www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 12: 947-950(2012) DOI: 10.4194/1303-2712-v12_4_22

SHORT PAPER

A Preliminary Study on Protease Activity of the Northern Pike (*Esox lucius* L. 1758) Larvae

Kaya Gökçek[1,*], Mehmet Naz[1], Tamás Szabó[2], Béla Urbányi[2]

[1] Mustafa Kemal University, Faculty of Fisheries and Aquaculture, Iskenderun, Hatay, Turkey.
[2] Szent Istvan University, Department of Fish Culture, Gödöllö, Hungary.

* Corresponding Author: Tel.: :+90.534 4150716; Fax: -;
E-mail: kayagokcek@yahoo.com

Received 30 July 2012
Accepted 21 October 2012

Abstract

The proteolitic activity of northern pike prelarvae was measured in yolk sac stage to reveal the critical first feeding time at relatively high incubation temperature. The differences observed among protease activities of larvae from day after hatching (DAH) 0 to 12 were statistically significant (P<0.05). Protease activities of larvae remained slightly increased from DAH 0 to DAH 5. A sudden raise in protease activities of larvae were observed from the DAH 6 to the DAH 8. Moreover, the conspicuous increase in protease activities of pike larvae was determined at the DAH 8 (595.22±9.07 U/mg protein). All starving larvae died four days after yolk sac consumption. In conclusion, to prevent tissue breakdown, larvae should be fed immediately just after yolk sac absorption at the DAH 8 at 13°C under controlled conditions.

Keywords: Pike, ontogeny of larvae, protease activity, yolk sac absorption, first feeding time.

Kuzeey Turna Bakığı (Esox lucius L. 1758) Larvasının Proteolitik Aktivitesi Üzerine Bir Öncalışma

Özet

Nispeten yüksek inkübasyon sıcaklığında kuluçkalanan Turna balığı pre-larvalarının proteolitik aktivitesi, besin keseli dönemden kritik ilk yem alma noktasına kadar ölçülmüştür. Yumurta açılım günü (DAH) olan 0. gün ile 12. günler arasında larvaların proteolitik aktiviteleri arasında istatistiksel olarak fark bulunmuştur (P<0,05). Turna larvalarının proteolitik aktivitesi DAH 0'da DAH 5'e kadar yavaşça yükselmiş, daha sonra DAH 6'dan DAH 8'e kadar ani bir yükseliş görülmüştür. Buna ek olarak, DAH 8'de bariz bir pik gözlenmiştir (595,22±9,07 U/mg protein). Lavaların tümü, besin kesesinin tükenmesini takiben 4. günde açlıktan ölmüştür. Sonuç olarak, doku yıkımını önlemek amacıyla, larvalar 13°C'deki kuluçkalama şartlarında, DAH 8'de besin kesesinin tükenmesini takiben hemen ilk yem verilmelidir.

Anahtar Kelimeler: Turna balığı, larval gelişim, proteaz aktivitesi, besin kesesi tüketimi, ilk besleme zamanı.

Introduction

Northern pike is the best known carnivorous freshwater fish species in Northern Hemisphere. Besides being a good bio-melorator, it is highly valuable species of commercial freshwater polyculture and angling. Although it's artificial propagation technics are well known (Szabo, 2003; Gokcek *et al*., 2012), larval nutrient requirements are still obscure. On the other hand, its intensive culture is still hindered by the high rate of cannibalism which starts at early larval stage (Kucska *et al*., 2006).

The digestion of a food for absorption in the digestive tract of the animal depends largely on the available enzymes (Noori *et al*., 2012; Cho, 1987). Studies on digestive enzyme activities can explain nutritional physiology and help resolve nutritional problems, such as digestibility of an artificial diet. One of the most important problems is to establish of the nutritional requirement of a candidate species for commercial production. Hofer and Köck (1989) suggested that, from the profile of the digestive enzymes, it is possible to predict the ability of a species to use different nutrients (Noori *et al*., 2012).

After yolk sac absorption of pike larvae in commercial hatcheries, fish are stocked to the earthen fertilized ponds for nursery in Hungary (Szabo, 2003). As known, after absorption endogenous energy sources, larvae need adequate exogenous energy sources. Although feeding trials were made by trout starter feed in pike (Kucska *et al*., 2005), the digestive enzyme activity of pike larvae is still unknown. The

aims of this study were to determine the relationships between yolk sac absorption and proteolitic activities of pike larvae and reveal the critical exogenous feeding time.

Material and Methods

Source of Larvae and Experimental Design

Pike larvae were produced from broodstock in captivity in Dinnyés Fish Farm, Hungary. Routine procedure of artificial propagation was applied for fertilization (Szábo, 2003). After 9 days of incubation period in the farm, larvae were transferred with oxygen supply to the research unit of the Department of Fish Culture at Szent Istvan University, Gödöllö, Hungary. Newly hatched larvae (9-day-old) were stocked into 100 L aquarium with aeration and the water temperature was 13±1°C during experiment. Larvae (approximately 100 individuals) were sampled daily into 3 ml eppendorf tubes without water and freezed in -80°C refrigerator from the day after hatching (DAH) till the day of death due to starvation (DAH 12). To make protease analyzes, samples were transferred from Hungary to Turkey in dry ice, and samples were put into -80°C refrigerator in 12 hours.

Prelarval Measurements

The total length of larvae and the digestive tract length were measured (n = 5) daily by Olympus SZX7 binocular microscope (Figure 1). The yolk sac volumes were calculated using formulae produced by Blaxter and Hempel (1966) and Cetta and Capuzzo (1982) as follows:

$$Vys = 4/3\pi(L/2)(H/2)^2 \ ($$

Vys = Volume of yolk sac, L = Major axis, H = Minor axis

Extracts of Pike Larvae

Pike larvae were randomly collected for 13 times, with day interval between sampling (from DAH 0 to DAH 12). Samples were rinsed in distilled water and stored at -80°C until protease analyses. Extracts of pike larvae were prepared by homogenization followed by centrifugation (16000 g, 30 min, 4ºC).

Determination of Protease Activities of Pike Larvae

Total protease activities of pike larvae was measured as described by Walter (1984), using casein (10 mg ml^{-1}) in 50 mM Tris-HCl buffer at pH 8.5 as the substrate. The mixtures including extracts of larvae were incubated with the substrate and then the reaction was stopped by addition of 500 µl trichloroacetic acid (TCA) (120 g L^{-1}). The absorbance was recorded at 280 nm. One unit of enzyme activity was defined as 1 µg of tyrosine released per minute. All measurements were carried out in triplicate. The soluble protein concentrations of pike larvae were determined according to Brasford (1976).

Statistical Methods

Data were analyzed using SPSS statistical software (SPSS, 1993). Comparisons were made using a one-way ANOVA test. Then, Post-Hoc Duncan multiple-comparison test was used for significant differences at the $P<0.05$ level.

Results

Protease activities of pike larvae are summarized in Table 1. The differences observed among protease activities of larvae from DAH 0 to DAH 12 were statistically significant ($P<0.05$). Protease activities of pike larvae remained slightly increased from DAH 0 to DAH 5. A sudden raise in protease activities of larvae were observed from the DAH 6 to the DAH 8. Moreover, the conspicuous increase in protease activities of pike larvae was determined on the DAH 8 (595.22±9.07 U/mg protein). Protease activities of larvae tended to decrease day by day after from the point of the end of the yolk sac absorption DAH 8.

The total length of larvae, yolk sac volume and the digestive tract length were given in Table 2. The

Figure 1. Northern pike prelarvae (L = Major axis, H = Minor axis, DTL = Digestive Tract Length)

Table 1. The changes observed in protease activities from hatching to the day of death due to starvation (mean± standard error (SE))

DAH	Protease Activities (U/mg protein)	DAH	Protease Activities (U/mg protein)
0	5.67 ± 0.29^{ab}	7	93.4 ± 0.34^{d}
1	3.21 ± 0.09^{a}	8	595.22 ± 9.07^{g}
2	3.23 ± 0.06^{a}	9	490.43 ± 8.29^{f}
3	6.5 ± 0.1^{ab}	10	115.53 ± 0.61^{e}
4	8.23 ± 0.08^{ab}	11	24.33 ± 0.21^{c}
5	13.98 ± 0.12^{abc}	12	16.38 ± 0.53^{bc}
6	20.49 ± 0.24^{c}		

In all lines, means with different superscripts are significantly different from each other ($P<0.05$)

Table 2. The larvae length, yolk sac volume and digestive tract length of pike larvae

DAH	Larvae Length (mm±SD)	Volume of Yolk sac (mm^3±SD)	Length of Digestive Tract (mm±SD)
0	3.92±0.15	1.84±0.71	0.96±0.05
1	4.22±0.19	1.26±0.19	1.01±0.10
2	4.84±0.10	1.00±0.21	1.13±0.10
3	5.08±0.13	0.90±0.19	1.23±0.06
4	5.22±0.24	0.82±0.11	1.27±0.09
5	5.75±0.18	0.26±0.05	1.32±0.06
6	6.10±0.12	0.16±0.13	1.36±0.06
7	6.23±0.25	0.10±0.01	1.41±0.05
8	6.56±0.03	0.05±0.06	1.56±0.24
9	6.64±0.16	-	-
10	6.74±0.20	-	-
11	7.03±0.69	-	-
12	7.28±0.45	-	-

total length was increased from 3.92 to 6.74 mm during sampling period. Digestive tract was 0.96 mm at the beginning of the hatching and 1.56 mm at the end of the yolk sac absorption. Yolk sac was totally consumed on the DAH 8.

Discussion

Yolk sac absorption duration depends on abiotic factors such as light and temperature (Blaxter, 1991; Saka *et al.*, 2001; Suzer *et al.*, 2007). By sudden increase of temperature, yolk sac consumption velocity is also increased and this may cause inadequate development of digestive enzymes at the beginning of exogenous feeding, weakness of larvae, and results in death. On the other hand, pike is a cold-water reproducer species and ontogenic development of larvae should be slow under controlled conditions (Szabo, 2003). In this study, yolk sac was totally consumed on the DAH 8 at 13° C, even though the optimum temperature is around 10° C for pike. Although mouth opening was observed in DAH 3, yolk sac reserves was adequate for larval development to the DAH 8.

Digestive enzyme activity can be used as an indicator of larval food acceptance and to some extent can serve as an indicator for digestive capacity in relation to the type of feed offered (Suzer *et al*., 2007; Nolting *et al*., 1999; Zambonino Infante and Cahu, 2001). The present study results reveal that proteolitic activity was low during mouth opening period. Therefore, there is no need to feed larvae exogenously till the yolk sac absorption. Protease activity was increased slightly to DAH 6. By yolk sac absorption, a sharp increase of proteolitic activity was observed. After this point, the proteolitic activity of larvae tended to decrease till the death point due to starvation. Zambonino Infante and Cahu (2001) indicated that the decline observed in specific activities of enzymes is not due to a diminution in enzyme synthesis but it is the result of an increase in tissue proteins.

In conclusion, the present study provides information about critical exogenous feeding time of pike larvae in the relatively high incubation temperature. Larvae should be fed immediately just after yolk sac absorption to prevent tissue breakdown. On the other hand, future research should focus on the effects of different temperatures on the development of proteolitic activities and the consumption duration of yolk sac reserves of pike larvae.

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