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Lack of metabolic thermal compensation during the early life stages of ocean pout *Zoarces americanus* (Bloch & Schneider) : a benthic, cold-water marine species

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The present study investigated the metabolic response of young ocean pout *Zoarces americanus* to temperature acclimation (3 *v.* 11° C), and to acute changes in water temperature from 3 to 17° C. The Q_{10} value for standard metabolic rate between acclimation temperatures was 5·3, warm-acclimated fish displayed higher rates of oxygen uptake at all temperatures during the acute thermal challenge, and changes in whole-body citrate synthase activity were qualitatively similar to those seen for metabolism. These results indicate that, in contrast to temperate species, young ocean pout from Newfoundland do not show thermal compensation in response to long-term temperature changes.

Key words: acclimation; metabolism; temperature; thermal compensation; *Zoarces americanus*.

Temperature is one of the most important factors influencing the metabolism of fishes. The physiological response to temperature change, however, depends on whether the change is of short or long duration. During an acute change in water temperature, fishes generally show large deviations in metabolic rate from that measured at their acclimation temperature: cold-acclimated individuals typically showing a large increase in metabolic rate when exposed to warm temperatures, while warm-acclimated fish often show a considerable decrease when acutely exposed to colder temperatures (Hazel & Prosser, 1974; Steffensen, 2005). In contrast, after prolonged exposure to the new environmental temperature, many fish species adjust their physiology, such that their metabolic rate rebounds toward its original value until it reaches a new, relatively constant state. If metabolic rate after acclimation is equivalent to that measured at the original temperature, the fish is said to have experienced complete compensation, whereas partial compensation is when post-acclimation metabolic rate is

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in-between that measured at the original temperature and that recorded shortly after the acute thermal challenge (Jobling, 1994).

These patterns of thermal acclimation appear applicable to most fish species (Jobling, 1994; Steffensen, 2005). Most work in this area, however, has focused on temperate species that display moderate to high levels of activity (Sidell, 1980; Jones & Sidell, 1982; Evans, 1990; Rodnick *et al.*, 2004), and there have been few investigations into the thermal physiology of non-polar cold-water species, or inactive species. Cold-water or inactive species are likely to be highly adapted to their specific environment and lifestyle, and may therefore show a reduced capacity to adjust their metabolism in response to temperature fluctuations. In support of this hypothesis, Zakhartsev *et al.* (2003) found that the ability of adult boreal eelpout *Zoarces viviparous* (L.) to acclimate to temperature change varies among populations according to their geographical distribution; eelpout from the cold Norwegian Sea show no metabolic compensation to long-term increases in water temperature, whereas compensation is seen in those from warmer, more southern regions. Based on these results, Zakhartsev *et al.* (2003) speculated that eelpout in the Norwegian Sea may be 'permanently adjusted' to life at colder temperatures, as they do not need to respond to large temperature fluctuations in their native habitat.

Understanding thermal acclimation in fishes is important because environmental temperature has a profound influence on energy allocation within individuals (Mehner & Weiser, 1994; Brodte *et al.*, 2006). For example, at temperatures beyond species-specific thermal ranges (defined by upper and lower 'pejus' tempertures; Pörtner, 2002), increased maintenance costs cause a reduction in aerobic scope that constrains growth, activity and reproduction (Pörtner *et al.*, 2006). By influencing these processes, global climate change could affect population abundances and their geographical distributions (Pörtner, 2002; Pörtner & Knust, 2007). Interestingly, however, while most work examining thermal acclimation in fishes has been performed on adult individuals, it is the delicate early life stages that may be most sensitive to changes in temperature (Rombough, 1988; Johnston & Hall, 2004; Killen *et al.*, 2007). Furthermore, survival through the early life stages is believed to be a critical factor influencing the population size and distribution of adult fishes (Houde, 1997).

The present study examined the metabolic response of newly hatched ocean pout *Zoarces americanus* (Bloch & Schneider) to acute and chronic temperature changes. Ocean pout were selected because populations of this species inhabit cold-waters off the coast of Newfoundland, and these fish produce elevated levels of antifreeze proteins year-round (Fletcher *et al.*, 2001). The latter characteristic, in particular, suggests that these fish are highly-adapted to life in cold-water. Further, ocean pout are a benthic, relatively inactive species, and show almost no movement when in respirometers (Killen *et al.*, 2007). This aspect makes them ideal for studying the effects of temperature on metabolism since measurements of oxygen uptake are representative of standard metabolic rate.

Ocean pout eggs (four masses in total, with each egg mass from a different family) were collected from the wild by scuba divers and placed in laboratory incubators at the Ocean Sciences Centre, Memorial University of Newfoundland, until hatching. The incubators were supplied with flow-through sea water

at 3° C. After hatching, groups of ocean pout were acclimated to either 3 or 11° C and fed a diet of enriched *Artemia* sp. nauplii; 3° C was the ambient water temperature in the natural habitat of young ocean pout at the time that the study was performed. The fish remained at these temperatures for a minimum of 4 weeks prior to experimentation. After fasting for at least 36 h, individual ocean pout (mean ± S.E. wet mass 280 ± 19 mg) were transferred to a small Blazka-type respirometer (57 ml volume; described in detail by Killen *et al.*, 2007) set at their acclimation temperature, and left undisturbed overnight. The next morning, oxygen uptake was measured using a fibre-optic oxygen measurement system (PreSens GmbH, Regensburg, Germany; Killen *et al.*, 2007). For fish acclimated to 11° C, temperature within the respirometer was then gradually reduced to 3° C over the course of 30 min. These fish were then left at this temperature for an additional hour, at which time oxygen uptake was measured at 3° C. Preliminary experiments showed that this time course allowed oxygen uptake to stabilize in young ocean pout after this drop to 3° C. For both groups, the water temperature was then increased by 2° C every 1·5 h until the temperature reached a maximum of 17° C, with oxygen uptake measured at every 2° C increment. While it is probably rare that this species experiences such high temperatures in the wild, shallow Newfoundland bays can turnover in the summer, and acute changes of $\geq$10° C can be seen over the course of several hours down to 10 m in depth (Gollock *et al.*, 2006).

Decreases in respirometer O_2 content (mg O_2 ml^{-1}) were converted to mass-specific oxygen uptake using the wet mass of each fish. The effect of variations in body size within treatments were accounted for by scaling all absolute oxygen uptake measurements to the power of 0·834 (the metabolic scaling exponent for this species determined by Killen *et al.*, 2007). The ocean pout were almost always motionless in the respirometers, and measurements of oxygen uptake therefore approximate standard metabolism. At 17° C, however, some individuals from both acclimation temperatures did display increased levels of activity. This consisted of only occasional movements within the respirometer, and thus activity-related increases in metabolic rate were expected to be minor.

Arbitrarily selected ocean pout were also sampled from the holding tanks for analysis of whole-body citrate synthase activity. Individuals were carefully netted from the tanks, immediately flash-frozen in liquid nitrogen, and then stored at −80° C until analysis. Citrate synthase activity was quantified using a spectrophotometric assay similar to that described by Sidell *et al.* (1987), with whole-body homogenates of 3- and 11° C-acclimated fish being assayed at both 3 and 11° C. Q_{10} values (which represent the factor by which a physiological rate increases or decreases with a temperature change of 10° C) based on acclimation temperatures and acute changes in temperature (3$\leftrightarrow$11° C) were calculated for oxygen uptake and citrate synthase activity using the equation provided by Schurmann & Steffensen (1997). All parts of the present study were conducted in accordance with the guidelines of the Canadian Council on Animal Care, and with the approval of the Institutional Animal Care Committee of Memorial University of Newfoundland (Protocol # 04-02-KG).

Mean ± S.E. oxygen uptake at the acclimation temperatures of 3 and 11° C were 76·26 ± 12·48 and 301·24 ± 38·33 mg O_2 $kg^{-0{\cdot}834}$ h^{-1}, respectively ($P < 0{\cdot}05$; $Q_{10} = 5{\cdot}3$) (Fig. 1). This Q_{10} value is much larger than would normally be

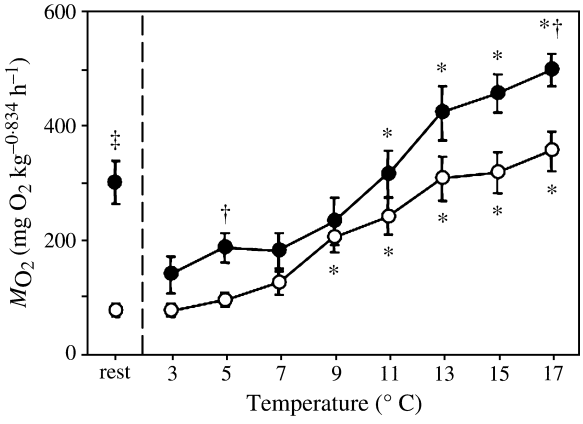

FIG. 1. Mean ± S.E. oxygen uptake (MO_2) of young ocean pout acclimated to either 3° C (○) or 11° C (●) (rest), and during an acute change in water temperature ($n = 9$ for all data points). MO_2 was significantly higher in 11 *v.* 3° C acclimated ocean pout when tested at their respective acclimation temperatures (†, $P < 0{\cdot}05$; one-way ANOVA). There was a significant interaction between the effects of acclimation temperature and the acute temperature increase on MO_2 (repeated measure ANOVA, $P < 0{\cdot}05$). Thus: (1) Dunnett's tests were used to establish, within each treatment, when MO_2 was elevated as compared with that measured at 3° C (*, $P < 0{\cdot}05$) and (2) separate one-way ANOVAs were used to test for significant differences in MO_2 between groups (3 *v.* 11° C acclimated) at each temperature during the acute challenge (‡, $P < 0{\cdot}01$ after Bonferroni correction for multiple tests). Finally, paired *t*-tests were used to compare MO_2 following acclimation with the equivalent temperature during the acute increase test. These values, however, were not significantly different ($P > 0{\cdot}05$). The Q_{10} value at rest between fish acclimated to 3 and 11° C was 5·3. Acute Q_{10} values from 3 to 11° C were 5·28 ± 1·33 for the cold-acclimated fish, and 5·22 ± 1·78 for the warm-acclimated fish; while acute Q_{10} values from 11 to 17° C were 2·07 ± 0·44 for the cold-acclimated fish, and 2·55 ± 0·35 for the warm-acclimated fish.

expected over this temperature range, as Q_{10} values of acclimated adult fishes are generally between 1 and 2 (Steffensen, 2005). One potential explanation for this elevated Q_{10} is that young ocean pout experience torpor or metabolic depression at 3° C. Such is the case for American eels *Anguilla rostrata* (Lesueur) ($Q_{10} = 4{\cdot}1$ between 5 and 10° C; Walsh *et al.*, 1983) and goldsinny wrasse *Ctenolabrus rupestris* (L.) ($Q_{10} = 15{\cdot}8$ between 4 and 10° C; Sayer & Davenport, 1996), both of which enter torpor at decreased water temperatures. The possibility that young ocean pout enter torpor, however, seems unlikely. Previous behavioural observations on newly hatched ocean pout indicate that, although they decrease overall activity at 3° C, young ocean pout still forage at this temperature and therefore do not appear to be in a torpid state (Killen & Brown, 2006). Furthermore, the standard metabolic rate of young ocean pout acclimated to 3° C (76·26 ± 12·48 mg O_2 $kg^{-0{\cdot}834}$ h^{-1}) is not unusually low when compared with juveniles of other marine teleost species at similar temperatures that do not enter torpor, when adjusted for differences in body size using appropriate metabolic scaling exponents (Bokma, 2004; Killen *et al.*, 2007) *e.g.* shorthorn sculpin *Myoxocephalus scorpius* (L.) = 46·2 mg O_2 $kg^{-0{\cdot}83}$ h^{-1} at 3° C (Killen *et al.*, 2007) and Øresund population Atlantic cod *Gadus morhua* L. = 26·07 mg O_2 $kg^{-0{\cdot}86}$ h^{-1} at 5° C (Schurmann & Steffensen, 1997).

An alternative explanation for the increased Q_{10} value for acclimated ocean pout is that their standard metabolism at 11° C (301·24 ± 38·33 mg O_2 $kg^{-0·834}$ h^{-1}) is higher than would be expected. Measurements of resting metabolism (*i.e.* standard or routine metabolic rate) are highly variable for fishes, but standard metabolism for ocean pout at 11° C in the present study was considerably higher than values reported for the juvenile stage of other teleost species at similar temperatures when adjusted for differences in body size [*e.g.* Atlantic cod = 43·23 mg O_2 $kg^{-0·86}$ h^{-1} at 10° C (Schurmann & Steffensen, 1997); whitefish *Coregonus lavaretus* (L.) = *c.* 130 mg O_2 $kg^{-0·86}$ h^{-1} at 14° C (Karjalainen *et al.*, 2003) and lumpfish *Cyclopterus lumpus* L. = 212·86 mg $kg^{-0·82}$ h^{-1} at 11° C (Killen *et al.*, 2007)]. Standard metabolic demands can increase greatly in fishes exposed to temperatures which exceed their upper pejus temperature (upper and lower pejus temperatures define the range beyond which aerobic scope begins to decrease due to a mismatch between oxygen supply and demand; Pörtner, 2002; Pörtner *et al.*, 2006). Although little is known about the ecology of young ocean pout off the shores of Newfoundland, their year-round production of antifreeze proteins suggests that they are a stenothermal species which are highly adapted to a narrow range of cold temperatures. In support of this hypothesis, Newfoundland populations of ocean pout have plasma antifreeze proteins that are five to 10 times higher than more southern New Brunswick populations (Fletcher *et al.*, 2001). If 11° C is higher than the temperature range to which this species is adapted, exposure to this temperature could greatly elevate the energy required for maintenance. This is especially probable since the early life stages of fishes are generally more sensitive to changes in temperature as compared with juveniles and adults (Rombough, 1988; Johnston & Hall, 2004; Pörtner *et al.*, 2006), with Q_{10} values for acclimated larvae often being in the range of 3–5 (Rombough, 1988). Although ocean pout are relatively large at hatch and are not considered altricial larvae (Methven & Brown, 1991), newly hatched individuals may not yet possess the metabolic machinery required to cope with large temperature fluctuations.

It has been reported that increased proton leakage across mitochondrial membranes can affect baseline oxygen demands (Brand, 1990), and it is believed that this is a major contributor to elevated oxygen demands in fishes that are above their upper pejus temperature (Pörtner *et al.*, 2006). Indeed, the extent of proton leakage has been shown to be highly temperature-dependent in cold-adapted fish species (Hardewig *et al.*, 1999). During acclimation to colder temperatures, the cellular and mitochondrial membranes of fishes typically show decreases in the degree of fatty acid saturation in order to maintain fluidity (Prosser, 1991). Conversely, the membranes of warm-acclimated fishes generally have higher proportions of saturated fatty acids. A re-examination of the fatty acid data presented by Killen & Brown (2006) for young ocean pout reveals that the ratio of whole-body saturated to monounsaturated fatty acids does not differ significantly in fish acclimated to 3 and 8° C (Table I). Similarly, these fish did not show any differences in the percentage of total fatty acids that were saturated. Although the study by Killen & Brown (2006) did not examine the lipid content of pout ocean acclimated to 11° C, these results at 3 and 8° C suggest a reduced capacity for homeoviscous adaptation, and an inability to maintain membrane integrity with increases in temperature. Such

TABLE I. Ratios of whole-body saturated to monounsaturated fatty acids, and the percentage of total fatty acids that are saturated in young ocean pout acclimated to either 3 or 8° C. Mean ± S.E. ratios and percentages were calculated using the data presented in the control treatments of Killen & Brown (2006) ($n = 10$ for each temperature). There were no significant differences for either ratio of saturates to monounsaturates (unpaired t-test, d.f. = 18, $P > 0{\cdot}05$) or percentage of saturated fatty acids (unpaired t-test, d.f. = 18, $P > 0{\cdot}05$) between temperatures

Temperature (° C)	Saturates:monounsaturates	Percentage saturated
3	0·58 ± 0·03	21·15 ± 0·64
8	0·64 ± 0·03	21·86 ± 0·39

a situation could contribute to increased mitochondrial proton leakage at 11° C (and a concommitant increase in oxygen uptake).

This hypothesis, however, does not explain why the standard metabolic rate of the warm-acclimated fish was elevated as compared with cold-acclimated individuals at all temperatures during the acute temperature increase (although it was only significantly higher at 5 and 17° C; Fig. 1). In many temperate species, acclimation to warm temperatures results in a decrease in aerobic enzyme activity, and thus warm-acclimated fishes are normally unable to match the metabolic rate of cold-acclimated fishes when exposed to cool temperatures (Hazel & Prosser, 1974; Prosser, 1991; Steffensen, 2005). Conversely, acclimation to cold-temperatures involves an increase in aerobic enzyme activity in many species. These compensatory responses to temperature change are thought to be achieved by either adjusting enzyme concentrations (by changing the number of enzyme copies per mitochondria or by altering mitochondrial density) or by producing alternate enzyme isoforms that are more efficient at a particular temperature (Guderley, 1990; Prosser, 1991). The present results for standard metabolic rate at various temperatures (Fig. 1), however, suggest that young ocean pout do not show this typical compensatory response to long-term temperature change, and perhaps even display inverse thermal-compensation (Hazel & Prosser, 1970). Ocean pout are a relatively inactive benthic species, and previously it has been suggested that inactive or 'lethargic' species may not require thermal compensation due to their decreased standard metabolic demands (Hazel & Prosser, 1970). It also is thought that adaptation to life in cold-water may diminish a species' ability to metabolically compensate to warmer temperatures (Hazel & Prosser, 1970; Zakhartsev *et al.*, 2003). In this regard, sea raven *Hemitripterus americanus* (Gmelin) show no thermal compensation of intrinsic heart rate *in situ* (Graham & Farrell, 1985). The cellular mechanisms responsible for the lack of thermal compensation are not known; however, the ability of stenothermal cold-adapted fishes to increase enzyme activity per mitochondrion appears limited (Hardewig *et al.*, 1999). The trends observed in the present study might also be expected if young ocean pout only possess a single, cold-water adapted isoform of various metabolic enzymes, the production of which is increased during acclimation to warm temperatures (*i.e.* they show an inverse compensation due to decreased enzyme efficiency at higher temperatures). A drop to colder temperatures in

these warm-acclimated fish would thus result in a higher than expected metabolic rate because of the excess concentration of enzymes that are optimized for function at cold temperatures. This mechanism could account for the elevated standard metabolic rate of the warm-acclimated individuals during the acute exposure to the cooler temperatures, however, much more research is needed to substantiate this hypothesis.

The analysis of citrate synthase activity showed qualitatively similar trends to those observed for oxygen uptake (Fig. 2). Citrate synthase is an oxidative enzyme which is part of the Krebs cycle, and its activity is often used as an indicator of aerobic capacity (Torres & Somero, 1988). Once again, the results suggest that young ocean pout do not thermally compensate, as citrate synthase activity in cold- and warm-acclimated fish was not different at either assay temperature (3 or 11° C). Quantitatively, however, the present findings for citrate synthase differ somewhat from those for oxygen uptake data, in that the activity in warm-acclimated fish was not significantly higher than measured in cold-acclimated individuals. Further, both the chronic Q_{10} (2·17) and acute Q_{10} (1·81 ± 0·25 for cold-acclimated fish and 1·76 ± 0·25 for warm-acclimated fish) for citrate synthase were lower than those calculated for oxygen uptake. These results are not necessarily surprising, however, because whole-body citrate synthase activity was analysed instead of measuring the activity present in specific organs or tissues (whole-body analyses are often the only option when working with extremely small animals). Whole body measurements of oxidative enzyme activity may become confounded because tissues and organs vary greatly in the degree to which they thermally compensate, and there can

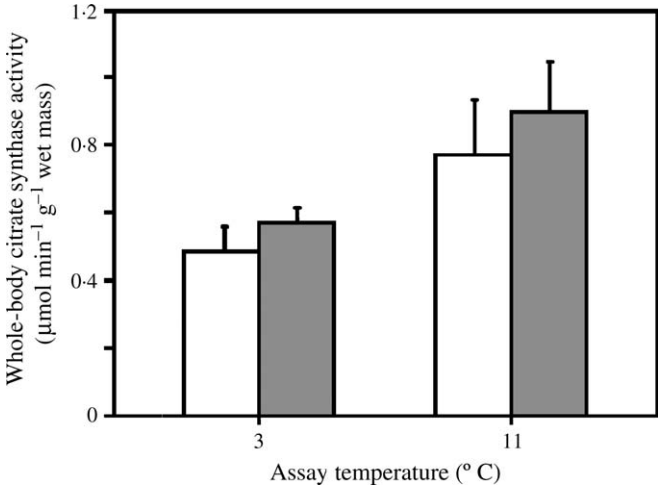

FIG. 2. Mean + S.E. (n = 7) whole-body citrate synthase activity for young ocean pout, *Zoarces americanus*, acclimated to either 3 (□) or 11° C (■) when assayed at both temperatures. The Q_{10} value for fish at their acclimation temperatures was 2·17, whereas the Q_{10} values for acute thermal change were 1·81 ± 0·25 for the 3° C acclimated fish and 1·76 ± 0·25 for the 11° C acclimated fish. The interaction term between acclimation temperature and assay temperature was not significant (two-way ANOVA, $P > 0·05$), the main effect of acclimation temperature was not significant ($P > 0·05$), but the main effect of assay temperature was significant ($P < 0·05$).

be shifts in the proportions of oxidative enzyme activity between tissues with thermal acclimation (Jones & Sidell, 1982; Hardewig *et al.*, 1999). Further, some tissues that comprise a large proportion of total body mass may posses relatively little citrate synthase activity (*e.g.* white muscle). These effects may overwhelm any significant changes in oxidative tissues with elevated citrate synthase activity such as the heart, red muscle or liver. Irrespective of the fact that whole-body enzyme activity was measured, the finding that citrate synthase activity of the warm-acclimated fish was not significantly lower than that of the cold-acclimated individuals at either temperature supports the hypothesis that young ocean pout do not show metabolic thermal compensation.

In summary, the present study shows that young ocean pout do not show typical thermal compensation when exposed to changes in temperature. Whether this is due to a reduced ability to compensate metabolically for changes in water temperature amongst cold-adapted/inactive species, inverse metabolic compensation, or the fact that early life-stages of fishes are particular sensitive to thermal change, is unclear and will require further investigation. These results, however, illustrate that researchers must use caution when attempting to predict the consequences of climate change on cold-water fish species, because their response to temperature change may vary substantially from that observed in temperate species. In addition, future work should examine how maximal aerobic metabolism and aerobic scope vary with temperature throughout the early development in fishes, and how these aspects of their thermal biology impact growth and survival early in life.

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