Hydrocephalus in burbot (*Lota lota* L.) larvae

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Abstract

During an experiment aimed at elucidating the nutritional requirements of burbot (*Lota lota* L.) larvae, the latter displayed a dorsal swelling of the cranium. Histological examination revealed dilated brain ventricles (hydrocephalus internus). Bacterial culture of the content of the swelling was negative. Slightly elevated copper concentrations were found in the culture water resulting in an increased larval body copper content. This is the first reported case of hydrocephalus in burbot larvae.

Introduction

The burbot (*Lota lota* L.) is the only freshwater member of the cod family *Gadidae*. In several European countries, this fish species is endangered. The exact cause of its declining populations is unknown but several factors such as pollution, global warming and destruction of the habitats play an important role (Harzevili et al., 2003). Larval rearing and weaning is needed to supply juveniles for restocking programmes and farming activities. Hitherto, little information is available on the nutritional needs of burbot larvae, a necessary prerequisite to be able to develop commercial feeds for these larval stages. Commercial dry feeds would decrease the labour cost and need for space and avoid the introduction of infectious agents as opposed to live feed (Jensen et al., 2008). It is in this context that an experiment was set up to characterize the nutritional requirements of burbot larvae. During this experiment, a cranial swelling was noted in the burbot larvae which is described in the present study. It is possible aetiologies are listed and discussed.

Materials and methods

The experimental facilities of the Laboratory for Aquaculture & ARC (Ghent University) obtained 32,000 burbot larvae (14 days post hatching (dph)) from the Research Institute for Nature and Forest (INBO) in Linkebeek, Belgium. They were fed *Artemia* nauplii since 8dph. The larvae were divided over four 90l upwelling tanks and

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were kept under continuous lighting. The tanks were disinfected beforehand by a combination of hydrogen peroxide (210g/l) and peroxyacetic acid (55g/l). Aerated tap water was used which was supplied through copper pipe lines. The hydraulic retention time was 1.5h. The levels of ammonia and nitrite did not exceed 0.05mg/l and 0.01mg/l, respectively. The average dissolved oxygen concentration was 7.02mg/l and was never lower than 6.67mg/l. The temperature of the water was 12.1°C±1.3°C during the first four weeks. Then the temperature was gradually increased to 14.9°C±1.3°C. The pH of the water ranged from 7.58 to 7.70. The carbonate hardness was 9°d (161.1mg$CaCO_3$/l).

In the experimental facilities, the larvae were fed plain *Artemia* nauplii Instar I (SepArt OF 500µm, Inve Aquaculture, Belgium) at an initial feeding intensity of 50 nauplii/larva (44 nauplii/10ml). After one week, the feeding intensity gradually was increased during the next three weeks to 80 nauplii/10ml. This feeding intensity was maintained for another two weeks. The larvae of tank 1 were fed discontinuously, twice a day. The larvae of tank 2, 3 and 4 were continuously fed *Artemia* nauplii with a peristaltic pump. After two weeks, only the larvae of tank 4 received nauplii enriched with highly unsaturated fatty acids (HUFA) (S.presso, INVE Aquaculture, Belgium) for the remaining live feed phase.

Three weeks following their arrival, a cranial swelling was seen in a small number of burbot larvae. One week later about 20% of the population in tank 1, 2 and 3 displayed this abnormality and most of the other larvae got affected during the next three weeks. Initially, the swelling was only visible upon close examination of the larvae but gradually increased in size to become easily discernible (Figure 1). The incidence of the cranial swelling of the larvae fed enriched *Artemia* nauplii (tank 4) was less than 5%. Most of the affected larvae died a few days later. A minority fully recovered after some weeks with complete disappearance of the swelling.

At the same time, larvae from the same batch were reared at two other institutes in Belgium (Aqua-ERF and INBO). In these larvae no abnormalities were encountered.

The content of the cranial swelling of the affected larvae was sampled aseptically for bacteriological examination. Samples were inoculated on Columbia agar with 5% sheep blood (Difco, Germany) and plates were incubated at 23°C for five days. Affected and clinically normal larvae were sacrificed using an overdose of a benzocaine solution (1g/10ml ethanol), fixed in neutral buffered formaldehyde or Bouin's solution for histological examination and processed as described previously by Rekecki et al. (2009).

Two weeks following the first observation of the cranial swelling in the larvae, the water and whole larval bodies were analysed for their copper and lead content by means of inductively coupled plasma mass spectrometry (ICP-MS) (Chan et al., 1998).

For comparative purposes, burbot larvae from one of the other institutes exhibiting no abnormalities were also sampled for histological, copper and lead analysis.

Figure 1. Burbot larva (42dph) displaying a cranial swelling.

Results

No bacterial colonies were observed upon inspection of the incubated agar plates.

Histological examination of the affected larvae revealed a dilation of the forebrain ventricles (hydrocephalus internus). The ventricles protruded dorsally and were only covered by the skin. The dilated ventricles were filled with protein-like material and aggregates of red blood cells occasionally present (Figure 2). Haemorrhagic zones were seen in the neural tissue. The nervous tissue itself and the skin, gills and internal organs displayed no abnormalities, as was the case for all organs in the clinically normal burbot larvae.

The concentration of copper and lead in the water of the affected larvae was 4.6µg/l and below 0.2µg/l, respectively. The concentration of copper in the affected larvae and the clinically normal larvae of the other institute amounted to 710µg/kg and 414µg/kg wet weight, respectively.

Discussion

Up until now, only a few cases of hydrocephalus in fish have been reported and their aetiology discussed.

Rodger and Murphy (1991) described Atlantic salmon (*Salmo salar* L.) alevins with a congenital

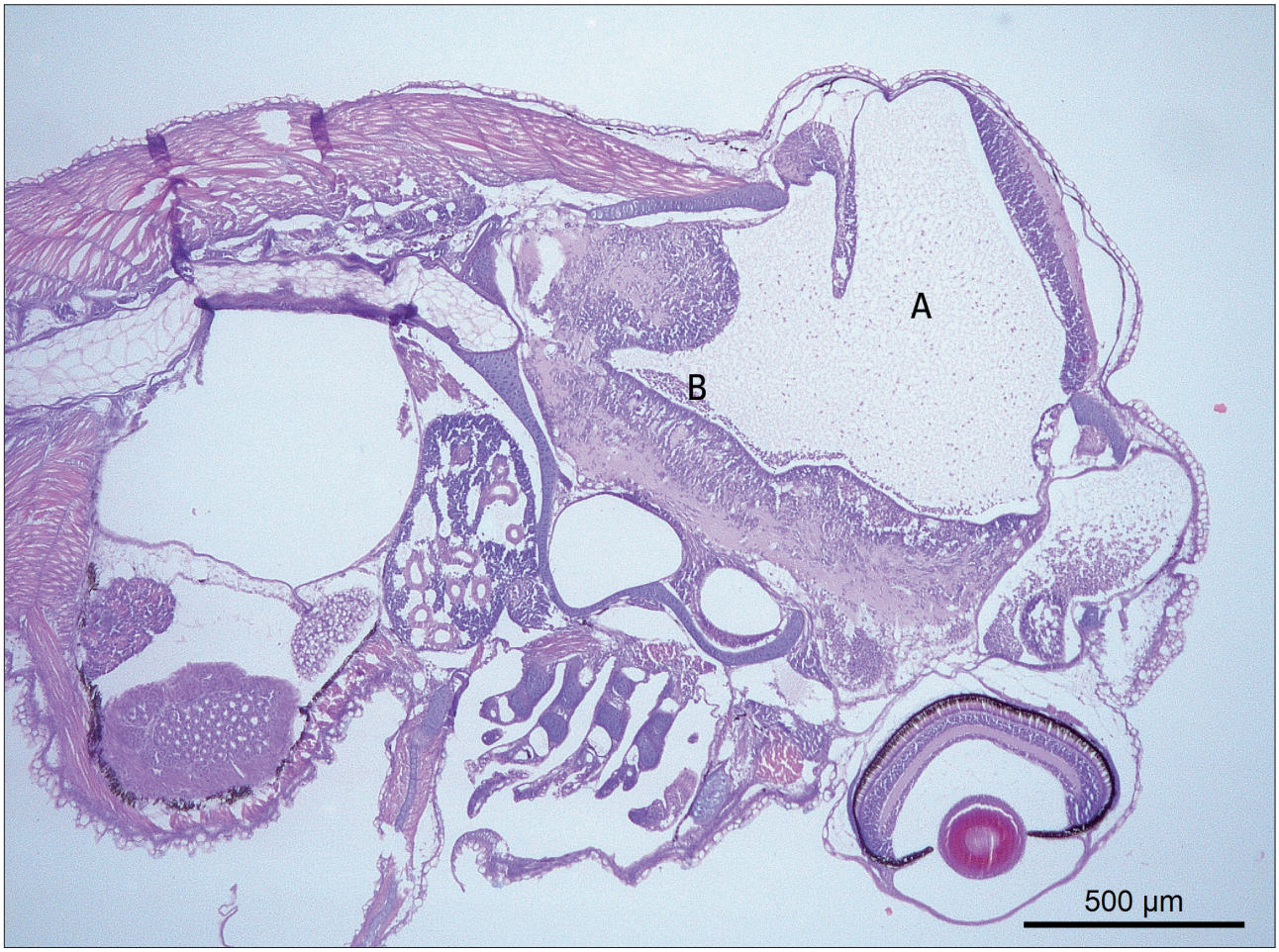

Figure 2. Longitudinal histological section (H&E) of a deformed head of an affected burbot larva (38dph). Aggregates of proteins (A) and red blood cells (B) can be detected in the cerebrospinal fluid of the dilated ventricle.

hydrocephalus (externus). Necrotic cells were found in the meninges and on the outer surface of the optic lobes. Some of the alevins had haemorrhages in the subarachnoidal space or in the optic lobes. Muench et al. (1997) reported hydrocephalus internus in four week old channel catfish (*Ictalurus punctatus* L.). Only 0.5 to 3% of the population was affected. In some of the larvae a pale eosinophilic fluid containing red blood cells was found in the distended fourth ventricle. One week later, the incidence dropped below 0.05%. Both anomalies were diagnosed as congenital, because no other causal agents could be detected. In our study, burbot larvae of the same batch were also reared and weaned at two other institutes. However, no similar deformities were encountered, urging us to speculate that in our case, the main aetiological factor was not genetic.

It is reported that hydrocephalus in pike (*Esox lucius* L.) fry can be caused by two different rhabdoviruses: Pike Fry Rhabdovirus (PFR) and Viral Haemorrhagic Septicaemia Virus (VHSV) (Speare and Frasca, 2006). Both viruses cause a generalized state of disease with high mortality. PFR causes an excess of fluid accumulation in the third ventricle, including brain haemorrhages. In the liver and kidney, necrotic lesions can be found (Muench et al., 1997). VHSV is

known to cause serious problems in European rainbow trout (*Oncorhynchus mykiss* L.). Non-salmonid species like pike can also be infected. Hydrocephalus is the main sign (Meier et al., 1994). In the present case, a possible viral aetiology was excluded because no signs of a generalized state of disease were noted and upon histological examination, no necrotic lesions were seen. The fact that no anomalies were observed in the larvae raised at the two other institutes supports this assumption. Indeed, the tanks in which the larvae were housed, were disinfected before use. If a virus were to have played a role, the larvae would have been carriers upon entering the experimental facilities and one would expect all larvae from this batch (including those kept at the other institutes) to be affected albeit with varying degrees due to their different environment.

In larval salmonids, hydrocephalus can also be the result of inadequate thiamine levels. These low levels can be due to a thiamine degrading factor present in prey fish and consumed by adult salmonids leading to lower thiamine levels in the eggs. Contaminants like PCBs may also play a role (Fitzsimons et al., 2001). Three similar syndromes linked with insufficient thiamine levels are reported: early mortality syndrome (EMS, in salmonids in the Great Lakes), the 'Cayuga syndrome' (in larval landlocked Atlantic salmon) and M74 (in Atlantic salmon from the Baltic Sea) (Fitzsimons et al., 2001). Histological investigation of these larvae revealed the presence of haemorrhages and necrotic zones in the brains (Elliot, 2005). The former could also be seen in the present larvae, but not the necrotic zones. The thiamine level in the burbot eggs was assumed to be sufficient, because larvae of the same origin, but reared at two other institutes did not suffer from similar disease signs.

Bruno (2005) reported the presence of mostly unilateral cranial nodules in Atlantic salmon fry with unknown aetiology. The cerebellum was displaced dorsally, but no histological abnormalities were detected. In coho salmon (*Oncorhynchus kisutch* L.) fry, a focal swelling on the head due to a cranial defect was described. The aetiology is unknown, but exposure to teratogenic chemicals such as malachite green is suggested as the most likely cause. Malachite green is known to target cranial tissue. A similar sign is seen in salmon fry, with an acquired external hydrocephalus (Speare and Frasca, 2006). The burbot larvae in the present study were not previously exposed to malachite green.

Heavy metals are known to induce a wide range of deformities, including hydrocephalus. For lead, the maximum acceptable toxic concentration for trout fry is 14.6µg/l (Davies et al., 1976). This is far beyond the level found during this study.

For copper, chronic toxicity concentrations for freshwater fish vary between 2µg/l and 14µg/l (Brix et al., 2001). The copper concentration in the water of the present rearing unit was 4.6µg/l. The copper concentration in the larval tissue was 710µg/kg wet weight after three weeks of exposure and with a dry weight content of 15%, the calculated body copper content thus amounts to 4.7µg/g dry weight. Copper exposure of rainbow trout fry to 0.19µg/l, 4.6µg/l and 9.5µg/l for 20 days resulted in a body copper concentration of 4.42µg/g, 5.82µg/g and 6µg/g dry weight, respectively (Marr et al., 1996; Hansen et al., 2002). All exposures resulted

in reduced growth. Deformations were only seen at higher concentrations. Incubation of common carp (*Cyprinus carpio* L.) eggs in water containing 0.2mg/l copper induced mostly vertebral malformations in the larvae, while only some larvae displayed craniofacial deformities (Jezierka et al., 2000). Copper is known to disrupt osmoregulation by influencing the Na^+/K^+-adenosine triphosphate pump and the cupric ion replaces the calcium ion in the tight paracellular junctions of the gill cells, leading to increased cell membrane permeability (Zahner et al., 2006). Whether a similar phenomenon took place in the brain of the burbot larva leading to fluid accumulation is unknown. Hence, we cannot confirm nor exclude the slightly elevated copper level of 4.6µg/l having a causative role in the noted hydrocephalus. Important to keep in mind is that burbot is known to be a very sensitive species to polluting agents. Industrial pollution of the aquatic environment is indeed indicated as a reason of the declining populations (Polinski et al., 2010).

When the larvae were fed *Artemia* nauplii enriched with HUFA's, the prevalence of the cranial deformity was markedly lower. Supplementing HUFA's can improve the larval quality and increase their stress resistance, as was seen in several other marine fish species (Sorgeloos et al., 2001; Wilson, 2009; Vizcaino-Ochoa et al., 2010). However, the exact role of HUFA's in these observations remains to be elucidated.

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