Temperature dependency of early growth of turbot (Scophthalmus maximus L.) and its implications for developmental progress

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Abstract

Growth in developing turbot larvae can be expressed independently of temperature when using effective day-degrees ($D^{\text{eff}}$) as an index of development. The calculation of $D^{\text{eff}}$ is based on the principle of thermal summation whereby the rate of development is linearly related to the ambient temperature above a species-specific threshold temperature ($T_\text{th}$) at which development is theoretically arrested. The $T_\text{th}$ for turbot embryos is 5.3°C. The increase in wet body mass of developing turbot larvae at 14, 18, and 22°C can be aligned into a single exponential relationship regardless of incubation temperature when using $D^{\text{eff}}$ as the index of development. Previous attempts to relate physiological processes in poikilothermic animals, including fish, to incubation temperature are discussed. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

The duration of any developmental period in a poikilotherm, measured in chronological time units (e.g. days), is closely related to temperature; increasing with lower temperatures and decreasing with higher temperatures. Within a tolerable temperature range, an organism can carry out normal activities although the ambient temperature exerts a major influence on the pace of various biological processes (Fry, 1947). A
quantitative expression of the temperature influence is necessary in order to fully assess the effect of temperature on physiological and anatomical features in poikilothermic animals. Therefore, an index that describes developmental progress independently of temperature has been searched for during the past hundred years (see reviews by Winberg, 1956; Blaxter, 1969; Kamler, 1992).

The object of this paper was to test the hypothesis that effective day-degrees is an index that expresses developmental progress in a fish independently of temperature. Two experiments were carried out on turbot eggs and yolk-sac larvae: (1) the incubation period of eggs from fertilisation to hatching was recorded at 15°C. This experiment, together with literature data, allowed the calculation of a biological zero point at which development theoretically is arrested in developing turbot embryos. (2) Another experiment was set up wherein the wet body mass of fed turbot larvae was followed at three temperatures. Wet body mass is often used as a measure of developmental progress in fish (e.g. Blaxter, 1969; Kamler, 1992). The results allowed a comparison of effective day-degrees, with other temperature indices such as chronological time (days), day-degrees and physiological day-degrees.

2. Materials and methods

2.1. Rearing of eggs and larvae

2.1.1. Experiment 1

About 2000 fertilised turbot (Scophthalmus maximus L.) eggs were maintained in six 10 dm³ floating glass beakers containing filtered and UV-treated seawater at 33‰ salinity. About 80% of the water was changed once per day. Eggs were maintained at an incubation temperature of 15.0±0.1°C and to a 12:12 h light/dark photoperiod, and the experimental period lasted from fertilisation to hatching, about 5 days. The exact time at which 50% of the developing eggs hatched was determined by counting the share of hatched larvae under a binocular every second hour around the hatching time.

2.1.2. Experiment 2

Newly hatched turbot larvae were divided into three groups and reared in 60 dm³ tanks using seawater at 34‰, with temperatures maintained at 14.0±0.5, 18.0±0.5 and 22.0±0.5°C. The experiment lasted from first-feeding to 9–14 days post-hatching, depending on the experimental temperature. The larvae were fed two species of rotifers, Brachionus rotundiformis and B. plicatilis, and newly hatched nauplii of the brine shrimp, Artemia franciscana. In the feeding procedure, B. rotundiformis was offered from feeding commencement and gradually exchanged with B. plicatilis, which again was gradually exchanged with Artemia nauplii towards the end of the experiment (Planas and Cunha, 1995). Rotifers were enriched for 3–6 h with Isochrysis galbana and Tetraselmis suecica before being offered to the larvae. Additional prey organisms was added twice a day to maintain concentrations of 5 prey cm⁻³ (Brachionus) and 0.25 prey cm⁻³ (Artemia) (Weltzien et al., 1999). This allowed the larvae to feed ad libitum (Planas and Cunha, 1995).
2.2. Sampling of larvae

As a measure of developmental progress, turbot larval wet body mass (wm) was determined daily (four replicates of 12–25 pooled individuals). Larvae were collected in a plankton mesh and rinsed 5–10 s in distilled water, before being transferred onto aluminium trays and weighed immediately on a Sartorius R 180 D top balance to the nearest 0.01 mg.

2.3. Calculations

To reach a temperature-independent index of development, three different time indices were compared. Day-degrees \( (D^o) \) is often used as a measure of developmental progress in the early life stages of poikilotherms:

\[
D^o = \Delta t \cdot T
\]

where \( \Delta t \) is developmental time in days and \( T \) is the temperature in °C.

Physiological day-degrees \( (PD^o); \) Huisman, 1974) is another approach to reach a temperature-independent index of developmental progress:

\[
PD^o = \frac{(\Delta t \cdot T)}{q}
\]

where \( \Delta t \) and \( T \) are as in Eq. (1), and \( q \) is Winberg’s correction factor for temperature, using 20°C as a reference (Winberg, 1956):

\[
q = Q^{(20-T)/10}
\]

where \( T \) still is the temperature in °C. The factor \( q \) is derived from van’t Hoff’s general \( Q_10 \) approximation (van’t Hoff, 1884, cited in Belehradek, 1930):

\[
Q_10 = \left( \frac{V_2}{V_1} \right)^{(10)/(T_2-T_1)}
\]

where \( V_1 \) and \( V_2 \) are the rates of the studied process at temperature \( T_1 \) and \( T_2 \) (in °C), respectively.

A third approach to reach a temperature-independent index of developmental progress is the effective day-degrees \( (D^o_{eff}); \) Kamler, 1992), which is based on a species-specific threshold temperature \( (T_o) \) at which development is theoretically arrested. From \( T_o \), a biologically effective temperature \( (T_{eff} = T - T_o) \) can be calculated. By exchanging \( T \) with \( T_{eff} \) in Eq. (1), the number of day-degrees over the threshold temperature (i.e. \( D^o_{eff} \)) can be calculated:

\[
D^o_{eff} = \Delta t \cdot (T - T_o)
\]

2.4. Statistics

Regressions were calculated using the method of geometric mean (functional, model
II) regression analysis. To linearise data, the temperature index values were log$_{10}$ transformed, and the growth data transformed according to:

$$1/(8.65 - 7.29x + 2.13x^2)$$

where $x$ is wet body mass raw data. The resulting growth curves were compared in SYSTAT 5.02 for Windows using randomised design analysis of covariance (ANCOVA). Individual temperature groups were statistically analysed with Tukey’s honestly significant difference method (Sokal and Rohlf, 1995). The first sampling point at all temperatures in Fig. 3 was omitted from the statistical analysis since larval feeding at this point had not yet started.

3. Results

The turbot eggs in Experiment 1 hatched at 4.8 days post-fertilisation. Together with recalculated data from the literature, this result was used to quantitate the relation between temperature and developmental rate ($V=1$/incubation time) in turbot eggs and larvae (Fig. 1). The data fit a linear regression ($r^2=0.994$, $n=15$) and give a $T_0=5.3\pm0.5^\circ$C (95% confidence interval) for developing turbot embryos. This regression was used to calculate the $Q_{10}$ for rate of development to hatch (Eq. (4)) as a function of temperature (Fig. 2). The $Q_{10}$ for developing turbot embryos increase steeply.
below 15°C. In the middle (14–18°C) and upper (18–22°C) range of their viable temperatures the average $Q_{10}$ is 2.6 and 2.0, respectively.

The $wm$ of turbot larvae reared at 14, 18 and 22°C is presented as a function of chronological time, $D^\circ$, $PD^\circ$, and $D^\circ_{eff}$ for comparison of the different indices (Fig. 3). Prey organisms were observed in the larval gut in all temperature groups at all times throughout the experiment. When expressing developmental progress in $D^\circ_{eff}$ (Fig. 3D), the three growth curves are not significantly different ($p > 0.280$). However, the three growth curves are significantly different when expressing developmental progress in chronological time ($p < 0.001$), in $D^\circ$ units ($p < 0.045$), or in $PD^\circ$ units ($p < 0.001$).

4. Discussion

Developmental rate ($V$, Fig. 1) is defined as the reciprocal of the time necessary to reach a certain developmental stage (Johansen and Krogh, 1914). In the present work, the reciprocal of the incubation time (time, in days, from fertilisation to 50% hatching) of turbot eggs have been used as a measure of developmental rate (Fig. 1). Ferguson et al. (1985) found a good correlation between incubation time, ontogeny of enzyme expression and morphological development in rainbow trout ($Oncorhynchus mykiss$) at different temperatures. To characterise the effect of temperature on physiological and anatomical features in an embryonic or larval poikilotherm, a way of expressing development independently of temperature is essential. Various attempts have been made
Fig. 3. Wet body mass of developing turbot (*Scophthalmus maximus* L.) larvae reared and fed ad libitum at 14, 18 and 22°C, and related to four different time indices: (A) chronological time, (B) day-degrees, (C) physiological day-degrees, and (D) effective day-degrees. The curves (*n* = 16–20) are exponential regression lines.

over the years to define a temperature independent index of developmental progress. These attempts include *Q*_10, *D*_10, *PD*_10 and *D*_eff.

4.1. *Q*_10 approximation

One of the most widely adopted methods for comparing the effect of temperature on the velocity of a biological process is van’t Hoff’s *Q*_10 approximation (Eq. (4)). When *Q*_10 is solved from the Arrhenius equation, which is thermodynamically based on the frequency of molecular collisions, *Q*_10 shows to be a function of temperature and decreases with increasing temperature (Schmidt-Nielsen, 1995):

\[
Q_{10} = \frac{K_{T+10}}{K_T} = e^{\frac{\mu}{R(T+10)}}
\]

where *K* is the rate constant, *T* the thermodynamic temperature in degrees K, *μ* the activation energy for the reaction in question, *R* the universal gas constant, and *exp* is
the base of the natural logarithm. Despite a lack of theoretical foundation in thermodynamics, many specific biological rate functions tend to have fairly characteristic and uniform values of $Q_{10}$ within narrow ranges of temperature. Whether $Q_{10}$ values are uniform over broader ranges of temperature and for features such as respiration, growth and feeding in whole organisms has, however, been subject to much debate (see reviews by Duncan and Klekowski, 1975 and Kamler, 1992). The present work shows that the $Q_{10}$ values for growth in developing turbot embryos between 10–23°C decreases from about 7 to about 2 with increasing incubation temperature (Fig. 2). This is in agreement with the Arrhenius equation (Eq. (6)) and the findings of Rombough (1988) and Kamler (1992).

4.2. Day-degrees

Day-degrees ($D^\circ$, Eq. (1)) was originally believed to be a temperature-independent index and has been used as a measure of developmental progress in the early life stages of fish (Grodzinski et al., 1975). It states that the time necessary to reach a certain stage of development multiplied by the incubation temperature is constant. Thus, there should be a linear relationship between time and incubation temperature rather than an exponential relationship as suggested by the van’t Hoff’s $Q_{10}$ approximation. Furthermore, the relation implies that 0°C is the true zero at which development is theoretically arrested. However, several studies on fish (Kokurewicz, 1970; Tashiro et al., 1974; Leitritz and Lewis, 1976; Penaz et al., 1983) have demonstrated a decrease in $D^\circ$ between two developmental stages with increasing temperature, indicating that $D^\circ$ is indeed temperature-dependent, and that the temperature dependency decreases with increasing temperature. The latter suggests that $D^\circ$ is an acceptable approximation when comparing developmental progress over small intervals at the higher end of the tolerable temperature range of a species.

4.3. Physiological day-degrees

Ege and Krogh (1914) produced an empirical relationship between respiratory rate and ambient temperature for various animals, a relationship that has since been called ‘Krogh’s normal curve’. Winberg (1956) approximated values of ‘Krogh’s normal curve’ with the help of a factor $q$ (Eq. (3)), in another approach to reach a temperature-independent index of developmental progress: the physiological day-degrees ($PD^\circ$, eq. 2). Although used in several works (e.g. Huisman, 1974; Hogendoorn and Vismans, 1980; Segner et al., 1994; Verreth, 1994; Terjesen, 1995), $PD^\circ$ is based on two controversial assumptions: that the acceleration of developmental rate with temperature (a) equals that of metabolic rate, and (b) conforms with that expected from ‘Krogh’s normal curve’. Kamler et al. (1994) found that these assumptions are not fulfilled in developing African catfish (Clarias gariepinus). Similarly, the results of the present work show that $PD^\circ$ cannot align body mass into a single relationship in developing turbot at different temperatures.
4.4. Effective day-degrees

The most promising approach to reach a temperature-independent index for developmental progress in poikilotherms was reinvented by Blaxter (1969) and Kamler (1992), based on earlier approaches by Reibisch (1902) (cited in Johansen and Krogh, 1914), Krogh (1913, 1914a,b) and Johansen and Krogh (1914). In the years 1910–1915, Krogh and co-workers made several investigations concerning the effect of temperature on development of physiological and anatomical features in various animals. They found that respiratory rates in a broad range of animals were related to different temperatures as described by the nearly exponential relationship of Krogh’s normal curve (Ege and Krogh, 1914). However, they (Krogh, 1914a; Johansen and Krogh, 1914) also concluded that developmental rate \( V \) in poikilotherms increased linearly with temperature:

\[
V = \frac{1}{\Delta t} = a + bT
\]  

where \( T \) is the temperature in °C and \( \Delta t \) the developmental time in days. The empirical constants \( a \) and \( b \) are assumed to be species-specific (for turbot, see Fig. 1). The linear correlation in Eq. (7) has been confirmed in several studies (Hayes, 1949; Blaxter, 1969; Gnaiger, 1980; Kamler, 1992; Kamler et al., 1994). The linearity seems to break at the extremes of the tolerable temperature range, and the curve may be sigmoidal (Penaz et al., 1983). Its lower left concave and upper right convex sections are correlated with asynchronous development, high percentage of deformities and low viability. The linear correlation between \( V \) and \( T \) implies that the law of thermal summation can be applied, i.e. the increase in \( V \) for a certain temperature interval within the tolerable range is constant regardless of the test temperature. Thus, the relationship between \( \Delta t \) and \( T \) is mathematically in the form described in Eq. (1). Kamler (1992) solved Eq. (7) for \( T \) and found a threshold temperature \( (T_0) \) at which development is theoretically arrested \( (V=0) \) when:

\[
T = T_0 = -\frac{a}{b}
\]  

The threshold temperature seems to be species-specific and can be determined by extrapolating the linear relationship between \( V \) and \( T \) to intersect the abscissa. In developing turbot embryos, \( T_0 \) is 5.3°C (Fig. 1). This \( T_0 \) agrees with the results of Knudsen (1983) who found that turbot juvenile development was arrested at experimental temperatures below 7°C, and also with Kuhlmann and Quantz (1980) who experienced a 100% mortality in turbot eggs incubated at 6°C. From \( T_0 \), the number of day-degrees over the threshold temperature, the effective day-degrees \( (D_{\text{eff}}^\circ) \) (Kamler, 1992) can be calculated (Eq. (5)). When applied to the growth data of turbot embryos, \( D_{\text{eff}}^\circ \) are superior to other temperature indices in expressing development independently of temperature (Fig. 3). Similarly, Cunha and Planas (1997) found that lipid catabolism in turbot larvae could be described independently of temperature when plotted against \( D_{\text{eff}}^\circ \).

From Eqs. (5), (7) and (8), the number of \( D_{\text{eff}}^\circ \) from fertilisation to hatching can be derived as the inverse of the constant \( b \) \( (D_{\text{eff, hatch}}^\circ = 1/b) \). Thus, \( T_0 \) and \( D_{\text{eff}}^\circ \) can be
used to predict 50% hatching time at any temperature. In turbot, this can be estimated to occur at 44 $D^s_{\text{eff}}$ post-fertilisation.

In conclusion, $D^s_{\text{eff}}$ is shown to be a temperature-independent index of development in turbot larvae. We recommend the use of $D^s_{\text{eff}}$ as the best index for describing developmental progress during the early life stages of fish reared under different temperature conditions.

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