

Review

Metabolic cold adaptation of polar fish based on measurements of aerobic oxygen consumption: fact or artefact? Artefact!☆

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Abstract

Whether metabolic cold adaptation in polar fish, based on measurements of aerobic standard metabolic rate, is a fact or an artefact has been a dispute since Høleton asked the question in 1974. So far polar fish had been considered to be metabolically cold adapted because they were reported to have a considerably elevated resting oxygen consumption, or standard metabolic rate, compared with oxygen consumption values of tropical or temperate fish extrapolated to similar low polar temperatures. Recent experiments on arctic and Antarctic fish, however, do not show elevated resting aerobic oxygen consumption values, or standard metabolic rate, and hence it is concluded that that metabolic cold adaptation in the traditional sense is an artefact. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

In 1914 Ege and August Krogh measured the metabolism of one goldfish, *Carassius auratus*, at temperatures varying from 0 to 28 °C. Based on the measurements the calculated Q_{10} value increased from 2.2 at temperatures between 23 and 28 °C to a value of 9.8 at temperatures between 0 and 5 °C. These data later formed the basis for Krogh's standard or normal metabolism curve (Krogh, 1916). Scholander et al. (1953) used this normal metabolism curve to extrapolate and compare the oxygen consumption of tropical fishes measured at 30 °C with Arctic fishes measured at 0 °C. The oxygen consumption of the tropical species extrapolated to 0 °C were 30 to 40 times

lower than the actual measured values of the arctic fish at a similar temperature, and it was concluded that 'the arctic forms show a very marked relative adaptation to cold'. Wohlschlag supported these ideas and published data showing that the resting metabolism of Antarctic Notothenioid fish was indeed higher than of tropical and temperate fish extrapolated to similar low temperatures (Wohlschlag, 1960, 1964). The concept 'metabolic cold adaptation' (MCA)—that polar fish have a considerable elevated standard metabolic rate relative to expected metabolic rates of temperate or tropical fish extrapolated to similar low temperatures—can be ascribed to Wohlschlag.

In 1974, however, Høleton questioned whether metabolic cold adaptation in polar fish was a fact or an artefact. He measured the oxygen consumption of several Arctic fish species to test the validity of metabolic cold adaptation and evaluated

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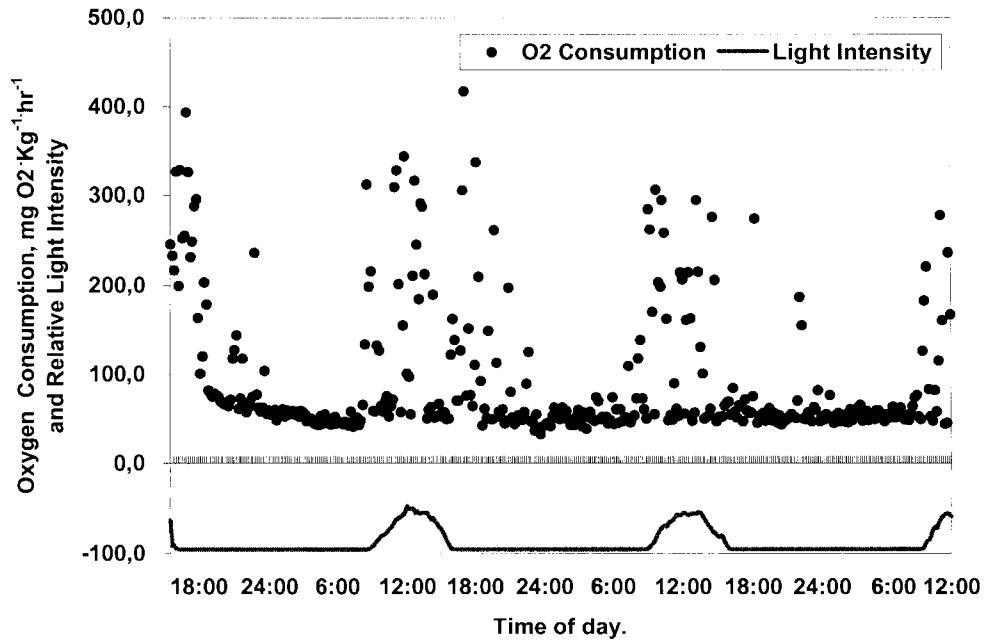


Fig. 1. Example of oxygen consumption of rainbow trout (*Oncorhynchus mykiss*) with a body weight of 392 g measured at 10 °C from December 23rd to 26th. The solid line indicates the relative light intensity. See text for further details (Steffensen, unpublished).

the experimental methods previously used. He concluded that of the 11 Arctic species measured only the arctic cod, *Boreogadus saida*, showed oxygen consumption higher than expected from temperate species and similar to previously published values for Arctic fish (Scholander et al., 1953). He also concluded that the increased oxygen consumption rates in the earlier studies maybe could be ascribed to handling stress and or spontaneous activity—and hence was not resting but routine oxygen consumption. Another problem was that fish with different body weights were compared in the earlier literature. Holeton started to correct oxygen consumption values to a standard size fish. Finally he pointed out that Krogh's otherwise widely accepted standard metabolism curve from 1916 probably is not valid at the lowest temperatures. Ege and Krogh (1914) themselves even referred to the high Q_{10} values at low temperatures as 'obviously wrong'. Krogh's standard metabolism curve was none the less used to extrapolate oxygen consumption rates of tropical and temperate fish to polar temperatures by Scholander et al. (1953), Wohlschlag (1964) and Wohlschlag (1963) to support the concept of metabolic cold adaptation.

2. After Holeton (1974)

With the common use of PCs in the 1980s, automated respirometers became available (Steffensen et al., 1984). The use of automated intermittent flow through respirometry allows repeated measurements of oxygen consumption during short time intervals (i.e. 10 min) for long periods (i.e. days) even without the researcher present in the laboratory. In addition it eliminates washout problems affiliated with the traditionally flow-through respirometry (Steffensen, 1989).

During the development and use of intermittent flow through respirometers I became aware of problems in determining standard metabolic rate due to: (1) increased metabolism after transfer to the respirometer due to handling; and (2) rather large fluctuations of metabolic rate at varying times of the day in many teleosts. Fig. 1 illustrates an example of an experiment in which oxygen consumption of a rainbow trout (*Oncorhynchus mykiss*) was measured during a Christmas vacation without any people in the laboratory. Immediately after the rainbow trout was transferred to the respirometer in the afternoon on December 23rd it had an oxygen consumption of almost 400 mg

$\text{O}_2 \text{ kg}^{-1} \text{ h}^{-1}$, but during the following 9 h it decreased to approximately $50 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$. From midnight between December 23rd and 24th until 08.30 h it had a relatively constant oxygen consumption of approximately $50 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$. This level can be considered the resting or standard metabolic rate. From 08.30 h to 22.30 h, however, oxygen consumption fluctuated with values ranging between 50 and $400 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$. If this experiment had been carried out with traditional flow-through respirometry the fish would have been transferred to the respirometer as in the present experiment, and the researcher would have started measuring oxygen consumption next day during normal working hours. The result would have been an average of all measurements from 08.30 h to 22.30 h and an erroneous overestimation of standard metabolic rate. The average oxygen consumption during this period was $124 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ($n=83$) compared with approximately $50 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ the night between December 23rd and 24th as well as between the 24th and 25th. Even if intermittent flow-through respirometry had been used, but with a lower time resolution, i.e. 1 or 2 h between flushing, it would have resulted in values similar to a moving average of 6 or 12 points, and the result would have been that the fish only during very few periods showed a low metabolic rate, and never during the day.

With the above observations in mind and Holton's publications from 1974 in which he suggested that the arctic cod (*Boreogadus saida*) was the only arctic fish that possibly showed metabolic cold adaptation, we decided to test this. University of Copenhagen's Arctic Station in Godhavn, Greenland is ideal for this type of investigation since three species of Gadoids are present in the area. It is the northernmost area of the otherwise temperate Atlantic cod (*Gadus morhua*), and the southernmost area of the Arctic polar cod (*Boreogadus saida*). In addition the low arctic Greenland cod (*Gadus ogac*) is common in the area. Comparing the standard metabolic rate of these three species, measured at the same temperature, as they were collected at, namely 4.5°C , and with automated intermittent respirometry, we could avoid some of the problems that probably flawed earlier experiments. All three species showed initial increased metabolic rate due to handling, but none showed a large variation as described for the rainbow trout. The results did not, however, show any significantly higher standard oxygen consump-

tion of the two Arctic species, Greenland cod ($72.8 \pm 4.6 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (mean \pm S.D.) and arctic cod ($84.2 \pm 14.5 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (mean \pm S.D.)), compared with the Atlantic cod ($66.3 \pm 4.7 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (mean \pm S.D.)), and hence there was no support for the traditional metabolic cold adaptation theory (Steffensen et al., 1994). All oxygen consumption values were weight corrected to a 100-g fish with a weight exponent of 0.8 as described in Steffensen et al. (1994).

Recently a population of the high Arctic stenothermal Gadoid, the East Siberian Icecod or arctic cod, *Arctogadus borisovi*, was discovered in Greenland in Uummanaq Fjord. Oxygen consumption at a temperature of 2°C was found to be $40.9 \pm 5.9 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (mean \pm S.D.) for eight fish with a body mass of $601.5 \pm 147.6 \text{ g}$, or $59.0 \pm 6.6 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ when adjusted to a standard mass of 100 g, a value not unusually higher than the three other Gadoids measured previously, and hence neither metabolically cold adapted (Drud Jordan et al., 2000).

The remaining candidates supporting the theory of metabolic cold adaptation are the Notothenioids from Antarctica. Earlier investigation of fish from McMurdo has shown that several species have higher oxygen consumption than usual when compared with temperate species (Wohlschlag, 1964; Forster et al., 1987; Wells, 1987). One distinct difference in habitat for the fish living in the Arctic compared to McMurdo is that the environment is much more stable and with temperatures rarely increasing above -1.2°C . At the Arctic Station in Greenland water temperatures can reach 5°C in the summer. Recently oxygen consumption of several species of Notothenioids from McMurdo Sound was measured. Fig. 2 is an example of the oxygen consumption of a *Trematomus bernacchii* with a body mass of 110 g, measured at a temperature of -1.1°C . This fish showed the usual initial increased oxygen consumption due to handling starting with a value of $85 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$, but it continued to fluctuate with only brief periods of standard oxygen consumption. This fish was considered to have a standard metabolic rate of $17.3 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ using the method described by Steffensen et al. (1994). The use of slow responding flow-through respirometry, or closed respirometry for long periods would have averaged the measurements. Oxygen consumption as an average for the initial 9 h was $44.6 \text{ mg O}_2 \text{ kg}^{-1}$

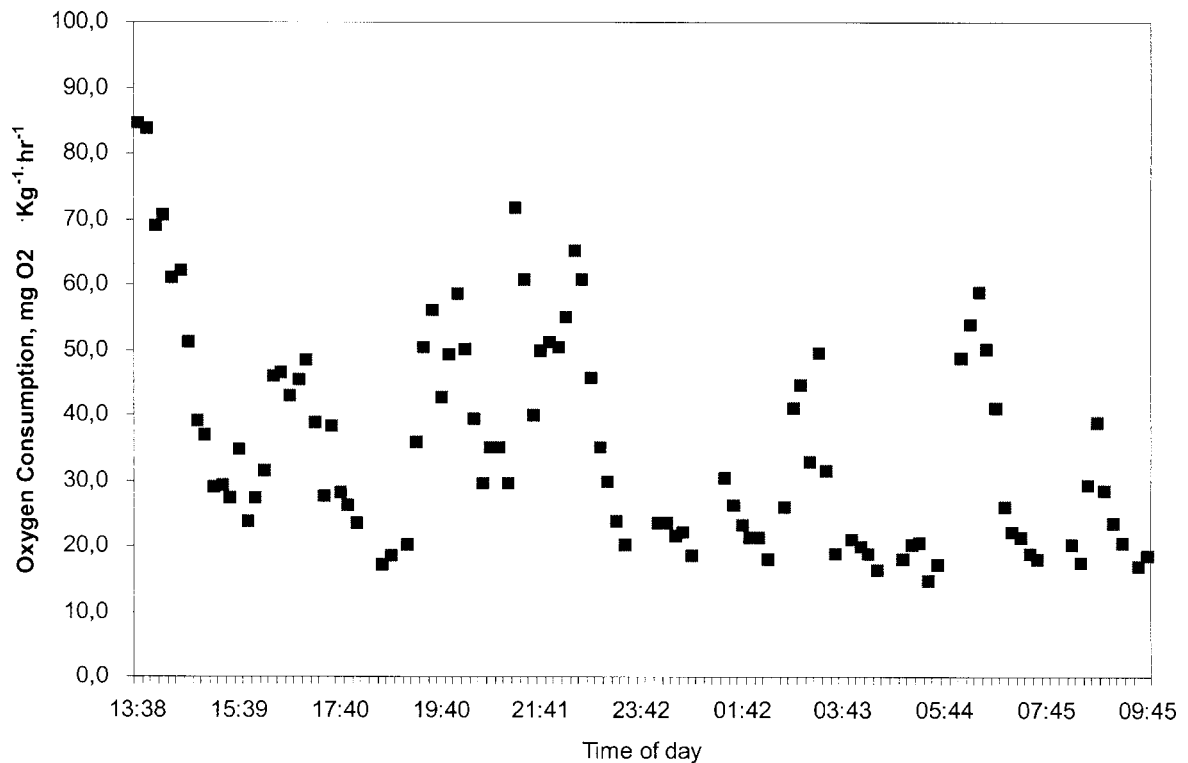


Fig. 2. Example of oxygen consumption of the Notothenioid *Trematomus bernacchi* at an experimental temperature of -1.0°C . Body weight = 110 g. Data from Steffensen and DeVries (in preparation). See text for further details.

h^{-1} , or more than twice as high as the standard metabolic rate.

The standard metabolic rate of 10 *Trematomus bernacchi* with an average body weight of 115.7 g was found to be 25.6 , or $27.4 \pm 6.9 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ weight corrected to a 100-g fish (Steffensen and DeVries, in preparation). Wohlschlag (1960) reported a value of $84.8 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (weight corrected to 100 g), or more than three times higher than Steffensen and DeVries (in preparation). Wells (1987) reported a value of $55.7 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ (weight corrected to 100 g). The reason for these previously rather high values can probably be ascribed entirely to the type of respirometry used and/or long periods of measurements. Likewise *Trematomus hansonii* was reported to have a standard metabolic rate of 65.9 and $70.2 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ by Wohlschlag (1964) and Wells (1987), respectively (weight corrected to 100 g), while Steffensen and DeVries (in preparation) reported a value of only $22.4 \pm 4.3 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ($n=10$). *Pagothenia borchgrevinki* was reported to have a standard oxygen consumption of 105.1 and 49.2 by Wohlschlag

(1964) and Wells (1987), but only $28.2 \pm 5.4 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$ ($n=10$) by Steffensen and DeVries (in preparation) (all weight corrected to 100 g). The conclusion of the study of the Antarctic Notothenioids was that they do not have significantly higher oxygen consumption than temperate species measured at similar low temperatures, and hence neither support the traditional metabolic cold adaptation theory based on aerobic oxygen consumption measurements (Steffensen and DeVries, in preparation).

The experiments referred to above rule out that any of the fish measured so far with intermittent flow-through or previous intermittent flow-through respirometry show any sign of metabolic cold adaptation with respect to whole organism aerobic metabolism based on oxygen consumption measurements. There is, however, neither any doubt that Polar fishes are physiologically adapted to the cold environment with respect to several other parameters such as antifreeze proteins (DeVries and Cheng, 1989), mitochondrial activity (Crockett and Sidell, 1990), enzyme activity (Somero, 1991) etc. These adaptations, however, do not

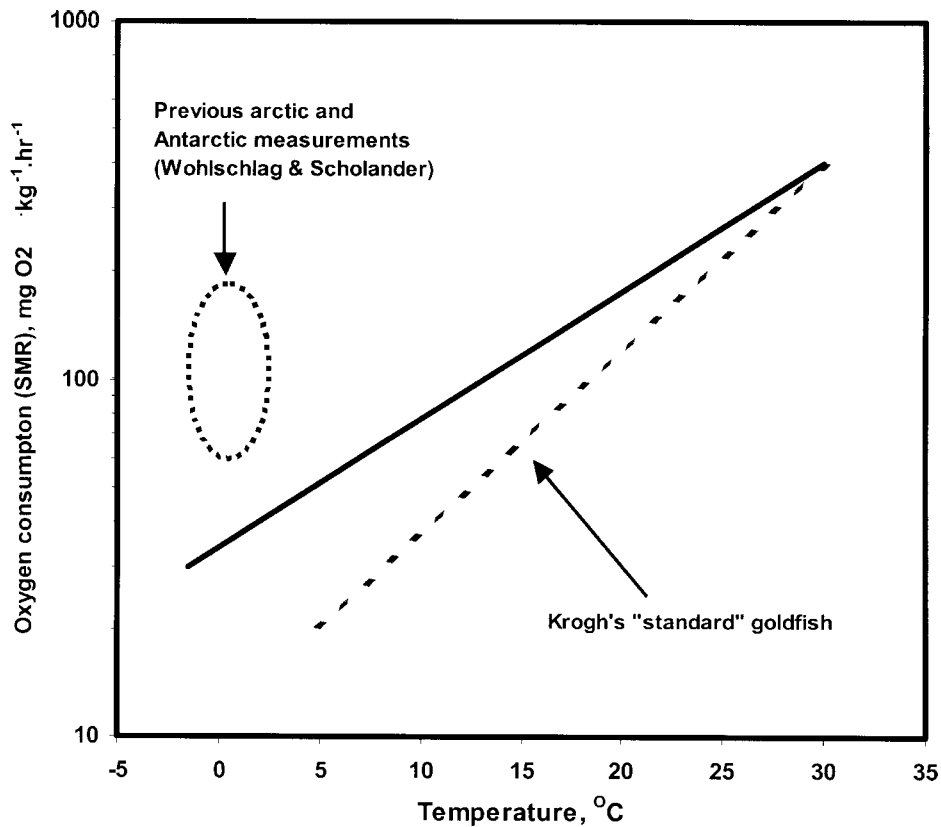


Fig. 3. Traditional interpretation of metabolic cold adaptation—that polar fishes have a higher than expected metabolic rate. Solid line represents tropical and temperate species at their normal temperatures and extrapolated to -2°C . Dotted oval represents previous Arctic and Antarctic measurements by Scholander (1953), Wohlschlag (1960, 1964), Wells (1987), Macdonald et al. (1987). Dotted line represents Krogh's standard goldfish (Krogh, 1916; Holeyton, 1974). See text for further details.

result in an increased standard metabolic rate in the fish referred to in this study.

Crockett and Sidell (1990) suggested that polar fish might have an expanded aerobic scope, based on their enzyme studies. Do polar fish show this? In a study by Bushnell et al. (1994) the metabolic scope of the temperate Atlantic cod ($N=6$) was compared with the arctic Greenland cod ($N=6$). Both species collected in Greenland. The results showed that there was no significant difference in neither extrapolated standard metabolic rate, active or maximum metabolic rate and hence not in scope for activity Bushnell et al. (1994).

As a consequence of the presented results the classical perception of metabolic cold adaptation of polar fish having three to five times higher oxygen consumption than temperate fishes extrapolated to similar low temperatures has to be abolished. Figures similar to Fig. 3 with values of

oxygen consumption of Arctic and Antarctic fish high above the extrapolated values of tropical and temperate fish appear in many review articles and textbooks, even recent ones, but should hereby be stopped. The Arctic and Antarctic fish do not have significantly higher standard metabolic rates, based on aerobic oxygen consumption measurements, from what would be expected from the extrapolation from temperate and tropical fishes.

3. Conclusion

I believe that Holeyton (1974) was correct when suggesting that metabolic cold adaptation was an artefact. No measurements of whole organism aerobic metabolism with computerised high resolution intermittent flow-through respirometry as used by Steffensen et al. (1994) of resting teleosts from the Arctic (four gadoids) or Antarctic (four

Notothernioids) have shown higher values than temperate species at similar temperatures.

Why have earlier experiments on some of the identical species hence reported higher oxygen consumption values? Probably due to a combination of several factors. The major problem is most likely the use of flow-through respirometers with the affiliated problem of not being able to distinguish between resting and routine metabolism. Another problem that has been neglected in most cases is that fish at low temperatures have an increased metabolic rate for up to 3 weeks after food intake, compared with a starved fish, due to the specific dynamic action. Finally the fish might not have been allowed an adequate length of time to settle in the respirometer after being handled, also with an increased rate of oxygen consumption as a result.

In addition the extensive use of the measurements of Ege and Krogh (1914) the metabolism of a single goldfish exposed to rather abrupt acute temperature changes nearly 100 years ago has fuelled and even polarised the long lasting controversy. In conclusion, however, I support Holeton's 27-year-old idea that metabolic cold adaptation of polar fish, based on aerobic oxygen consumption measurements, is an artefact. On the other hand I fully acknowledge that the some fish species are cold adapted with respect to enzyme kinetics, mitochondria, etc.—it just does not result in an increased aerobic oxygen consumption in the Arctic and Antarctic species we have studied so far. We must redefine metabolic cold adaptation.

4. Addenda

The East Siberian Icecod (*Arctogadus borisovi*) referred to in this study has very recently been shown to be genetically identical to the Arctic cod (*Arctogadus glacialis*), and hence should be only be referred to as the latter according to Møller, P.R., Jordan, A.D., Gravlund, P., Steffensen, J.F., 2002, Phylogenetic position of the cryopelagic codfish genus *Arctogadus* Drjagin, 1932 based on partial mitochondrial cytochrome b sequences, Polar Biology, in press.

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