ELSEVIER

Comparative Biochemistry and Physiology, Part C 140 (2005) 97–102

CBP

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Hematology patterns of migrating European eels and the role of EVEX virus

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Received 25 May 2004; received in revised form 10 January 2005; accepted 16 January 2005

Abstract

We show that European eels infected with the rhabdovirus EVEX (Eel Virus European X) virus, developed hemorrhage and anemia during simulated migration in large swim tunnels, and died after 1000–1500 km. In contrast, virus-negative animals swam 5500 km, the estimated distance to the spawning ground of the European eel in the Sargasso Sea. Virus-positive eels showed a decline in hematocrit, which was related to the swim distance. Virus-negative eels showed a slightly increased hematocrit. Observed changes in plasma lactate dehydrogenase (LDH), total protein and aspartate aminotransferase (AAT) are indicative of a serious viral infection. Based on these observations, we conclude that eel virus infections may adversely affect the spawning migration of eels, and could be a contributing factor to the worldwide decline of eel.

Keywords: European eel; *Anguilla anguilla*; Virus; Simulated migration; EVEX; Blood chemistry; Lactate dehydrogenase; Total protein; Aspartate aminotransferase

1. Introduction

Worldwide, eel populations have been dwindling over the last decade. Steep declines of 90–99% have been reported for European eel (*Anguilla anguilla*), Japanese eel (*Anguilla japonica*), and American eel (*A. rostrata*) (Stone, 2003). Eels are very vulnerable to environmental factors because of their complex life cycle. As a catadromic fish species, they migrate several thousand kilometers to their spawning areas. Possible adverse effects on the adults include contamination with PCBs – which are released from fat stores during their long-distance migration (Castonguay et al., 1994) – and infection with the parasitic swim bladder nematode *Anguillicola crassus* (Haenen et al., 1994). Furthermore, diminished fat stores due to insufficient food supplies in the inland waters (Svedäng and Wickström, 1997), blockage of migration routes by power stations and power plants, and over-fishing, are all possible causes (Castonguay et al., 1994). Changes in oceanographic currents may interfere with transport of eel larvae to the European coast, and this too may contribute to the decline in eel populations (Knights, 2003). However, no conclusive evidence on any of these causes has been presented yet (Dekker, 2004).

A factor, that has not received much attention to date, is the worldwide occurrence of eel viruses (van Ginneken et al., 2004). Viruses are known to affect blood-forming tissues in fish, and typically become virulent during stress (Wolf, 1988). In salmon for example, Infectious Haematopoietic Necrosis Virus (IHNV) and Viral Haemorrhagical Septicemia Virus (VHSV), both rhabdoviruses, can affect hematopoietic tissues, leading to severe anemia (Wolf,

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1532-0456/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.cca.2005.01.011

1988). The most prominent cases of rhabdovirus infections in eel populations, described in literature, are infections with EVA (Eel-Virus-America) and EVEX (Eel-Virus-European-X). Both viruses are serologically related (Kobayashi and Miyazaki, 1996). EVA was first discovered in Japan in 1974, in a shipment of American elvers, which had been stocked in Cuba (Wolf, 1988). Another virus, which was isolated in a shipment from France to Tokyo, was named EVEX because of its European origin (Sano et al., 1977). So EVEX was described for the first time in 1977, in the period when the European eel populations started to decline. At this moment it is not known if EVEX is a virus endemic to the European eel population or that it substantially spread over the past 50 years due to aquaculture practices. EVEX virus has recently been observed in several countries worldwide (van Ginneken et al., 2004) in European eel (*A. anguilla*) in the Netherlands, Italy and Morocco, but also in New Zealand longfin eel (*Anguilla dieffenbachi*). In this respect it is worrying that also *Herpesvirus anguillae* is isolated and identified in eel populations all over the world. In cultured eel in Taiwan (Ueno et al., 1992; Chang et al., 2002), in cultured eels in the Netherlands (van Nieuwstadt et al., 2001; Davidse et al., 1999; van Ginneken et al., 2004) but also (this study) in adult European eels from Lake Grevelingen. In the comprehensive study of Jørgensen et al. (1994) elvers and eel of *A. anguilla* were sampled on 306 occasions in Denmark, United Kingdom, France and Sweden several eel viruses were isolated like EVEX, EVA, IPN, and herpes like viruses. This study also supports our view that viruses are widespread in the eel population.

For eels, long-term migration can certainly be considered a major stressful event. Therefore, one may assume that an outbreak of a virus infection in infected individuals can take place during this journey. Based on the work of Schmidt, who caught leptocephali (the larvae of the eel) in the ocean, it is assumed that the spawning grounds of the European eel are 6000 km removed from the European continent in the Sargasso Sea (Schmidt, 1923; Miller and McCleave, 1994). It is generally assumed that the silver eel does not feed during its journey to the spawning grounds, which it reaches 4 to 6 months later (Tesch, 1977; Fricke and Kaese, 1995).

In order to test this hypothesis we simulated the 5500-km journey to the Sargasso Sea in large Blazka swim tunnels of 127 l, comparing virus-positive and -negative European eels.

2. Material and methods

2.1. Rationale of the experiment, selection of the animals

It was initially our intention to simulate the 5500-km migration of European eel (*A. anguilla* L., Anguillidae, Teleostei) to the Sargasso Sea in 22 Blazka swim tunnels. We used silver eel (1500 g; ±85 cm), caught in the Grevelingen (Netherlands) during their seaward migration in September 2000. Fish were kept in seawater (33 ppt) for 1 month before use in the experiment. The recirculation system and swim tunnels were placed in a climatized room with a constant temperature of 15 °C. The water temperature was kept at 14 °C. Animals were kept under constant dark conditions. Of these animals, four, five and four animals stopped with swimming after approximately 500, 1000 and 1500 km, respectively (Fig. 1).

The animals were sampled live, and blood was collected while organs were investigated for virus infections. All animals were infected with the EVEX virus (Eel-Virus-European-X (unknown). This group was called the *Virus-positive group*. Of the swimgroup only the 1000 (N=5) and 1500 km sample (N=4) were used for blood analysis (see Tables 1 and 2).

In a second trial, to simulate the 5500-km migration of European eel to the Sargasso Sea, we used hatchery animals (700–900 g, ±75 cm). The experiments with virus-negative eels from a hatchery were performed in freshwater at a temperature of 19 °C. The animals were sampled alive after 6 months and blood was collected while organs were investigated for virus infections. All animals were virus free. This group was called the *Virus-negative group*. Blood plasma of all animals was investigated afterwards for blood chemistry at the CKCL-laboratory of the Academic Hospital, Leiden University, The Netherlands.

2.2. Blazka swim tunnel

The Blazka swim tunnel has a length of 200 cm, with a diameter of the outer swim tunnel tube of 28.8 cm and a diameter of the inner swim tunnel tube of 19.0 cm. The volume was 127.14±0.90 L (n=5). It was calibrated with a Laser Doppler technique at the Delft Hydraulics Laboratory, Technical University Delft. The experimental set-up is described elsewhere (van den Thillart et al., 2004). The swimming speed of the water in the swim tunnels was set at 0.5 body lengths per second.

2.3. Experimental protocol

The Virus-positive group consisted of one Swim group (N=13), one Rest group (N=13), and one Initial group (N=10). The Swim group was put in Blazka swim tunnels. The Initial and Rest group were kept in flow boxes (Overtoom BV.) of 40 l connected to the same water recirculation system. The Initial group was sampled at the start of the experiment as a zero sample. Both Swim and Rest groups were kept at the same water quality conditions in the tunnels and flow boxes during the experiment. The *Virus-negative group* consisted of one Swim group (N=9), one Rest group (N=12), and one Initial group (N=9). All animals from virus-positive and virus-negative groups were healthy at the start of the experiment. The presence or absence of viruses in all animals was determined after