Aquaculture 281 (2008) 158–161

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Short communication

Pulsed expression of growth hormone mRNA in the pituitary of juvenile Pacific bluefin tuna under aquacultured conditions

Kohsuke Adachi [a,1], Keitaro Kato [a,*], Masayoshi Yamamoto [a], Katsuya Ishimaru [a], Toru Kobayashi [b], Osamu Murata [a], Hidemi Kumai [a]

[a] Fisheries Laboratory, Kinki University, Shirahama 3153, Wakayama, Japan
[b] Laboratory of Aquatic Biology, Department of Fisheries, Faculty of Agriculture, Kinki University, Nara 631-8505, Japan

ARTICLE INFO

Article history:
Received 11 April 2007
Received in revised form 27 May 2008
Accepted 5 June 2008

Keywords:
Pacific bluefin tuna
Growth hormone
Daily rhythm
Somatic growth
mRNA expression
Pituitary gland

ABSTRACT

The daily expression profile of growth hormone (GH) mRNA in juvenile Pacific bluefin tuna (*Thunnus orientalis*) was investigated under aquacultured conditions. Total RNA from pituitaries ($n=5$) was sampled from 10 am to 12 pm the next day at 1 h intervals. The expression levels of GH mRNA were evaluated using real-time PCR normalized against the β-actin gene. The expression level of GH transcripts reached a peak at 3–4 am at which time it was about 10 times higher than at any other time period. Considering their high growth rate compared to red seabream (*Pagrus major*) and other aquacultured species which shows continuous GH mRNA expression patterns, it can be concluded that the pulsed expression of GH mRNA just before daybreak is a key for the extremely high somatic growth rate of Pacific bluefin tuna.

1. Introduction

Pacific bluefin tuna (*Thunnus orientalis*) is one of the largest teleosts which can grow to more than 3 m in body length and 300 kg in body weight. The speed of somatic growth, especially of juveniles, is extremely high and they reach 215.5±14.5 mm in total length and 149.0±30.2 g in body weight within 60 days after hatching, which is almost a 5% growth rate per day in total length (Fig. 1). It is generally accepted that the key regulator for somatic growth is growth hormone (GH) which is expressed in somatotroph cells of the pituitary and is secreted into the blood to stimulate the growth of bone and muscle by promoting cell division, differentiation and enlargement (Copeland and Nair, 1994; Corin et al., 1990).

It has been recognized for almost 40 years that GH levels in mammals fluctuate during the day. Human GH levels in plasma show a major pulse shortly after sleep onset which accounts for almost half of the daily GH production (van Cauter et al., 1998) although quantitative analysis for daily GH mRNA expression is limited. In mammals it has already been reported that this pulsatile GH expression pattern is more effective than continuous expression of GH in stimulating somatic growth (Isgaard et al., 1988). There is, in contrast, limited knowledge about daily GH expression profiles and the effects of pulsed expression of GH on somatic growth in fish, especially for fish with extremely high growth rates such as Pacific bluefin tuna, although there have been some reports about the daily GH mRNA expression patterns of aquacultured fish (Ayson and Takemura, 2006; Mori et al., 2001).

In the Fisheries Laboratory of Kinki University, Pacific bluefin tuna have been successfully hatched and reared from artificially hatched parent fish (Sawada et al., 2005). This provides an opportunity to examine the expression pattern of GH mRNA by periodical sampling of juvenile fish over the course of a day. In this study, we measured the daily GH mRNA expression profile of juvenile Pacific bluefin tuna and compared that to red seabream (*Pagrus major*), which has a much slower growth rate, in order to examine the relationship between growth performance and pulsatile mRNA expression of GH in fish.

2. Materials and methods

2.1. Sample animals

Juvenile Pacific bluefin tuna (42 days after hatching; 15.2±4.2 g body weight and 144.3±8.9 mm total length) were bred in water tanks (20,000 L) under a natural photoperiod at the Ohshima Station of Fisheries Laboratory at Kinki University. The water temperature was maintained at 26.8–29.0 °C, the temperature commonly used for sea water in the hatchery. Fish were fed to satiation at 1 h intervals during

* Corresponding author. Tel.: +81 739 42 2625; fax: +81 739 42 2634.
E-mail address: kei-kato@kindaisuiken.jp (K. Kato).
[1] Present address: Central Research Laboratory, Nippon Suisan Kaisha, Ltd., Kitanomachi, Hachioji, Tokyo 192-0906, Japan.

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.06.021

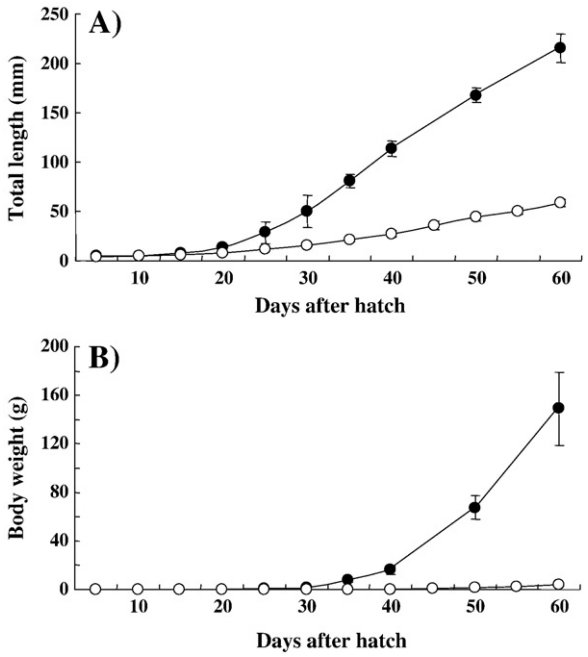

Fig. 1. Growth curves for juvenile Pacific bluefin tuna and red seabream under aquacultured conditions in the Fisheries Lab of Kinki University. A) Total length (mm). B) Body weight (g). Closed circles represent Pacific bluefin tuna (*Thunnus thynnus orientalis*) and open circles represent red seabream (*Pagrus major*). Details for rearing conditions are summarized in Sawada et al. (2005).

the day (from 5 am to 6 pm) with Japanese sand lance (*Ammodytes personatus*). Food was withheld for a 24 h period prior to 10 am on the first day of the study. To minimize stress during sampling, fish were captured using a lure with six hooks, and then were immediately sacrificed in ice-cold water in order to isolate total RNA from their pituitaries as detailed below. For nighttime sampling, a dim electric bulb (20 W) was used to illuminate the hatchery and the effects of that light on the sea water tank were also negligible (<0.01 μmol photon m^{-2} s^{-1} in the tank). Twilight ended at 6:06 pm and began at 5:43 am on the sampling day. The details for rearing conditions of Pacific bluefin tuna are summarized in Sawada et al. (2005). Additionally red seabream (94 days after hatching; 21.6±4.3 g body weight) were bred in water tanks (500 L) under natural photoperiod conditions, and were fed to satiation three times a day with a formulated diet. Food was withheld for 24 h prior to 10 am on the first day. Red seabream were caught by net and then were sacrificed immediately on ice. Pituitaries were dissected, then RNAs were extracted, reverse transcribed and analyzed by real-time PCR in both types of fish as detailed below. Cortisol levels in all fish were below detection limits indicating that the animals used in this study were under little or no stress.

2.2. Preparation of reverse transcripts from pituitary glands

Pituitary glands were continuously sampled for 27 h at 1 h intervals (n=5 at each time) after which total RNA was isolated by the AGPC method (Chomczynski and Sacchi, 1987). After dissolving total RNA in 50 μl water, it was treated with 10 units of DNase I (TAKARA Bio INC, Ohtsu, Japan) for 30 min at 37 °C. In order to check for contamination by genomic DNA, PCR (10 μl) was carried out using 1 μl DNA-digested solution with 0.5 unit Ex Taq (TAKARA Bio INC), 1× Ex Taq Buffer, 200 μM each dNTP and 0.5 μM tuna β-actin-F-R primers. The reaction was carried out at 94 °C for 2 min, 25 cycles at 94 °C for 15 s, at 55 °C for 15 s, and at 72 °C for 20 s, followed by a final extension for 5 min at 72 °C. If a signal was found in the mixture upon electrophoresis, the sample was digested by DNase I again and was rechecked by PCR. This process was repeated until no amplification could be observed. Reverse transcription (20 μl) was performed with random hexameric priming using 2 μl total RNA solution, 50 units MMLV RTase (TAKARA Bio INC), 500 μM dNTP, 50 pmol random hexamers and 10 units RNase Inhibitor (TAKARA Bio INC). Reactions were carried out at 42 °C for 15 min.

2.3. Confirmation of specific amplification

PCR was performed using 1 μl DNA-digested solution with 0.5 unit Ex Taq (TAKARA Bio INC), 1× Ex Taq Buffer, 200 nM each dNTP and 0.5 μM of tuna GH-F-R or tuna β-actin-F-R. Reactions were carried out at 94 °C for 2 min, then 25 cycles at 94 °C for 15 s, at 55 °C for 15 s, and at 72 °C for 20 s, followed by a final extension for 5 min at 72 °C. The products were separated on 2% agarose gels and were subcloned into the TOPO vector to validate their DNA sequences.

2.4. Real-time PCR

Before real-time PCR, we checked for contamination of genomic DNA in the reverse transcripts to exclude the possibility of non-specific amplification. The primers designed for GH and for the internal control (β-actin) do not cross-react with any other genes and the DNA sequences were validated (data not shown). In each standard curve, the multiple correlation coefficient showed a reliable value (data not shown). The daily expression profile of GH mRNA was examined by real-time PCR normalized against the copy number of β-actin (Bustin, 2000) using specific primers for the GH and β-actin genes. Primers for the Pacific bluefin tuna are as follows. tuna GH-F; 5′-AGCAGCGTCAGCTCAACAAA-3′, tuna GH-R; 5′-AACTCCCACGATTCCACCAA-3′, tuna β-actin-F; 5′-ACCCACACAGTGCCCATCTA-3′, and tuna β-actin-R; 5′-TCACGCACGATTTCCCTCT-3′. Primers for the red seabream are as follows. rsb GH-F; 5′-ATCATCAGCCCCATCGACA-3′, rsb GH-R; 5′-CAGAACCGCCAGACAGAGAA-3′, rsb b-actin-F; 5′-CCAAAGCCAACAGGGAGAAG-3′, and rsb b-actin-R; 5′-AGAGGCATACAGGGACAGCA-3′. Real-time PCR was performed using SYBR Green Premix Ex Taq (TAKARA Bio INC) in a Smart

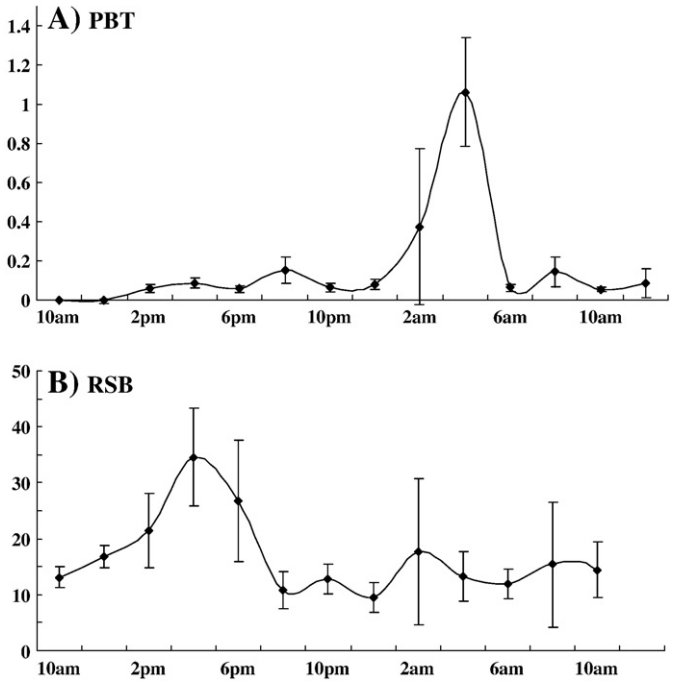

Fig. 2. Daily GH mRNA expression patterns in the pituitary. A) Pacific bluefin tuna. B) Red seabream. The peaks at 1–2 and 3–4 am in Pacific bluefin tuna are significantly higher than those at other time periods by one-way ANOVA and post-hoc Bonferroni's multiple comparison tests (p<0.05). Twilight ended at 6:06 pm and began at 5:43 am on the sampling day. Data are presented as means±SE.