Rapid Somatic Growth and Muscle Damage in a Salmonid Fish

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Abstract

A novel category of extensive and distinct muscle damage within the white swimming muscle of Arctic charr (*Salvelinus alpinus*, Salmonidae) is described. Based upon myomorphological characters and somatic growth patterns displayed by individual fish, it appears that this type of muscle damage is associated only with healthy and rapidly growing fish.

Key words: salmonid fish, histomorphology, septal muscle damage, rapid somatic growth.

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Degeneration of skeletal muscle fibres in mammals (including man) is usually considered to be a pathological phenomenon associated with disease [1] or physical exercise [2-4]. Muscle damage in fish has often been linked to malnutrition (e. g. vitamin E-selenium deficiency) and exposure to toxic agents [5-7] but whilst it is frequently reported that exercise leads to muscle fibre hypertrophy in fish [8], swimming activity is not known to induce muscle damage. Pathologically induced muscle damage (PMD) is characterized by an irregular, indistinctive and multifocal distribution of the damaged areas within the myotomes [2]. Based upon histomorphological analysis of the white swimming muscle in hatchery-reared Arctic charr (*Salvelinus alpinus*), we wish to suggest a novel category of muscle damage, which we shall designate growth-associated muscle damage (GMD). Unlike PMD, that seen in healthy and rapidly growing fish is not scattered within the myotomes but relates to the myosepta in a well-defined and systematic pattern.

Material and Methods

In this study, we examine skeletal muscle tissues sampled from sexually immature Arctic charr (30-60 g). At the start of the experiment, individually tagged fish were divided randomly into three homogeneous treatment groups each group containing 100 individuals at an initial size of approximately 20 g and 13 cm [9]. Fish were either allowed to perform routine activity (controls) or were forced to swim (exercised) continuously against different water current speeds corresponding to either 1 or 2 body lengths per second (BL/s) for a period of 63 days.

At the end of the experiment, the white swimming muscle of sub-samples of fish from each group were taken for histomorphological analysis of transverse sections using light-microscopy and a planimeter (Planix 7 P). All tissue samples were excised from the left mid-lateral region below the dorsal fin, immersion-fixed in buffered neutral formalin solution, and embedded in paraffin wax. Sections (3 μm) were routinely stained with Van Gieson and a combination of hematoxylin and eosin (HE) before being analysed [10]. Somatic growth of individual fish was calculated for each consecutive 21-day period (initial, intermediate and final) as (= ΔW x 100/Wd) where ΔW is weight gain (in g) in the 21-day periods 0-21, 21-42 or 42-63 and Wd is weight (g) on days 0, 21 and 42 respectively. Due to loss of tags etc. during the course of the experiment, only individual fish with complete growth data sets were used in this study. Consequently, the number of fish examined differed between treatment groups at the end of the experiment (Table). Further details concerning the experimental conditions and sampling procedures are given by Christiansen and Jobling [9] and Christiansen *et al.* [10].

Results

A remarkable variability was seen in myo-morphological characters such as the appearance of the myoseptal packing confining each myotome, and the localization and extension of damaged muscle fibres within the myotomes. When these characters were examined in the light of

Table. The number of Arctic charr examined. O denote control fish. BL/s denote the relative swimming speed expressed as body-lengths per second. PMD and GMD denote fish showing pathologically induced and growth-associated muscle damage respectively. Specific growth rate (SGR [18]) for individual fish was calculated over the 63day period. SGR is designated either as poor (SGR < 0 = neg), moderate (SGR = ≥ 0 ≤ 1 = +) or rapid (SGR > 1 = ++).

Swimming speed (BL/s):	0			1			2		
Growth:	neg	+	++	neg	+	++	neg	+	++
Category:									
NORMAL	1	1	-	-	2	-	1	2	-
PMD	3	1	-	1	-	-	-	-	-
GMD phases:									
Degenerative	-	1	-	-	-	-	-	1	2
Regenerative	-	1	2	-	1	1	-	-	7
Restitutive	-	-	1	-	-	1	-	-	1
Number of fish per treatment group		11			6			14	

somatic growth patterns displayed by individuals the fish could be classified as pertaining to one of three main categories, with each category revealing a unique combination of myo-morphology and somatic growth pattern:

The normal fish

Fish designated as NORMAL were found in both control and exercised groups (Table). The myo-morphology was characterized by intact myotomes and slim myosepta. Somatic growth was low to moderate (Fig. 1).

The PMD fish

Fish showing PMD were found, primarily, in the control group and this type of muscle damage was not observed amongst fish exercised at 2 BL/s (Table). The myo-morphology revealed a clear pathological pattern showing varying degrees of muscle atrophy. Within the central part of the myotomes intact muscle fibres were interspersed with severe discontinuous and multifocal damaged areas (Fig. 2). The myosepta were slim, but within the damaged areas proliferation of connective tissue was marked and blood cells were often seen. Fish showing PMD were all in poor condition, had failed to eat and had lost body weight during the course of the experiment (Table).

The GMD fish

In contrast to PMD fish, none of the fish showing evidence of GMD had lost weight and almost all displayed rapid somatic growth (Table). The GMD fish were found in all treatment groups, although predominantly amongst

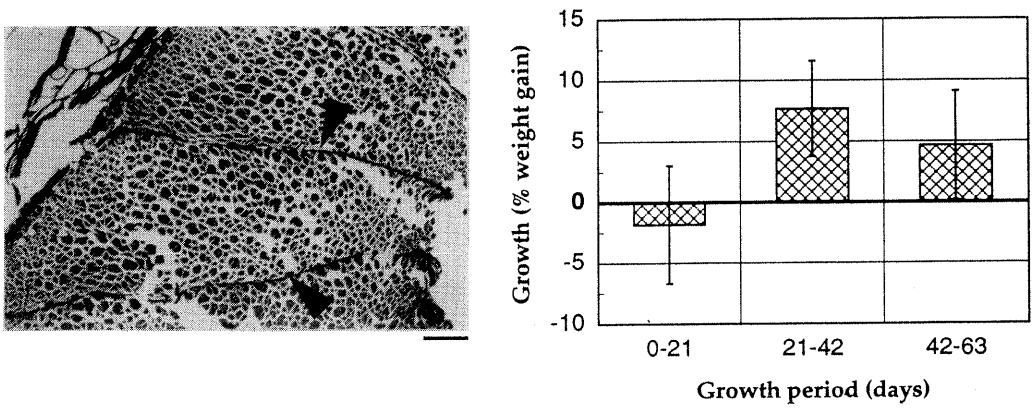

Figure 1. Myo-morphology and growth pattern of Arctic charr within the category designated as NORMAL. All transverse sections were routinely stained using HE and Van Gieson [10, 19]. Arrows denote myosepta. Scale bars below photomicrographs = 500 μm. Bars in histograms indicate SEM.

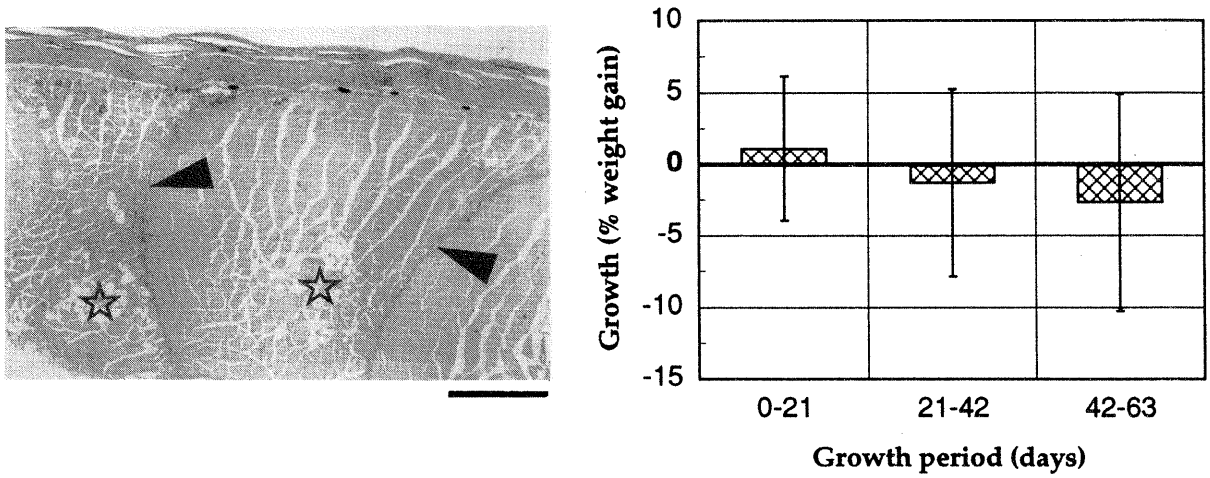

Figure 2. Myo-morphology and growth pattern of Arctic charr within the category designated as pathologically induced muscle damage (PMD). Asterisks denote multifocal muscle damage. For other abbreviations and symbols see figure 1.

fish exercised at 2 BL/s (Table). Generally, the fish appeared to be healthy, in good condition and muscle fibres displayed varying degrees of hypertrophy. In contrast to fish within the PMD-category, muscle damage in GMD-fish was very distinct and localized only in the region close to the myosepta (Fig. 3A). Examination of myo-morphology and somatic growth patterns of individual fish suggested that GMD could be divided into three sequential phases which we tentatively designate degenerative, regenerative, and restitutive phases respectively (Fig. 3).

In fish undergoing the degenerative phase, the bulk of the myotome was intact and myosepta were slim. Unlike PMD, damage was never seen scattered throughout the central part of a myotome but a continuous and well-defined damaged area, sharply separated from the intact muscle fibres, was seen close to one of the myosepta within each myotome. Septal muscle damage was never observed on both sides of a given myoseptum but was localized either distally or proximally towards the horizontal septum displaying a very regular and systematic pattern (Fig. 3A). The damaged areas consisted of disrupted muscle fibers and homogeneous myofibrillar material, but no intrusions of connective tissue and blood cells were observed. Planimetric measurements [10] revealed that muscle damage was substantial, amounting to 21.5-34.5 % of the total myotomal area. Fish displaying degenerative GMD had high somatic growth that increased continuously during the course of the experiment.

Fish in the regenerative phase of GMD, showed essentially the same pattern of muscle damage as fish in the degenerative phase but the damaged areas were much smaller (Fig. 3B). There was, however, considerable proliferation of connective tissue resulting in myoseptal pyknosis. During the initial growth period (days 0-21) fish showing regenerative GMD had somatic growth that was more than double that of fish undergoing the degenerative phase. Growth continued to increase during the intermediate growth period (days 21-42) but during the final growth period (days 42-63) growth was stabilized at the level observed during the intermediate growth period.

For fish undergoing the restitutive phase, the myotomes were intact and there was no evidence of disrupted muscle fibres. Septal muscle damage appeared to have been completely replaced by large intrusions of fat and myoseptal connective tissue. Consequently, myosepta were at their thickest in these fish (Fig. 3C). Somatic growth of fish displaying the restitutive phase of GMD were the highest observed for fish in any category during the initial and intermediate growth periods. During the final growth period, however, fish still grew well but growth was lower than in the preceding periods.

Discussion

Physical exercise is known to stimulate protein turnover [11], muscle fiber hypertrophy [8], somatic growth [9], and improve general welfare [9] in salmonid fish, and GMD was observed, predominantly, in the exercised fish. However, fish displaying GMD were also found amongst the most rapidly growing individuals held under control conditions. The swimming behaviour between control and exercised fish differed markedly. Whereas exercised fish exhibited regular and syncronized locomotion throughout the experiment, fish performing routine activity displayed great variability in locomotory activity, ranging from a state of more or less passivity to spontaneous bursts of high speed swimming [9]. Consequently, GMD does not result from the swimming mode displayed by the fish *per se* but appears to be limited to periods when individual fish undergo rapid somatic growth irrespective of exercising regime.

To our knowledge, the distinct septal muscle damage as shown in Fig. 3A, and evidence of GMD in the swimming muscle of fish and skeletal muscle damage in other fast growing vertebrates has not been reported previously. The

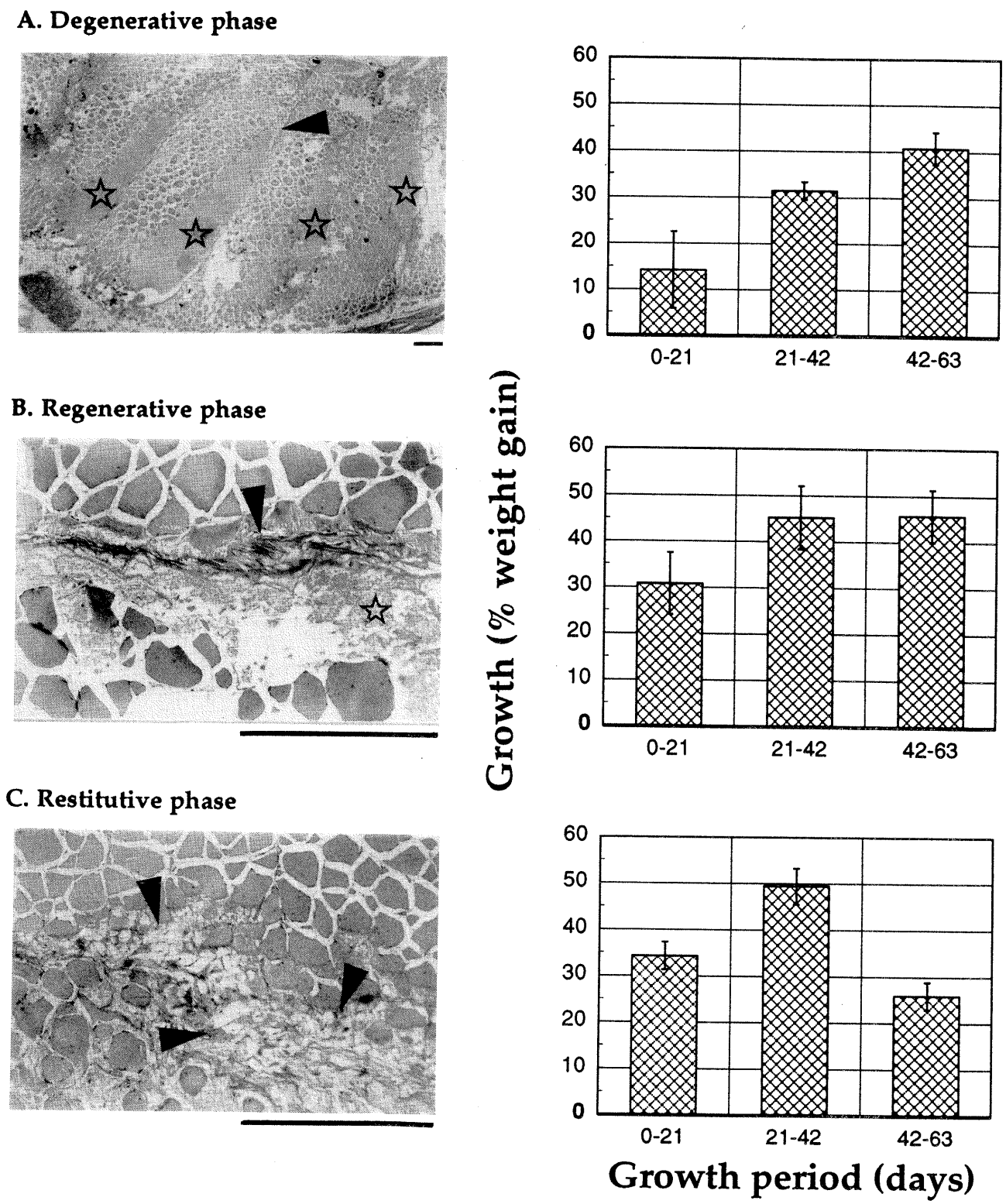

Figure 3. Myo-morphology and growth pattern of Arctic charr within the category designated as growth-associated muscle damage (GMD). The three suggested phases of GMD (A. Degenerative, B. Regenerative, and C. Restitutive) show a transition from distinct septal muscle damages (asterisks) and slim myosepta to intact muscle fibres and proliferation of fat and myoseptal connective tissue. For other abbreviations and symbols see figure 1.

simultaneous study of myo-morphology and somatic growth performance of individual fish is a prerequisite for the identification of GMD. Somatic growth of individual fish may either not have been recorded in earlier studies of muscle morphometrics [12], or muscle morphometrics may have been examined only for fish that displayed low to moderate somatic growth [13]. Slowly-growing fish would not be expected to develop GMD.

It is possible that GMD may be a common phenomenon in healthy and rapidly growing fish within the salmonid species, since myo-morphological characters comparable to those seen in the Arctic charr have been found in healthy non-exercised post-smolt of Atlantic salmon *(Salmo salar)* (Anil B. Amin personal communication). However, individual growth records for these fish were not available and, consequently, the possibility of there being a correlation between somatic growth pattern and myo-morphological characters could not be established.

The proximate causes *(in sensu* Mayr [14]) and the consequences of extensive GMD are obscure and require further investigation. A partial explanation for the development of GMD may, however, be sought in the unique spatial arrangement of muscle fibres within the white skeletal muscle of fish. Myotomes are constrained by collageneous myosepta into which muscle fibres are inserted [15]. High rates of somatic growth result in a rapid increase in muscle mass, and, due to lack of space (overgrowth) within the myoseptal packing, distinct regular patterns of septal muscle damage may develop as a result of mechanical disruption of muscle fibres. Following the degenerative phase collagen synthesis may be stimulated during the replacement of muscle fibres [16, 17] leading to thick myosepta and somatic growth is stabilized or even restrained (Fig. 3).

In conclusion, we suggest that the development of septal muscle damage may be a normal and non-pathological phenomenon in rapidly growing salmonid fish, and that GMD may result from a combination of a rapid increase in muscle mass and a delayed expansion of the myoseptal packing. Thus, the previous somatic growth patterns displayed by individual Arctic charr could be estimated from the appearance of simple myo-morphological characters.

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References

[1] Edwards RHT, Jones DA: in Peachey LD, Adrian RH, Geiger SR (eds): *Handbook of Physiology (Sec. 10),* Amer Physiol Soc, Bethesda, Maryland, 1983; pp 633-672.

[2] Kuipers H, Drukker J, Frederik PM et al: *Int J Sports Med* 1983; 4: 45-51.

[3] McCully KK: *Federation Proc* 1986; 45: 2933-2936.

[4] Pearson AM: *Food Science and Nutrition* 1990; 29:167-196.

[5] Poston HA, Combs Jr GF, Leibovitz L: *J Nutr* 1976; 106: 892-904.

[6] Huerkamp MJ, Ringler DH, Chrisp CE: *Laboratory Animal Science* 1988; 38: 178-182.

[7] Haensly WE, Neff JM, Sharp JR et al: *J Fish Diseases* 1982; 6: 365-391.

[8] Davison W: *Comp Biochem Physiol* 1989; 94A: 1-10.

[9] Christiansen JS, Jobling M: *Can J Zool* 1990; 68: 2186-2191.

[10] Christiansen JS, Jobling M, Amin AB et al: *Can J Zool* 1991; 69: 2450-2455.

[11] Houlihan DF, Laurent P: *Can J Fish Aquat Sci* 1987; 44: 1614-1621.

[12] Weatherley AH, Gill HS: *The Biology of Fish Growth.* Academic Press, London, 1987; pp 1-443.

[13] Weatherley AH, Gill HS: *Experientia* 1989; 45: 875-878.

[14] Mayr E: *Science* 1961; 134: 1501-1506.

[15] Bone Q: in Hoar WS, Randall DJ (eds): *Fish Physiology (Vol. 7).* Academic Press, London, 1978; pp 361-424.

[16] Myllylä R, Salminen A, Peltonen L et al: *Pflügers Arch* 1986; 407: 64-70.

[17] Kovanen V: *Acta Physiol Scand* 1989; 135: 1-56.

[18] Ricker WE: in Hoar WS, Randall DJ (eds): *Fish Physiology (Vol. 8).* Academic Press, London, 1979; pp 677-743.

[19] Geneser F: *Textbook of Histology.* Munksgaard, Copenhagen, 1986; pp 1-831.