Temperature and development in larvae of the turbot
Scophthalmus maximus

Abstract Turbot (Scophthalmus maximus L.) were reared at 12 and 16 °C until 26 d after hatching. At both temperatures, starting at the neural plate stage, somites were initially formed every 75 min. Expressed as a percentage of development time (DT, fertilisation to 90% larvae hatching) somite formation occurred relatively earlier during embryogenesis at 12 °C (45% DT) than at 16 °C (55% DT). At 12 °C, after the 32-somite stage the rate of somite formation decreased to one every 300 min. The larvae hatched after 6 d at 12 °C and 3 d at 16 °C at a relatively primitive stage of development, prior to the opening of the mouth and anus, with unpigmented eyes, and a straight gut. Temperature altered the relative timing of organogenesis in the larval stages. At 12 °C, the following characters appeared (in this order): swimbladder > loop in the gut (at the time of yolk exhaustion) > caudal fin. In contrast, at 16 °C, the caudal fin appeared at the same time as the loop in the gut. At 16 °C, spines formed on the head in the region of the otic capsule at the time the swimbladder formed and the yolk was exhausted, but were absent in 12 °C larvae. At both temperatures, in 1 d-old larvae the myotomes just behind the yolk-sac contained ~200 inner muscle fibres (presumptive white muscle). The initial growth of inner muscle was largely due to hypertrophy, but by 26 d at 12 °C and 11 d at 16 °C hyperplastic growth became important, as evidenced by a significant increase in the number of small fibres (<10 µm²). By 26 d the average number of inner muscle fibres had increased to 341 at 12 °C and 988 at 16 °C. New muscle fibres were added in distinct germinial zones at the dorsal and ventral apices of the myotomes. Metamorphosis was associated with a thickening of the superficial (presumptive red) muscle layer and the appearance of tonic muscle fibres.

Introduction

Turbot (Scophthalmus maximus L.) produce small (diam = 0.9 to 1.5 mm) pelagic eggs around the coast of Scotland from June to September (Russell 1976). Larvae remain pelagic until the onset of metamorphosis, at which time the body becomes laterally flattened and eye migration occurs, and they become demersal, settling onto shallow sandy beaches (Day 1884). Turbot are a relatively eurythermal species, with a geographic range from Norway (75°N) to the southern Mediterranean (30°N) (Wheeler 1978). Throughout this latitudinal range, sea temperature increases between spawning and the early juvenile phase. Eggs and larvae produced early in the season may therefore experience a different temperature regime from those spawned later.

The durations of the pelagic egg and larval phases vary with temperature (Jones 1972; Lasker 1981) and are important factors influencing the dispersal of flatfish (Hovencamp 1991; Riley et al. 1981). In addition to effects on the rate of development, temperature has been reported to alter the relative timing of the appearance of morphological characters. Fukuhara (1990) found, in the Japanese flounder Paralichthys olivaceus, that the relative timing of appearance of events such as eye pigmentation, mouth opening and pectoral fin formation varied between 15 and 21 °C. Temperature has also been found to alter meristic characteristics such as vertebral and fin ray number in a large number of species, including flatfish (Taning 1952; Fonds et al. 1974; Fahy 1981; Lindsey 1988). Somite formation and myogenesis proceed in a rostral to caudal sequence in fish embryos (Hannerman 1992). Johnston and co-workers found that in spring-spawning Atlantic herring (Clupea harengus), the initial synthesis of contractile proteins and the differentiation of distinct muscle-fibre

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types occurred relatively earlier with respect to somite stage at 12°C than at 5°C (Johnston et al. 1995). Temperature has also been shown to alter the number and diameter of muscle fibres at hatching in salmon (Stickland et al. 1988), herring (Johnston 1993), and plaice (Pleuronectes platessa) (Brooks and Johnston 1993). In herring, the volume density of mitochondria also varies with rearing temperature (Vieira and Johnston 1992). Changes in the relative timing of development occur at the molecular level, as well as at the morphological level. For example, Crockford and Johnston (1993) found that the muscle of 1 d-old Atlantic herring reared at 5°C contained a mixture of embryonic and larval toponin T isoforms, whereas only larval isoforms were expressed at 10°C.

Al-Maghazachi and Gibson (1984) devised a comprehensive morphological staging procedure for larval turbot based on that already described for plaice (Pleuronectes platessa) by Shelbourne (1957) and modified by Ryland (1963, 1966). However, staging systems based on morphological characters related to body length and/or age may not be applicable across the whole range of temperatures at which a species can reproduce.

The aim of the present study was to investigate the influence of temperature on development in turbot, from fertilisation to the beginning of metamorphosis, with particular reference to the growth of the myotomal muscles.

Materials and methods

Fish

Brood-stock turbot (Scophthalmus maximus L.) were obtained from a commercial fish farm (Golden Sea Produce Ltd., Hunterston, Argyll, Scotland) and brought into season by varying the photoperiod. Eggs from a single female were stripped and fertilized with milt from a single male. The fertilized eggs were split into two periods. Eggs from a single female were stripped and fertilized with Laverack’s solution (4% gluteraldehyde, 1 mM CaCl₂, 20 mM NaCl, 2.5% paraformaldehyde, 120 mM sodium cacodylate, 1% sucrose, pH 7.0). Samples of approximately 10 embryos/developmental stage from each temperature were fixed in either neutral buffered formalin (5% formalin, 0.03 M sodium dihydrogen phosphate, 0.05 M disodium hydrogen phosphate) or Laverack’s solution (4% gluteraldehyde, 1 mM CaCl₂, 20 mM NaCl, 2.5% paraformaldehyde, 120 mM sodium cacodylate, 1% sucrose, pH 7.0). Samples were taken at 09.00, 13.00 and 18.00 hrs until hatching. Embryonic development time (DT) was taken as the point at which 90% of live embryos had emerged from the eggs. Larvae were fed a diet of Artemia sp. nauplii enriched with a fatty acid diet supplement (Golden Sea Produce Ltd.). The early pelagic larval stages were sampled daily until 7 d after hatching. After 7 d sampling, frequency was reduced to every fourth day until 30 d post-hatching. Larvae were fixed in Bouin’s fluid for 24 h and then transferred to 75% ethanol.

Growth, morphology and yolk utilisation

Materials and methods

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