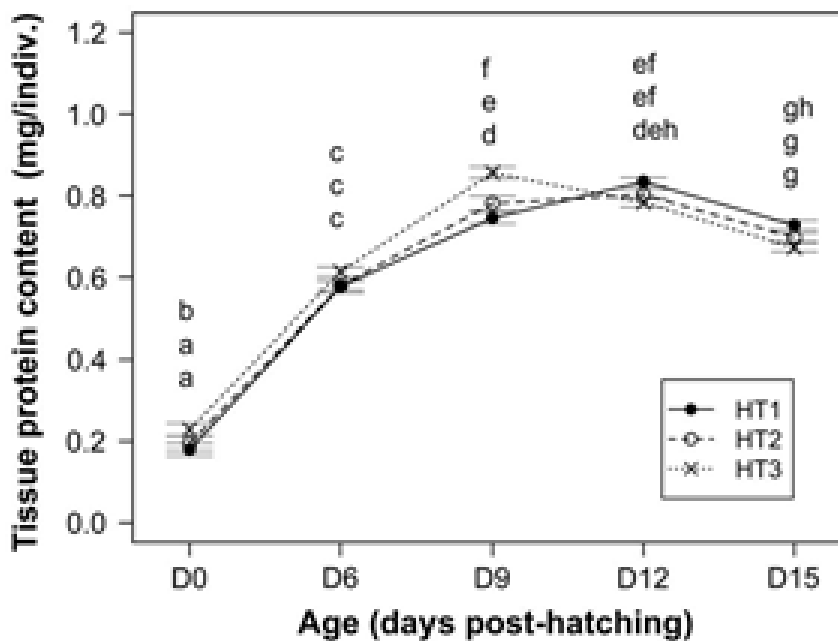


Figure 4. Effect of the interaction between hatching time and age on the tissue protein content (mean  $\pm$  SE) in northern pike larvae. HT1–HT3 =hatching times. The different letters denote significant results ( $p < 0.05$ )

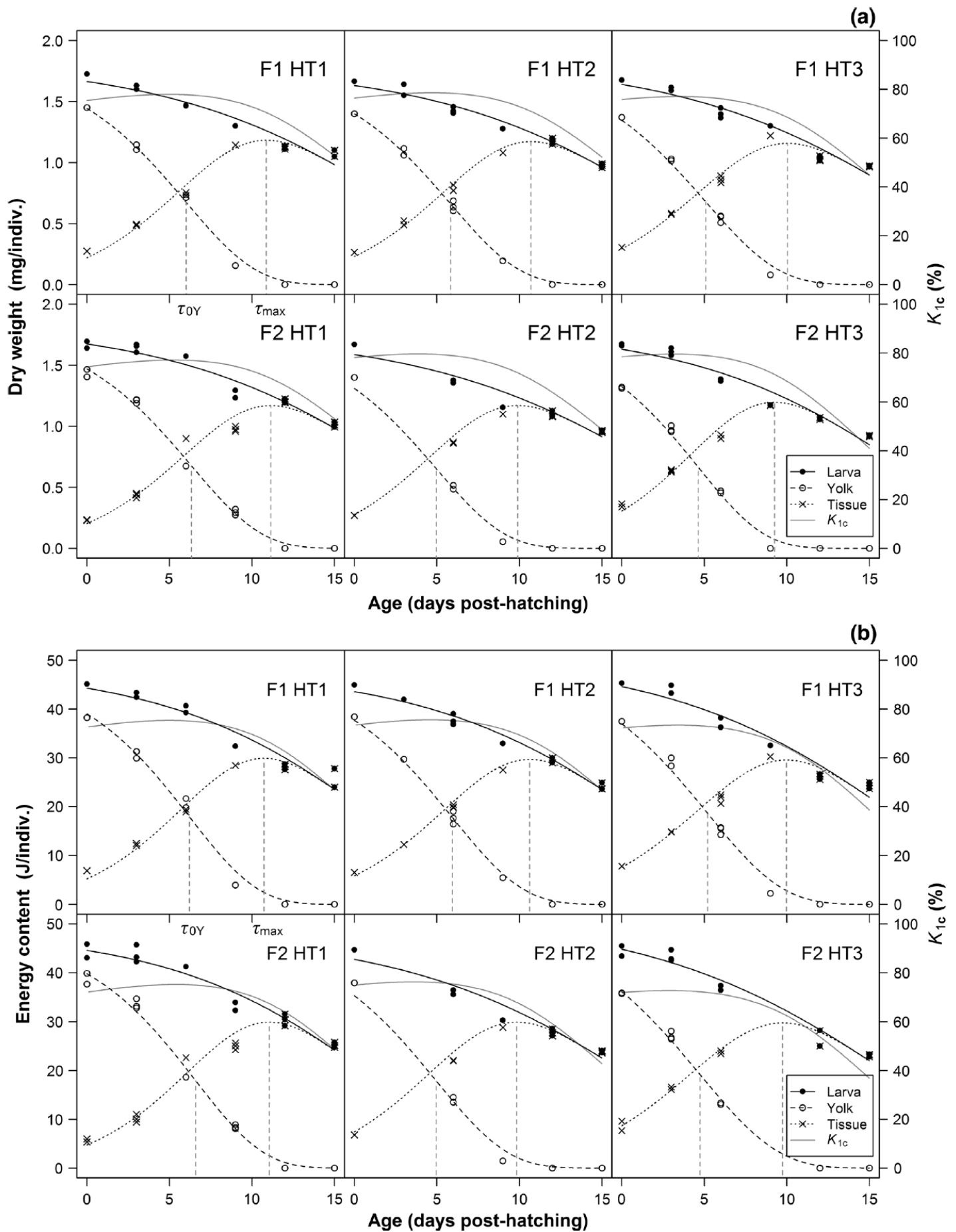


Figure 5. Cliquez ou appuyez ici pour entrer du texte.

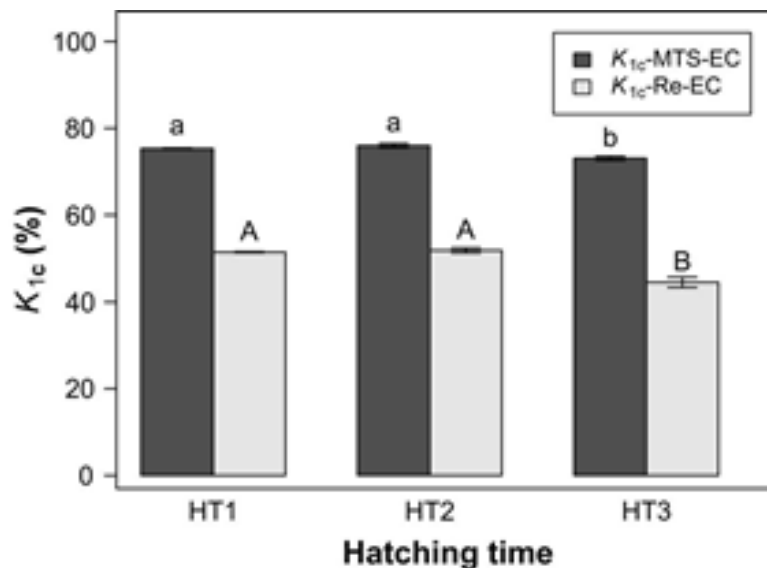


Figure 6. Hatching time effect on the gross yolk conversion coefficients ( $K_{c1}$ ) at the maximum tissue size (MTS) and complete yolk resorption (Re) in northern pike larvae, in terms of energy content (mean  $\pm$  SE). HT1–HT3 =hatching times. The different letters denote significant results ( $p < 0.05$ )

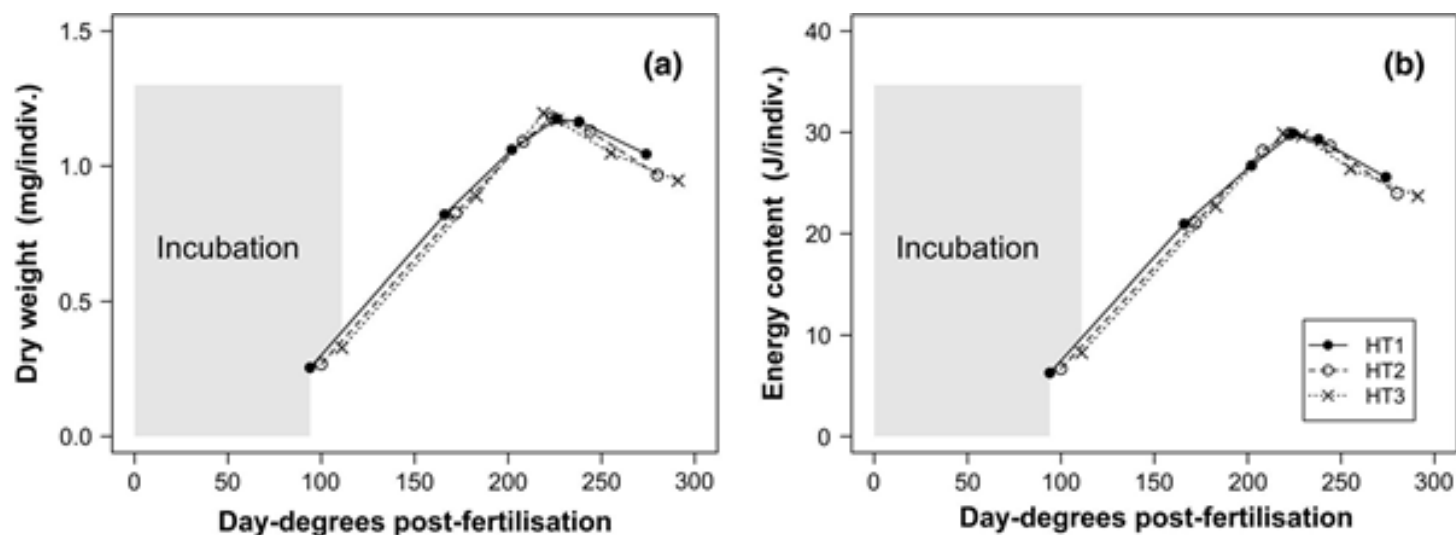


Figure 7. Dry weight (a) and energy content (b) evolutions in early (HT1), mid (HT2) and late (HT3) hatched larvae of northern pike with regard to the fertilization time

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## 6. Acknowledgements

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