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# Removal of the chorion before hatching results in increased movement and accelerated growth in rainbow trout (*Oncorhynchus mykiss*) embryos

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## Summary

**We investigated the effects of the chorion on movement and growth in rainbow trout (*Oncorhynchus mykiss*) embryos. To test if the chorion restricts movement and growth before hatching, we manually removed the chorion 3–6 days before the natural time of hatching (dechorionated) and compared movement, growth and oxygen consumption in dechorionated embryos and in embryos whose chorions remained intact until the time of hatching (chorionated). Dechorionated embryos exhibited 36 times more movement before hatching compared with intact embryos. By 10 h post-hatch there was no difference in the number of movements between the two groups. At the time of hatching [30 days post-fertilization (d.p.f.)], dechorionated embryos had a significantly greater embryonic body dry mass compared with chorionated embryos, which persisted up to 45 d.p.f. At first feeding (50 d.p.f.) there was no significant difference in embryonic body dry mass between the two groups. Dechorionated embryos had a significantly greater embryonic body protein content after hatching (32, 33 d.p.f.) compared with chorionated embryos. Despite the differences in movement and growth, there were no significant differences in oxygen consumption between chorionated and dechorionated embryos. Furthermore, there was no correlation between the number of movements and oxygen consumption in rainbow trout embryos (chorionated, dechorionated, and hatched). Taken together, the data indicate that rainbow trout embryos have the capacity to be relatively active before hatching, but that the chorion restricts or inhibits movement. Moreover, precocious activity in pre-hatch embryos is correlated with accelerated growth and higher protein content, suggesting that the exercise training effect observed in adult salmonids is also present in early developmental stages.**

Key words: exercise, protein content, oxygen consumption, yolk protein, embryo, yolk-sac larvae, hatching, development, rainbow trout, *Oncorhynchus mykiss*.

## Introduction

The majority of fish embryos develop surrounded by a fluid layer known as the perivitelline fluid encased in a tough acellular shell or chorion that is composed mainly of protein and glycoprotein. One of the major functions of the chorion is to physically and chemically protect and isolate the embryo from external environmental conditions (Cotelli et al., 1988). In addition, the chorion is involved in respiration, excretion of metabolic wastes and ionic and osmotic balance (Groot and Alderdice, 1985). Mechanical protection offered by the chorion is especially important in salmonid embryos as gravel movement and pressure changes within the redd can result in physical damage to the embryo (Groot and Alderdice, 1985). This is apparent in the relatively thick chorions of salmonid embryos compared with marine embryos, which are pelagic and must remain buoyant (reviewed by Blaxter, 1988).

Given the restrictions of living within a sphere, are embryos capable of movement? The development of locomotion varies widely between fish species depending on the timing of ontogenetic events, as well as life history of the species. In *Danio rerio* embryos [at 29°C hatching occurs at 48 h post-fertilization (h.p.f.)] spontaneous contractions begin as early as 17 h.p.f. followed by a coiling behaviour in response to touch at 21 h.p.f. and finally organized swimming in response to touch starts at 27 h.p.f. (Saint-Amant and Drapeau, 1998). In salmonids, embryonic development is protracted, with hatching occurring after several weeks, depending on the species and environmental conditions. In *Salmo salar* embryos (at 6°C hatching occurs after 81 days) weak sporadic contractions of the trunk were observed at 27 days post-fertilization (d.p.f.) followed by rhythmic movements at 32 d.p.f. (Johnston et al., 1999). In *S. trutta*, occasional body movements at 35 d.p.f. that increased in frequency up to hatching at 54 d.p.f. have been reported (Proctor et al., 1980). Thus, it appears that fish embryos have the appropriate 'machinery' and are capable of movement while still confined in the chorion before hatching.

Very few studies have considered the relationship between

activity and metabolism in embryonic or larval fish. Activity substantially influences the metabolic rate, and consequently oxygen consumption in all organisms (Fry, 1971), including adult and juvenile fish (Brett, 1964; Fry, 1971; van den Thillart, 1986). Davenport and Lönning (Davenport and Lönning, 1980) reported that oxygen consumption was significantly reduced in anaesthetized, and therefore inactive, *Gadus morhua* larvae compared with unanaesthetized larvae. Weiser reported that active yolk-sac rainbow trout increased their metabolic rate by 85–166% over and above the routine metabolic rate (Weiser, 1985). There is no data, to our knowledge, on the influence of activity on oxygen consumption at earlier developmental stages in salmonids, before the time of hatching. This is particularly interesting because of the increase in body movements prior to hatching (Proctor et al., 1980).

In addition to changes in metabolic rate, activity or exercise is known to influence many other physiological parameters, including changes in whole-animal growth. Growth in adult fish is plastic and is influenced by a forced moderate exercise regime over an extended period of time, also known as exercise training. When subjected to exercise training, adult fish show much more rapid growth (demonstrated by changes in weight gain) compared with non-exercised fish, and this is especially true for salmonid species, e.g. *S. trutta* (Davison and Goldspink, 1977); *Salvelinus fontinalis* (Johnston and Moon, 1980); *O. mykiss* (Houlihan and Laurent, 1987); *S. salar* (Totland et al., 1987). By contrast, very little is known about the effects of activity on growth during early life stages. No relationship was found between activity and growth in larval zebrafish (Bagatto et al., 2001), and it was suggested that any extra energy obtained was allocated to swimming and was unavailable for growth. To our knowledge the effects of activity on growth have not been further considered in the embryonic or larval stages of other fish species, including salmonids that are more differentiated at hatch relative to zebrafish (Blaxter, 1988).

Rapid growth in rainbow trout is associated with higher rates of protein synthesis (Valente et al., 1998). The same is true in adult fish subjected to exercise training. The rate of protein synthesis must exceed the rate of protein degradation for growth to occur. Swimming stimulated growth and protein synthesis and, to a lesser extent, protein degradation in muscle tissue of trained adult rainbow trout compared with controls (Houlihan and Laurent, 1987). The effects of activity on protein synthesis have not been explored in the early life stages of fish. Protein is the major yolk constituent of embryonic and early larval salmonids. In addition to supplying energy *via* catabolic processes, yolk protein is broken down providing amino acids for tissue growth in the developing embryo (Heming and Buddington, 1988). Thus, an increase in activity levels in embryonic or larval fish may lead to a more rapid conversion of yolk protein into embryonic tissue.

The first objective of our study was to ascertain if the chorion restricts movement before hatching. We predicted that by removing the chorion several days before hatching, dechorionated embryos would exhibit more movement than embryos whose chorions remained intact (chorionated). Our preliminary findings indicated that dechorionated embryos did in fact show more movement. Our second objective, therefore, was to measure the effects of activity on metabolic rate in embryonic rainbow trout. We predicted that the more active, dechorionated embryos would consume more oxygen compared with chorionated embryos. Given that in adults, there is a positive relationship between activity and growth, our third objective was to measure the effects of activity on growth in embryonic rainbow trout. We predicted that because dechorionated embryos move more than chorionated embryos, they will grow faster. The last objective of our study was to test if dechorionated embryos convert yolk protein into embryonic tissue at a faster rate than chorionated embryos. We predicted that the protein content would be higher in the embryonic body of dechorionated embryos compared with chorionated embryos at any given time. To test these predictions, the number of movements, embryonic body dry mass, and protein contents were measured before, during and post-hatch in chorionated and dechorionated rainbow trout embryos.

## Materials and methods

### *Experimental animals*

Fertilized rainbow trout (*Oncorhynchus mykiss* Walbaum) embryos were obtained from Rainbow Springs Trout Farm (Thamesford, ON, Canada). Embryos were maintained in continuous flow Heath trays (52.1 cm×62.9 cm×6 cm; width×length×depth) with mesh bottoms (10°C, pH 8.1, water hardness 411 mg $l^{-1}$ as $CaCO_3$; $Ca^{2+}$, 5.24 mmol $l^{-1}$; $Cl^{-1}$, 1.47 mmol $l^{-1}$; $Mg^{2+}$, 2.98 mmol $l^{-1}$; $K^{+}$, 0.06 mmol $l^{-1}$; $Na^{+}$, 1.05 mmol $l^{-1}$) at Hagen Aqualab, University of Guelph, ON, Canada for the duration of all experiments. Incubation trays were shielded from light during the entire incubation period, unless otherwise specified.

### *Experimental protocol*

There were four series of experiments conducted. Series I: recording of movement; Series II: recording of oxygen consumption; Series III: estimation of growth rate; Series IV: estimation of total protein.

Separate batches of embryos were used in each series, and hatching time was consistent between each batch of embryos (30–34 d.p.f.). For each batch, embryos were obtained from three or more different females. In each series of experiments, embryos were removed from the incubation trays prior to the time of hatching and briefly placed in shallow Petri dishes. Embryos were either dechorionated under a light microscope by manual removal of the chorion using fine forceps (experimental group or dechorionated) or handled with fine forceps without removal of the chorion (control group or chorionated). Following this procedure, unless otherwise stated, embryos were placed into mesh-bottom chambers (~25 embryos per chamber) and returned to the incubation tray.

There was no mortality associated with the dechorionating procedure.

*Series I: recording of movement*

In order to measure the degree of activity or movement in chorionated and dechorionated embryos, embryos were randomly chosen from the incubation tray on 27 d.p.f. and were separated into chorionated and dechorionated groups as described above. Following this procedure, chorionated and dechorionated embryos were then immediately placed into one of eight wells (1.2 cm×1.8 cm×0.6 cm; width×length×depth) in a custom-built plexiglass chamber (5 cm×18 cm) supplied with flow-through water. Embryos were kept under a 12 h semi-dark (lights on at ~50% normal intensity) and 12 h dark photoperiod for the duration of the experiment. A video camera mounted above the chamber was used to continuously record movement (24 h $day^{-1}$) for 80 h. Videotapes were later analyzed and the total number of movements per 5 h period was recorded to compare movement in chorionated and dechorionated embryos. Values are reported as number of movements $h^{-1}$.

*Series II: recording of oxygen consumption*

Closed respirometry was used to measure and compare oxygen consumption in chorionated and dechorionated rainbow trout embryos. On 27 d.p.f., embryos were randomly removed from the incubation tray and one-half of the embryos were dechorionated and the remainder were left intact (chorionated). Following this procedure, embryos were returned to the incubation tray and left undisturbed for ~5 h (in order to recover from the dechorionating procedure) after which time they were placed, in pairs, into one of six temperature-controlled respirometers. Two respirometers were left empty as controls. The chambers were filled with air-saturated (~10 mg $O_2$ $l^{-1}$), autoclaved water (~9 ml), and the temperature was maintained at 10°C. Embryos were left undisturbed in the chambers for about 1 h before sealing each chamber. The chambers were sealed for 1 h during which time the water oxygen content was continuously measured and did not fall below 70% saturation. Measurements were made as described previously (Ninness et al., in press). Measurements were made once daily for 7 days, with naïve embryos being used each day. During each trial, one to two respirometers were randomly chosen and the number of movements made by the embryos in the respirometer was recorded. Preliminary experiments were conducted to estimate the capacity of our system to detect changes in oxygen consumption (Ninness et al., in press).

*Series III: estimation of growth rate*

To measure and compare growth rate in chorionated and dechorionated embryos, embryos were randomly chosen from the incubation tray and one half of the embryos were dechorionated 24 d.p.f. Dechorionation occurred early in this series relative to other series in order to maximize the effects of activity on growth, and 24 d.p.f. was the earliest that the chorion could be removed without causing any ill effects to the embryo. Measurements were made 24, 28, 30, 32 and 33 d.p.f. Embryos ($N$=8) were anaesthetized in 0.15 g $l^{-1}$ ethyl 3-aminobenzoate methanesulfonate salt (MS-222) and if present, the chorion was removed. The embryonic body was separated from the yolk sac ($N$=6, each value represents a pooled dry mass of five embryos in order to increase the accuracy of measurements). Embryonic body dry mass was measured after a constant mass was achieved (~48 h at 50°C). A separate experiment was conducted to ascertain if the differences in growth between chorionated and dechorionated embryos persisted into later life stages. Embryos were dechorionated 24 d.p.f. and measurements were made 24, 30, 45, 50, 60, 75, 90 d.p.f. Fish were sampled as described above, except that in addition to the embryo body, the dry mass of the separated yolk sac also was measured. After 100% hatch (34 d.p.f.) fish were moved from the incubation tray into a divided floating container in a 900 l recirculating tank. Fish were 'over-fed' starting 50 d.p.f., using an automatic feeder (Sweeney Enterprises Inc., Boerne, TX, USA) set to dispense every hour for 12 h $d^{-1}$ for the duration of the experiment.

*Series IV: estimation of total protein*

To measure total protein concentration (mg protein $individual^{-1}$) in chorionated and dechorionated embryos, embryos were randomly chosen from the incubation tray and one half of the embryos were dechorionated 27 d.p.f. Measurements were made 27, 30, 32 and 33 d.p.f. Embryos were anaesthetized in 0.15 g $l^{-1}$ MS-222, the chorion was removed if present, the embryonic body was separated from the yolk sac, blotted dry on a Kimwipe and was frozen at –80°C for up to 4 weeks before analysis.

*Tissue analysis*

To measure total tissue protein, ~0.01 g of frozen tissue was ground to a fine powder under liquid $N_2$ using a mortar and pestel, dissolved in 10 volumes of ice-cold trichloroacetic acid solution (TCA; 10%) and centrifuged at 14 000 ***g*** for 10 min. The supernatant was removed, the pellet was washed with TCA and centrifuged again at 14 000 ***g*** for 5 min. The supernatant was again removed, the pellet was dissolved in 500 volumes of 1 mol $l^{-1}$ NaOH, vortexed and rocked for 24 h. Samples were then diluted to 0.5 mol $l^{-1}$ NaOH by the addition of water and centrifuged at 500 ***g*** for 10 min. Preliminary experiments were performed to ensure that the concentration of NaOH and incubation time were sufficient to completely dissolve the embryonic protein (data not shown). Total tissue protein was quantified as described by Lowry et al. (Lowry et al., 1951) with modifications outlined by Rutter (Rutter, 1967). The protein content (mg protein $individual^{-1}$) was calculated as the product of the protein concentration (mg $g^{-1}$ protein) and the wet mass (g $individual^{-1}$).

*Statistical analysis*

All statistical analyses were performed using the general linear models (GLM) procedure of the SAS system (version 8e; SAS Institute In, Cary, NC, USA). Differences in

movement between chorionated and dechorionated embryos (Series I) were analyzed using a repeated-measures analysis of variance (ANOVA). Differences in dry mass (Series II) and protein content (Series III) were analyzed using a two-factor ANOVA (treatment and time). The Tukey test was used to test for differences between chorionated and dechorionated embryos ($P$<0.05). Values are presented as means ± s.e.m.

## Results

### *Series I*

In embryos still encased in the chorion a movement consisted of a flexure into a 'C' shape with the nose and tail being brought into close proximity for a duration of ~1 s. In embryos not encased in the chorion (i.e. dechorionated and hatched) a movement consisted of two body flexions with a duration of ~0.5 s. The magnitude and duration did not appear to differ between the two groups.

The number of movements was 36-fold higher in dechorionated relative to chorionated embryos before hatching (–15 to –5 h) (Fig. 1). Similarly, the number of movements was ninefold higher in dechorionated compared with chorionated embryos during hatch (0 h). By 5 h post-hatch, the difference in movement between the two groups had decreased, but dechorionated embryos continued to move significantly more (~1.5-fold) than embryos whose chorions remained intact until hatching (chorionated). By 10 h post-hatch there was no significant difference in movement between the two groups (Fig. 1). Additionally we analyzed, but have not shown, movement up to 50 h before hatch and 30 h after hatch. It is worth noting that differences in movement between chorionated and dechorionated embryos over the longer term were similar to Fig. 1 (–15 h to –5 h). Differences in movement between chorionated and dechorionated embryos 15–30 h post-hatch were similar to the difference reported 10 h post-hatch in Fig. 1.

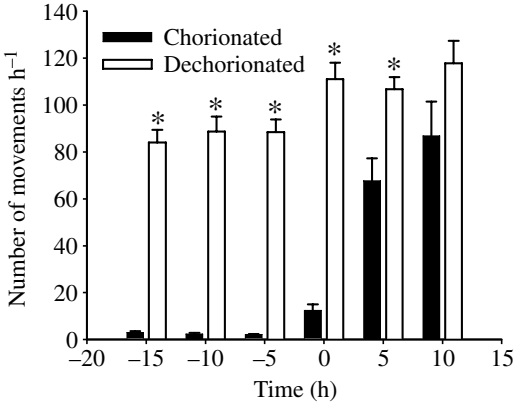


Fig. 1. Number of movements per hour in chorionated (chorion intact) and dechorionated (chorion removed) rainbow trout embryos over a period of 25 h. Negative numbers indicate times before hatch, time 0 indicates the 5-h interval in which hatching occurred (defined as when the embryo body was free of the chorion), and positive numbers indicate times post-hatch. Values are means ± s.e.m. ($N$=4). *Significantly different from chorionated values ($P$<0.05).

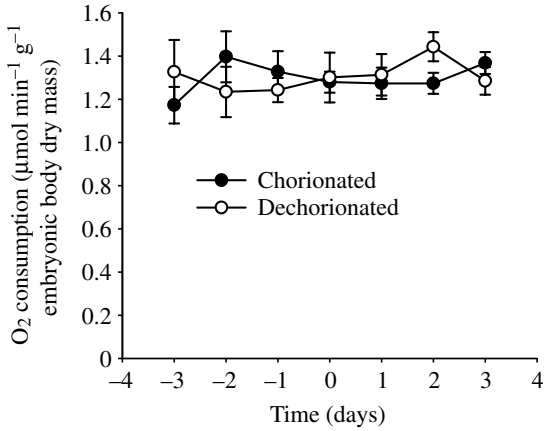


Fig. 2. Oxygen consumption (μmol min$^{-1}$ g$^{-1}$ embryonic body dry mass) in chorionated (chorion intact) and dechorionated (chorion removed) rainbow trout embryos 3 days before hatch to 3 days after hatch. Embryonic body dry mass is the dry mass of the embryo only, separated from the yolk. Negative numbers indicate days before hatch and, time 0 indicates when hatching started (hatching did not necessarily occur in the respirometer) and positive numbers indicate days post-hatch. Values are means ± s.e.m. ($N$=9).

### *Series II*

There was no difference in oxygen consumption between chorionated and dechorionated embryos (Fig. 2). Furthermore we found no correlation ($r^2$=0.016) between the number of movements and oxygen consumption in rainbow trout embryos around the time of hatch (Fig. 3).

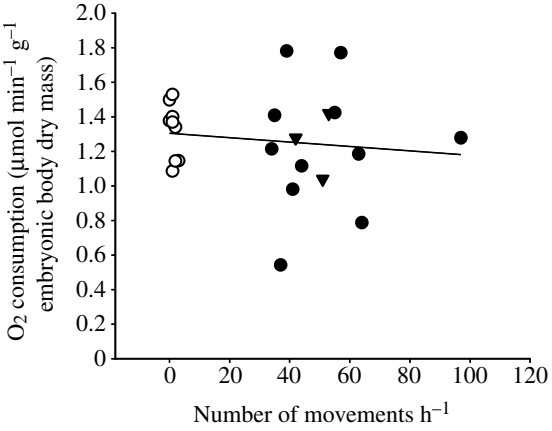


Fig. 3. Correlation between the number of movements (per hour) and oxygen consumption (μmol min$^{-1}$ g$^{-1}$ embryonic body dry mass) in chorionated (open circles, chorion intact), dechorionated (closed circles, chorion removed), and hatched (triangles) rainbow trout embryos around the time of hatch (3 days before hatch to 3 days after hatch). Embryos were dechorionated 3 days before hatching. Movements were measured while the embryos were in the respirometers.

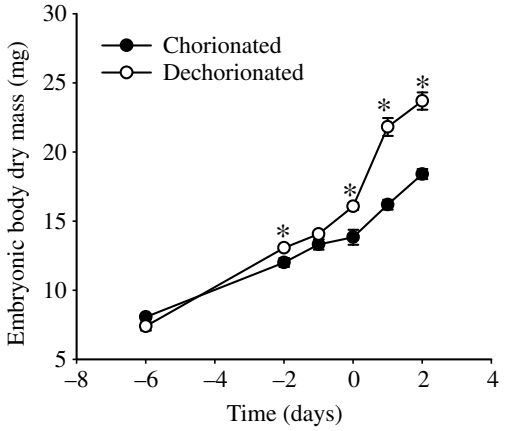


Fig. 4. Embryonic body dry mass (mg) in chorionated (chorion intact) and dechorionated (chorion removed) rainbow trout embryos 6 days before hatch to 3 days after hatch. Embryos were dechorionated 6 days before hatching (–6 days). Embryonic body dry mass is the dry mass of the embryo only, separated from the yolk. Negative numbers indicate days before hatch, time 0 indicates when hatching started and positive numbers indicate days post-hatch. Values are means ± s.e.m. ($N$=8, with each value consisting of a pooled dry mass of 5 embryos). *Significantly different from chorionated values ($P$<0.05).

### *Series III*

Dechorionated embryos at 28 d.p.f. had a small (9%), but significantly greater embryonic body dry mass compared with chorionated embryos (Fig. 4). The difference in dry mass between the two groups increased with time; on day 30, 32 and 33 the dry mass of the dechorionated group was 16–35% greater relative to the chorionated group. The differences in growth between chorionated and dechorionated embryos persisted up to 45 d.p.f., with the dechorionated group having a 25% higher body mass compared to the chorionated group (Table 1). Furthermore, the yolk mass of the dechorionated group was 28% lower relative to the chorionated group at 45 d.p.f. Interestingly, the dechorionated group had a decelerated growth phase between 45 and 50 d.p.f. (57% increase in body mass) compared to the chorionated group (82% increase in body mass). Consequently, from 50 d.p.f. onwards there were no significant differences between embryonic body or yolk mass between the chorionated and dechorionated groups.

Table 1. *Embryonic body and yolk dry mass of chorionated and dechorionated rainbow trout embryos 24–90 days post-fertilization*

| | Dry mass (mg) | | | |
|---|---|---|---|---|
| | Chorionated | | Dechorionated | |
| d.p.f. | Embryonic body | Yolk | Embryonic body | Yolk |
| 24 | 1.01±0.03 | 32.74±0.51 | 1.01±0.03 | 32.74±0.51 |
| 30 | 3.08±0.04 | 31.80±0.48 | 3.62±0.09* | 30.26±0.95 |
| 45 | 12.60±0.45 | 15.55±1.34 | 15.81± 0.38* | 12.09±0.81† |
| 50 | 22.91±1.56 | 8.24±1.52 | 24.84±1.41 | 5.78±0.73 |
| 60 | 46.55±2.47 | 2.53±0.67 | 47.33±2.57 | 1.86±0.51 |
| 75 | 118.93±8.01 | | 124.14±9.46 | |
| 90 | 230.55±14.25 | | 241.24±13.68 | |

d.p.f., days post-fertilization.
Values are means ± s.e.m. ($N$=8).
*Significantly different from chorionated embryonic body value; †significantly different from chorionated yolk value.

### *Series IV*

The majority of whole embryo (embryonic body plus yolk-sac) protein content (mg protein individual$^{-1}$) was present in the yolk fraction (~92%) of rainbow trout embryos around the time of hatching, as expected. Only a very small portion (~8%), of the whole embryo protein content was present in the embryonic body (Fig. 5A,B). In the embryonic body of chorionated and dechorionated embryos (highlighted in Fig. 5C), the difference in protein content between the two groups increased with time so that at 30, 32 and 33 days post-fertilization the protein content of the dechorionated group was 28–72% greater relative to the chorionated group.

## Discussion

### *Movement*

The findings of the present study agree with the prediction that the removal of the chorion before the natural hatching time in trout embryos results in increased movement compared with those embryos whose chorions remain intact. It is interesting to note that, once dechorionated, embryos demonstrated a high number of movements immediately (~60 movements h$^{-1}$). Furthermore, after natural hatching the number of movements increased very rapidly (fourfold increase 5 h post-hatch). Thus, it appears that the musculoskeletal system is sufficiently developed for frequent activity before hatching. These results are consistent with previous observations of spontaneous movements during late embryonic development in both mammals (Suzue, 1996) and fish (Proctor et al., 1980; Peterson and Martin-Robichaud, 1983; Saint-Amant and Drapeau, 1998).

The fact that movement increases so rapidly after hatch in rainbow trout embryos suggests that movement has an important physiological function. One possibility is that early movement is linked to further muscle growth and development. During larval growth (i.e. after hatch) in teleost fish, the red and white muscle layers of the myotome continue to develop by an increase in the number (hyperplasia) and diameter (hypertrophy) of muscle fibres (Nag and Nursall, 1972; Johnston, 2001). In birds and mammals, body movements create muscle force, which is necessary for the development of the muscle fibres (Vandenburgh et al., 1991). Hence, it is probable that the movement observed in rainbow trout immediately after hatch is an important contributor to normal skeletal muscle development. In contrast, van der Meulen et al. reported that the normal development of several features of the axial musculature, including the development and

segregation of red and white muscle layers occurred in mutant immobile zebrafish (van der Meulen et al., 2005). It would be interesting to know if salmonids are similar to zebrafish in this regard, or if indeed mobility is necessary for normal muscle differentiation and development.

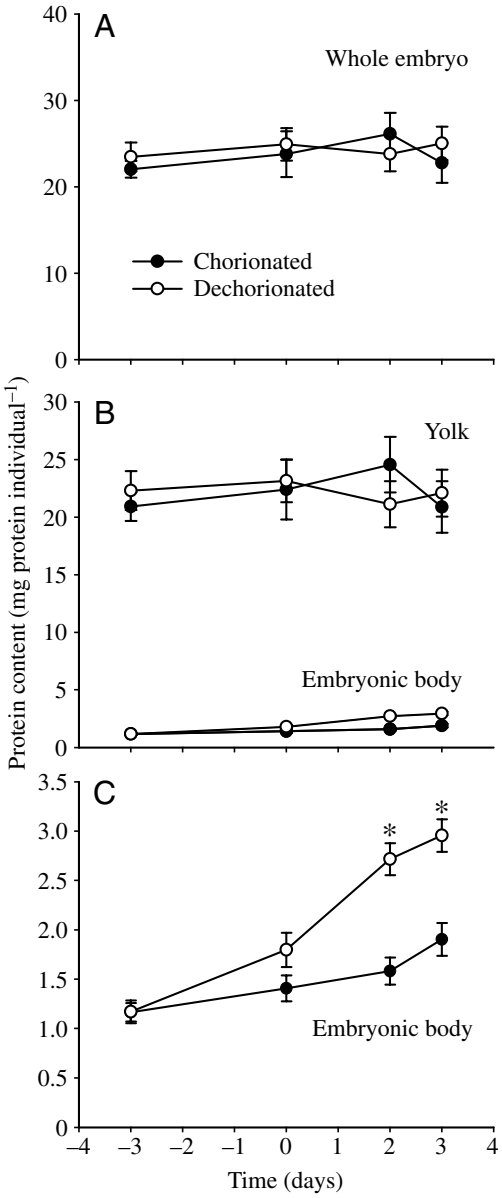


Fig. 5. Distribution of protein content (mg protein ind$^{-1}$) in whole embryos (embryonic body plus yolk sac; A), and in the embryonic body and yolk fractions (B) of chorionated (chorion intact) and dechorionated (egg case removed) rainbow trout 3 days before hatch to 3 days after hatch. (C) The values for the embryonic body shown in B but on a different scale, in order to highlight the differences in protein content (mg protein ind$^{-1}$) between chorionated and dechorionated embryos. Embryos were dechorionated 3 days before hatching (–3 days). Negative numbers indicate days before hatch, time 0 indicates when hatching started and positive numbers indicate days post-hatch. Values are expressed as means ± s.e.m. ($N$=8). *Significant difference from chorionated value ($P$<0.05).

Movement also may be important for respiration. Immediately after hatching, the skin is a major site of gas exchange with oxygen uptake occurring primarily by diffusion (Rombough and Ure, 1991). Surrounding the embryo is a semi-stagnant region of water, the boundary layer, where oxygen is depleted and metabolic wastes accumulate (Rombough, 1988a). Once out of the chorion, body movements may be an important mechanism for stirring the water layer immediately surrounding the larva, thereby replenishing the dissolved oxygen in the boundary layer. However, if movement was critical for respiration, then intact embryos would be expected to move more because of lower oxygen tensions surrounding the encapsulated embryo, although direct measurements have not been reported. Our movement data does not support this idea and it therefore seems unlikely that movement is related to respiration.

An additional factor that may play a role in the increased movement in dechorionated embryos relates to water currents. Rheotaxis is a behavioural orientation to water currents (Arnold, 1974). It has been reported that water velocities as low as 1 cm s$^{-1}$ can be detected and induce subsequent orientation in plaice (*Pleuronectes platessa*) (Arnold, 1969). Furthermore, positive rheotaxy has been documented in early stages of fish development, including zebrafish larvae (Bagatto et al., 2001). Although the water flow in the present experiment was low, it is possible that the swimming movements demonstrated by dechorionated embryos were a response to water flow over the embryo that would not be sensed by chorionated embryos.

### *Oxygen consumption*

Removing the chorion of trout embryos several days before hatching resulted in an increase in activity but not an increase in oxygen consumption. This is not consistent with what we predicted, nor does it agree with data in juvenile and adult fish where an increase in activity is correlated with an increase in oxygen consumption (e.g. Brett, 1964; Fry, 1971; van den Thillart, 1986). These unexpected results are probably not due to measurement error as the oxygen consumption values for newly hatched larvae in the present study (2.6±0.15 μmol g$^{-1}$ h$^{-1}$) are comparable to those reported by Weiser (Weiser et al., 1985) for newly hatched rainbow trout larvae (4.8±1.1 μmol g$^{-1}$ h$^{-1}$). Weiser's measurements (Weiser et al., 1985) were made at 12°C, compared to 10°C used in the present experiments, possibly accounting for the slightly higher values reported by Weiser (Weiser et al., 1985).

It is possible that at the stage of development (i.e. around hatch) used in our study, differences in oxygen consumption between active (dechorionated) and inactive (chorionated) embryos were difficult to resolve. Bagatto et al. reported no significant differences in mass-specific routine oxygen consumption between trained and untrained yolk-sac and swim-up zebrafish, but at the free-swimming stage, trained zebrafish had a significantly higher mass-specific routine oxygen consumption compared with controls (Bagatto et al., 2001). It is probable that around the time of hatching, the

production of new tissue constituted such a large proportion of the total energy expenditure that differences in activity between the two groups did not make a measurable contribution to metabolic rate. After hatch, larval fish often experience rapid growth (Kamler, 1992), associated with a high metabolic cost. Jaworski and Kamler reported that yolk energy was used mainly for tissue growth, while less energy was expended to fuel metabolism in several different species, including rainbow trout (Jaworski and Kamler, 2002). Indeed, growth in trout embryos accounted for more that half (59%) of the total amount of energy consumed between fertilization and 90% yolk absorption (Rombough, 1988b). Finally, van der Meulen et al. assayed the expression of several genes involved in energy metabolism in immobile zebrafish embryos (van der Meulen et al., 2005) and concluded the energy metabolism in immobile embryos was not greatly affected by a lack of muscle activity (i.e. cellular energy metabolism was similar in active and inactive, immobile zebrafish embryos). Taken together, our study and others, suggest that, movement in fish embryos has a trivial metabolic cost relative to the cost of growth.

It should be noted that there may be species differences. For example, Davenport and Lönning reported significantly higher oxygen concentration in active, unanaesthetized *G. morhua* larvae compared with inactive, anaesthetized larvae immediately after hatching (Davenport and Lönning, 1980). *G. morhua* larvae are pelagic, however and will swim to the top of the water column immediately after hatching. In contrast, trout will remain at the bottom of the water column for several weeks after hatching.

### *Growth*

We predicted that the increased movement of dechorionated embryos would result in accelerated growth and yolk utilization. Indeed, the dry mass of the embryonic body of the dechorionated embryos was significantly higher than that of chorionated embryos. This is consistent with the improved growth observed in adult salmonids during exercise training (Davison and Goldspink, 1977; Johnston and Moon, 1980; Houlihan and Laurent, 1987; Totland et al., 1987; Bugeon et al., 2003). Forced exercise is known to be a powerful stimulus for the hypertrophy of both red and white fibres in fish (Johnston and Moon, 1980; Totland et al., 1987) and an increase in fibre number in red muscle (Sänger and Stoiber, 2001). As a consequence, there is an increase in the total muscle cross-sectional area, thus contributing to whole-animal growth. It should be pointed out that in preliminary experiments, we found no significant differences in length between chorionated and dechorionated embryos and so length was not measured in subsequent experiments.

Only a few studies have considered the effects of movement on growth and muscle development in the early life stages of fish and to our knowledge our study is the first to report a positive relationship between activity and growth. In contrast, van der Meulen et al. reported that muscle development was not grossly affected by decreased muscle activity in immobile zebrafish embryos (van der Meulen et al., 2005). Two studies reported that growth was actually improved in 'immobile' *S. salar* larvae, compared with 'mobile' larvae (Hansen and Møller, 1985; Peterson and Martin-Robichaud, 1995); however, movement was not quantified. Swim training in larval zebrafish (*D. rerio*) did not improve growth (Bagatto et al., 2001). The differences between our findings and the above studies may relate to species differences, as well as differences in experimental protocols.

Factors other than exercise are known to contribute to growth in fish, and possibly contributed to the increased growth observed in dechorionated embryos and larvae in the present study. Some of these include hormones (Sumpter, 1992) and oxygen tension (Matschak et al., 1998). The lack of measured difference in oxygen consumption between chorionated and dechorionated embryos in the present study eliminates oxygen as a key factor. The most notable growth factors expressed during early development include insulin, IGF-I (insulin-like growth factor I), IGF-II, growth hormone and thyroid hormone (for a review, see Johnston, 2001). It may be possible that removal of the chorion initiates changes in the levels of one or all of these hormones, thus affecting growth.

The accelerated growth in dechorionated embryos persisted post-hatch up to 45 d.p.f., but by 50 d.p.f. (first-feeding in the present experiment) there were no differences in body mass between the two groups. These findings are consistent with others (Geffen, 2002), who found that smaller, early hatching herring larvae catch-up with larger, late hatching larvae by the time of first feeding. First feeding has been described as a 'critical period', associated with high mortality (Blaxter, 1988). Therefore, there is a high survival value placed on attaining the largest possible size at this time because larger fish are stronger swimmers, are better able to catch prey and are less susceptible to predation (Blaxter, 1988). Indeed, between 45 and 50 d.p.f. the growth rate was greater in the smaller 'chorionated' compared with the larger 'dechorionated' fish (2.06 mg $day^{-1}$ *vs* 1.81 mg $day^{-1}$, respectively). It is well established that developmental rate is under strong genetic control (Blaxter, 1988). It is therefore possible that once the activity level between the two groups was similar, the growth rate of dechorionated fish slowed relative to chorionated fish, resulting in an optimum body size at first-feeding.

The findings of the present study support the prediction that dechorionated embryos convert yolk protein into embryonic tissue at a faster rate than chorionated embryos. After hatching, the protein content of the embryonic body tissue steadily rises, whereas the protein content of the yolk decreases (Rønnestad et al., 1993; Terjesen et al., 1997). This process was accelerated in dechorionated embryos in the present study, as tissue protein content was significantly higher relative to chorionated embryos. Rates of yolk reabsorption and protein synthesis are influenced by extrinsic factors, such as temperature (Mathers et al., 1993; Peterson and Martin-Robichaud, 1995). The present study indicates that activity also influences the rate at which yolk proteins are converted into body tissue.

It should be noted that there were no differences detected in the protein content of the yolk between chorionated and

dechorionated embryos over the short time scale investigated (around the time of hatching). The yolk protein content was ~20 times greater than the protein content of the embryonic body. It would be difficult, therefore, to resolve small differences in yolk protein content if present, whereas it was much easier to distinguish small differences in the protein content of the embryonic body.

One obvious question arising from the present study is why have a chorion? Our results imply that early removal of the chorion may be beneficial given that larger fish are stronger swimmers, are better able to catch prey, and are less susceptible to predation (Blaxter, 1988). Salmonid embryos in the wild develop buried in gravel in flowing rivers and streams, where protection against mechanical damage provided by the chorion is especially important. In our laboratory study, embryos were not subjected to the same extrinsic conditions relative to their wild counterparts. It is possible, therefore, that if developmental time within the chorion were reduced, increased mortality may occur as a result of mechanical stresses in the natural environment. It is probable that the developmental time spent within the chorion is a trade-off between optimizing protection and attaining the largest possible size at hatch. Our results also showed that although there were short-term effects on growth by early removal of the chorion, these effects did not persist in the long-term (i.e. up to first feeding). In the long-term, therefore, early removal of the chorion may afford no advantage to the animal.

In summary, we have demonstrated that the chorion restricts movement before the natural time of hatching in rainbow trout embryos. The relatively high level of activity observed immediately after manual removal of the chorion indicates that trout skeletal muscle is sufficiently developed to sustain frequent exercise several days prior to hatch. Removal of the chorion and increased movement were correlated with increased growth rate and protein content, but not oxygen consumption. The influence of pre-hatch activity had a finite impact on larval growth, as the difference between the two groups (chorionated and dechorionated embryos) disappeared by 50 d.p.f. These findings demonstrate that early growth and development in trout is a complex interplay between intrinsic (e.g. genetic) and extrinsic (e.g. exercise) factors.

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