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**Family origin and the response of threespine stickleback, Gasterosteus aculeatus, to thermal acclimation**

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**Abstract** To establish whether family origin affects the response of the threespine stickleback (*Gasterosteus aculeatus*) to thermal acclimation, we examined the rates of feeding, growth, and food conversion, relative tissue and organ masses and activities of a mitochondrial and a glycolytic enzyme in pectoral and axial muscle of individually housed fish from six families during acclimation to 8 °C and 23 °C. Feeding rates differed among families but were consistently higher in warm-acclimated than cold-acclimated fish. Growth rates differed among families. In four families growth was greater at 8 °C; these families generally had higher conversion efficiencies at 8 °C than 23 °C. For two families, growth was greater at 23 °C than 8 °C and conversion efficiencies did not differ between 8 °C and 23 °C. Relative tissue and organ masses (percent axial muscle, hepatosomatic, gut and kidney indices) differed with gender and among families (hepatosomatic, gut and kidney indices) but little with acclimation status. In all families and in both muscles, activities of the mitochondrial enzyme, citrate synthase (CS), were increased by cold acclimation. Axial muscle levels of the glycolytic enzyme, lactate dehydrogenase (LDH), were not affected by thermal acclimation or family origin, but were strongly correlated with the hepatosomatic index and axial muscle protein content. Pectoral muscle levels of LDH were affected by family origin which also influenced the response to thermal acclimation. Similar patterns were observed for specific activities and total muscle contents of these enzymes. Stickleback family origin influenced rates of feeding and growth and the thermal sensitivity of growth rates but not the compensatory increase in muscle CS levels with cold acclimation. The differing thermal sensitivities of growth could reflect distinct strategies for the timing of juvenile growth.

**Key words** Threespine stickleback · Growth · Feeding · Food conversion · Muscle metabolic capacities

**Abbreviations** CS citrate synthase · DTNB 5,5′-dithiobis-2-nitrobenzoic acid · GSI gonadosomatic index · HSI hepatosomatic index · LDH lactate dehydrogenase

**Introduction**

For ectotherms, physiological rates are set by abiotic factors, principally temperature, and by biotic factors among which genetic parameters are central. Direct thermal effects accelerate biochemical reactions (up to a thermal optimum), stimulate compensatory responses and may lead to a variety of secondary responses. When temperature rises, the increased maintenance requirements will lead tissue reserves and metabolic capacities to deteriorate unless food ingestion rises accordingly (Vézina and Guderley 1991; Guderley et al. 1994). Therefore, given the genetic influences upon basal metabolic rates (Hawkins et al. 1986; Danzmann et al. 1987), responses of growth rates and tissue metabolic capacities to temperature may vary with genetic background.

Many species of temperate fish acclimate to changes in habitat temperatures by adjusting tissue metabolic capacities, presumably to enhance organismal performance at the new temperature. Cold acclimation markedly increases muscle aerobic capacity (Johnston et al. 1985) and sustained swimming capacity at low temperatures in carp (*Cyprinus carpio*; Rome et al. 1984) and striped bass (*Morone saxatilis*; Sisson and Sidell 1987). Burst swimming performance of mummichog (*Fundulus heteroclitus*), goldfish (*Carassius auratus*) and short-horned sculpin (*Myoxocephalus scorpius*) is improved by cold acclimation, presumably via changes in the myosin ATPase complex in glycolytic fibers (Beddow and Johnston 1995; Johnson and Bennett 1995; Ball and Johnston 1996). Cold acclimation enhances oxidative
capacity of muscle of channel catfish (Ictalurus punctatus) in all seasons, but the response of muscle glycolytic capacity differs seasonally (Seddon and Prosser 1997). Indirect thermal effects may confound compensatory responses of muscle metabolic capacity, particularly in white muscle which changes its biochemical composition considerably with shifts in food availability and body condition (Loughna and Goldspink 1984; Guderley et al. 1996; Dutil et al. 1998).

In wild populations, physiological variability among individuals is partly genetic and partly due to differential success at feeding, winning agonistic interactions, obtaining territories or avoiding parasitism. Considerable interfamilial variability in size at a given age, muscle enzyme levels and swimming performance occurs in laboratory-reared juvenile threespine stickleback Gasterosteus aculeatus (Garenc et al. 1998). As genetic factors influence basal metabolic rate and growth rates in a variety of organisms (Hawkins et al. 1986; Danzmann et al. 1987), we reasoned that stickleback from different families could vary in rates of feeding, growth and food conversion capacity. This, in turn, could influence muscle metabolic capacities.

Anadromous threespine stickleback from the Isle Vert population in eastern Canada migrate from the cold St. Lawrence Estuary to high and fluctuating temperatures (up to 30 °C) in the intertidal salt marsh pools in which they reproduce in May and June (Craig and FitzGerald 1982). Adults return to the Estuary after breeding (early July) whereas juveniles gradually depart during the summer (Picard et al. 1990). Cold acclimation of threespine stickleback increases muscle aerobic capacity, particularly in the 1-year-old fish which can live a 2nd year (Vézina and Guderley 1991; Guderley et al. 1994). We reasoned that the selective importance of eurythermality of swimming performance during the reproductive season is such that thermal compensation of muscle oxidative capacity occurs in all stickleback families. On the other hand, selection for distinct juvenile migratory strategies (timing and duration of stay in salt marsh pools) could be related to interfamily differences in the response of growth rate and tissue energetic status to thermal acclimation.

Therefore, in this study, we examined whether family origin influences the response of feeding rate, growth rate, food conversion efficiency, condition factor, relative tissue and organ masses and muscle enzyme levels of threespine stickleback to thermal acclimation. In early June, 1-year-old, laboratory reared stickleback from six families were acclimated to 8 °C and 23 °C. The fish were individually housed to facilitate measurement of rates of growth, feeding and conversion efficiency. Photoperiod was changed weekly according to local conditions. To assess muscle metabolic capacities, we measured citrate synthase (CS), a marker for the mitochondrial matrix, and lactate dehydrogenase (LDH), the terminal enzyme of glycolysis, in the pectoral and axial muscles. Activities were measured at 10 °C and 20 °C to approximate capacities at acclimation temperatures.

### Materials and methods

#### Fish rearing and acclimation conditions

Three-spine stickleback were obtained by crossing wild parental fish which had been captured after their spring reproductive migration in salt marsh pools in the National Wildlife Reserve of Isle Verte near Rivière du Loup, Quebec and rearing the progeny for 1 year in our laboratory at Université Laval. Once the wild stickleback had habituated to laboratory conditions, six male stickleback were placed in individual 40-l aquaria and allowed to establish their nests. Then each male was presented with a gravid female. Once the female had deposited her eggs in the nest, she was withdrawn and the male cared for the offspring until shortly after the larval fish started to feed. All crosses involved different parental fish. The stickleback from a given family (full siblings) were reared together at 23.9 ± 1.5 °C (mean ± SD) and salinity was adjusted to 20‰ using deionized water and an artificial sea-water mixture (Forty Fathoms “Bio-Crystals”; Hagen, Montreal, Canada). Rearing and feeding conditions for the first 6 months followed Garenc et al. (1999). Thereafter, adult Artemia sp. and blood worms were provided ad libitum 3-times a week. The photoperiod was changed weekly to correspond to seasonal values at our latitude.

For thermal acclimation, 14 fish were taken from the six families (families A to F), weighed, measured for total length and placed in individual 2-l tanks in late May (photoperiod 15L:9D). The tanks were haphazardly assigned to cold and warm acclimation. As it is difficult to determine gender unless stickleback are breeding, assignment of males and females to acclimation conditions was confirmed during dissection of the fish. In the warm-acclimated groups, the ratio of males to females was 2:3 for families A, B, C, E, and F and 3:4 for family D. In the cold-acclimated groups, the ratio was 4:3 for families D, E and F, 3:4 for family A, 2:5 for family B and 5:2 for family C. Although the sex ratio could not be shown to differ with acclimation condition or family (χ² test, P=0.978), gender was included as a factor in the statistical analyses.

Half of the fish were maintained at their rearing temperature (23±1 °C mean ± SD) for a further 8 weeks, while the others were placed at 8±1 °C for 11 weeks. The top of each aquarium was 25 cm below a fluorescent light (34 W). Photoperiod and salinity were controlled as described above. Every day, fish were fed ad libitum with blood worms and frozen shrimp. At all times, excess food remained after feeding. During the last 10 days of acclimation, the fish were fed with a pre-weighed amount of blood worms (0.3 g) and the worms remaining 15 min after feeding were collected, drained and weighed. The water in the tanks was changed at least 3-times a week. No parasites were apparent during acclimation or subsequent dissections of the stickleback. At the end of thermal acclimation, the fish were again weighed, measured, killed by decapitation and frozen at ~70 °C for later enzymatic determinations.

### Calculated parameters

**Rates of growth (%) mass day⁻¹, mg day⁻¹** and feeding (% mass day⁻¹) were calculated relative to the initial mass. The conversion efficiency was estimated as the daily growth in mass (over the entire acclimation period) relative to the daily feeding rate (during the last 10 days of acclimation), assuming that growth was constant throughout the acclimation period. We calculated Fulton’s condition factor:

\[ C = \frac{\text{mass}}{\text{length}^3} \cdot \text{1000} \cdot \text{1000} \]

Organ and tissue masses (liver, gonads, kidneys, gut, axial and pectoral muscles) were determined after dissection of the thawed specimens and were expressed relative to the total body mass. The gut was emptied of its contents before weighing.