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Diseases and Injuries Associated with Mortality of Hatchery Reared Baltic Cod *(Gadus morhua* L.) Larvae

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Buchmann, K., J. L. Larsen, I. Dalsgaard: Diseases and injuries associated with mortality of hatchery-reared Baltic cod (Gadus morhua L.) larvae. Acta vet. scand. 1993, 34, 385-390. – A cod hatching plant was established in 1992 on the island of Bornholm in the Baltic Sea in order to elucidate the possibilites for restocking of cod fry in this brackishwater system. The disease prevalence in 3 batches of hatchery-reared yolksac larvae from the Baltic cod *(Gadus morhua* L.) was monitored during the posthatch period. High prevalences of bacteriosis/mycosis, lordosis/scoliosis, injuries and protozoan endoparasitism were recorded. *Vibrio sp.* and *Vibrio anguillarum* serovar 04, 06, 08 in addition to nontypable strains and saprolegniaceous fungi were isolated from the larvae. The dinoflagellate-like endoparasites were located in the yolksac of the cod larvae.

Vibrio anguillarum; mycosis; protozoa; endoparasite; lordosis; scoliosis; aquaculture; Baltic Sea.

Introduction

Diseases of larger cod from the Baltic Sea have been the subject of several investigations (*Fagerholm* 1982, *Larsen & Jensen* 1982, *Køie* 1984, *Buchmann* 1986, *Möller & Anders* 1986, *Thulin et al.* 1989, *Dalsgaard et al.* 1992). Recently a protozoan endoparasite was recorded in cod larvae from this area *(Hedegaard Pedersen et al.* 1993), but otherwise information about pathogens in fish larvae from the Baltic Sea is sparse.

Due to the declining catches of cod *(Gadus morhua)* in the Baltic Sea from 1985 and until 1992, the Danish Ministry of Fisheries, in order to produce cod fry for stocking, established a pilot scale hatchery for Baltic cod eggs on the island of Bornholm in the Spring 1992. During the early posthatch period the yolksac larvae experienced heavy mortalities in the incubation system. The present paper reports on diseases and injuries associated with these mortalities.

Materials and methods

Cod larvae

Spawning male and female cod were caught by commercial trawlers in the Bornholm Basin from late May to late July 1992. Eggs were stripped and fertilized in seawater (16 ppt salinity, 5-10°C) immediately after catch. They were brought to the hatchery and incubated in a series of hatchery cones (55 l volume each). These were connected to a recirculated system containing a total of 3-4 m^3 chilled seawater (16 ppt salinity, 6-10°C). The water was collected from 70-80 meters depth in the

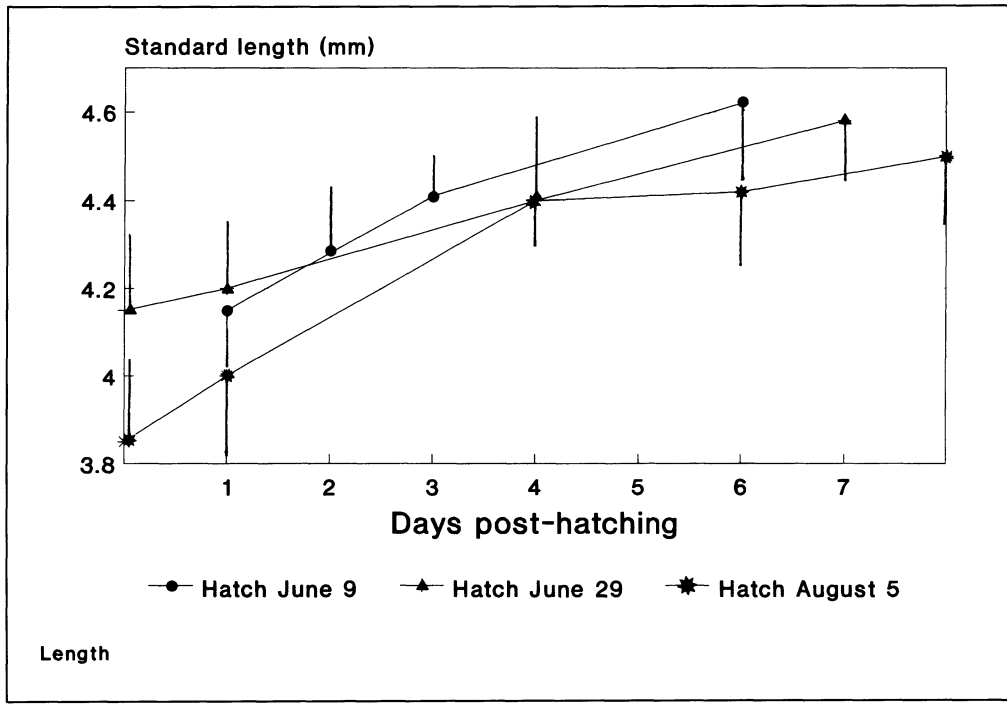

Figure 1. Standard length of yolk sac larvae from 3 batches in the week following hatching. Each point represents the mean of measurements in 20 specimens. Vertical bars indicate standard deviation.

Bornholm Basin below the halocline, which is the natural hatching zone *(Wieland* 1988). This salinity is sufficient for boyancy of Baltic cod eggs *(Nissling & Westin* 1991). The eggs hatched within 12 to 15 days.

Microscopy

Samples (each comprising 20 living cod larvae) were collected regularly from 3 batches of larvae (hatching date June 9, June 29, August 5) during the week following hatching. The standard length (length from upper lip to caudal tip of notocord) was recorded and by use of the dissection microscope (25-50 × magnification) and the compound microscope (100-1000 × magnification) the presence of injuries (yolksac herniae and scratches of the primordial fin), microbial infections (bacterial and/or mycotic infection foci), lordosis/scoliosis and the presence of parasitic dinoflagellates in the yolk sac *(Hedegaard Pedersen et al.* 1993) were noted.

Bacteriology

Larvae from the batches hatched at June 9 and June 29 were examined. The larvae were collected from the water using filtration on Millipore HA membrane filter (Millipore Corp. Bedford, Mass.) with a pore size of 0.45 μm. A volume of 100-250 ml water was filtrated to obtain 15 larvae which were removed from the filter and washed 3 times in sterile saline. Groups of 5 larvae were then incubated on blood agar (Blood Agar Base

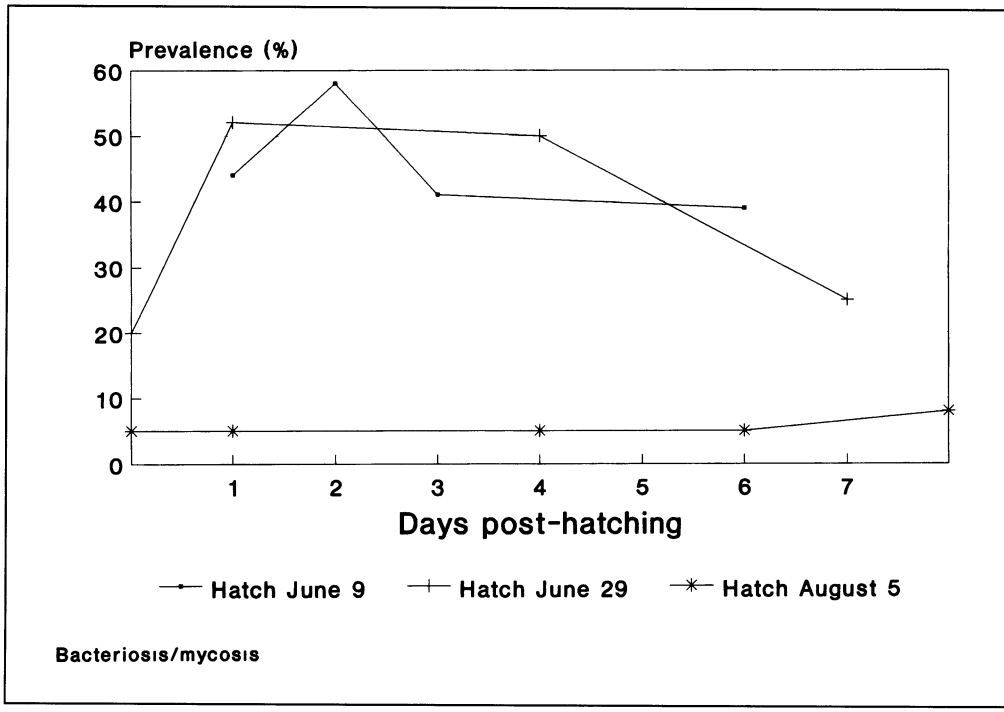

Figure 2. Prevalence of bacterial/mycotic infections in the three batches of larvae.

(Gibco)) with 5% citrated calf blood), on TCBS Agar (Difco) (20°C, 48 h) and Marine Agar (Difco) (15°C, 6 days), respectively. Only the dominant colonies were identified (5 from each batch). The biochemical and serological tests were performed as previously reported *(Larsen* 1983, *Sørensen & Larsen* 1986).

Results

During the periods of investigation a mortality of 100% in all 3 groups of cod larvae was recorded. The non-fed yolksac larvae experienced a significant length growth during the period of investigation (Fig. 1). The prevalences of injuries, bacteriosis/mycosis, lordosis/scoliosis and parasite infections during this period were notably high (Figs. 2-4).

With exception of the last batch of larvae the infections with bacteria/fungi were the most prevalent and generally the prevalences of injuries in the larval batches were almost as high. Lordosis/scoliosis were recorded in 5-20% of the larvae. The prevalence of the endoparasite infection declined from an initial level of 20-25% to a low level of 5% during the week following hatching.

Bacteria isolated from the larvae all belonged to *Vibrio anguillarum* or *Vibrio* sp. The serological characterization of *Vibrio anguillarum* showed that serovar 06 and 08 (hatching June 9) and serovar 04 and 08 (hatching June 29) were present. Some isolates of *Vibrio anguillarum* were non-typable.

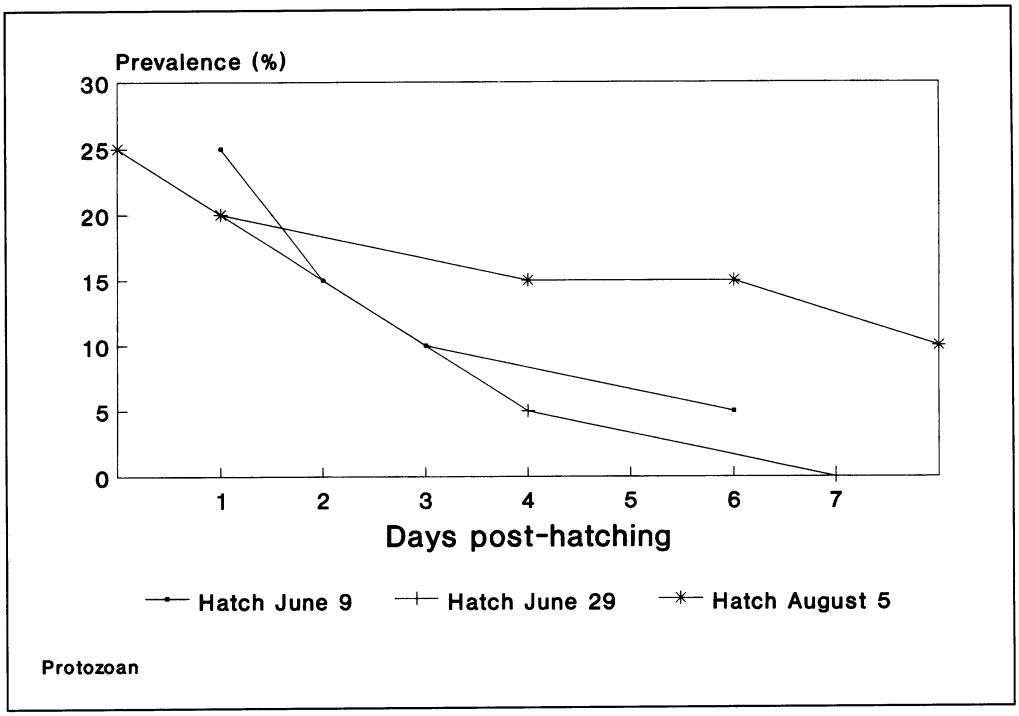

Figure 3. Prevalence of infections with the protozoan yolksac parasite in the 3 batches of larvae.

In the mycotic foci in the larvae non-septate hyphae were observed. Fungi from infected larvae were isolated on blood agar base and identified as *Saprolegnia* sp.

Discussion

The rearing of Baltic cod larvae in the above mentioned production plant faced a number of disease problems of which infections with *Saprolegnia* sp. were important. The fungal hyphae penetrated the larval yolk sac and were able to colonize all body surfaces. Saprolegniaceous fungi have mostly been known as pathogens in freshwater fishes. However, recently strains of these fungi were reported resistant to mesohaline conditions *(Shafer et al.* 1990). The relatively low salinity of the incubation water is obviously insufficient for inhibition of this fungal pathogen and it should be investigated if the use of higher salinity water for incubation is able to reduce the problem. We identified *Vibrio anguillarum* serovar 04, 06 and 08 on the cod larvae but a possible pathogenicity to the larvae of these bacteria is yet to be demonstrated. These serovars have all previously been isolated from diseased cod *(Sørensen & Larsen* 1986), and serovar 04 was recently implicated in a serious outbreak of vibriosis among juvenile artificially reared North Sea cod in Denmark *(Dalsgaard* unpublished).

The cause of the skeletal deformities is unknown but the role of contaminants in the cod should be considered as a possible influential

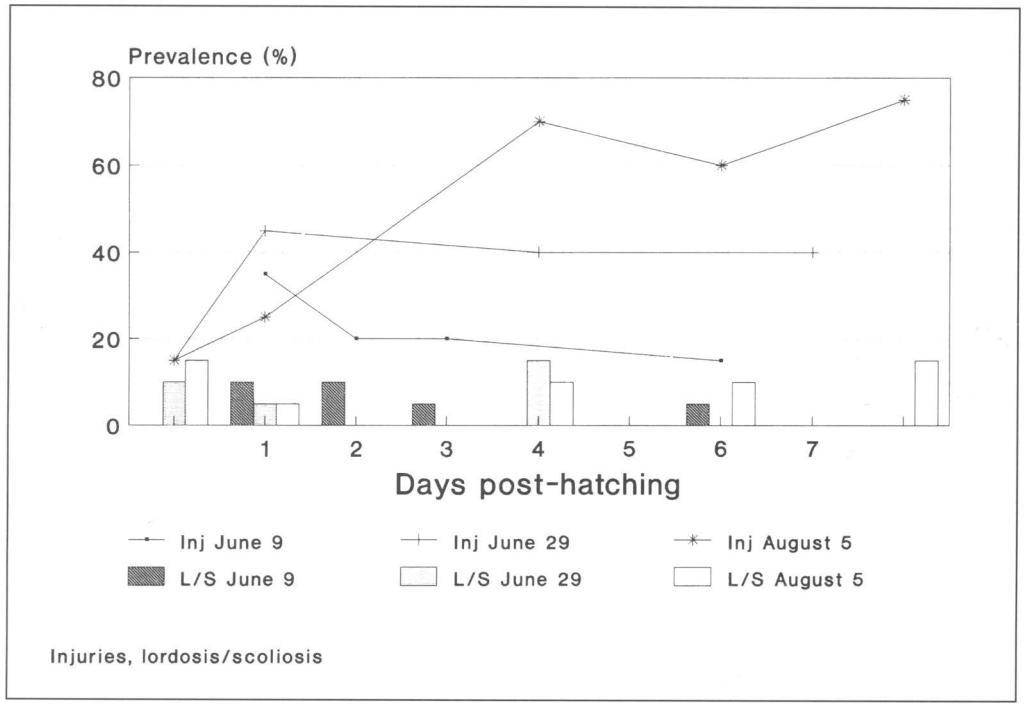

Figure 4. Prevalence of injuries (scratches of primordial fin, yolk sac herniae) and skeletal deformities (lordosis/scoliosis) in the 3 batches of larvae.

factor. It has been demonstrated that exposure of cod embryos to contaminated sea-surface microlayer from both the Atlantic and the Baltic Sea will elicit abnormal development in the cod larvae *(Kocan et al.* 1987). These authors found that the prevalence of skeletal deformities in cod larvae increased from 0-5% in the control group compared to more than 80% in the exposed groups.

The observed injuries in larvae are probably caused by a number of factors. Next to damages caused by the egg shell during hatching and mechanical molestation of larvae in the incubation cone one should consider the role of bacteria in wounding of the yolk sac epithelium. The yolksac herniae (yolk mass protruding through a wound in the yolk sac epithelium) could be associated with bacterial activity, corresponding to the lytic effect of *Flexibacter* sp. on fish eggs *(Bergh et al.* 1992). However, *Flexibacter* sp. was not isolated in the present investigation.

The endoparasitic protozoan detected in the yolksac of the cod larvae was described by *Hedegaard Pedersen et al.* (1993). TEM-studies (Transmission electron microscopy) suggested that both uni- and multicellular stages of this parasite exist and demonstrated the presence of mitocondria with tubular cristae and granular endoplasmatic reticulum in the cytoplasma. The parasite diameters range from 25 μm to 70 μm.

The prevalence of the endoparasitic protozoan in the yolksac larvae decreased mark-

edly during the posthatch period. Whether this was due to spontaneous parasite death or host mortality is unknown. A corresponding infection with the parasitic dinoflagellate *Ichthyodinium chabelardi* in sardine larvae was reported to elicit host death *(Hollande & Cachon* 1952). Therefore further studies should elucidate the pathogenicity of this protozoan endoparasite in the cod.

Acknowledgement

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Resumé

Sygdomme og skader forbundet med dødelighed for opdrættede torskelarver fra Østersøen

Med henblik på at undersøge mulighederne for udsætning af kunstigt klækkede og opdrættede torsk i Østersøen etablerede det danske fiskeriministerium i 1992 et torskeopdrætsanlæg på Bornholm. Forekomsten af bakterielle, parasitære og mykotiske infektioner i torskelarver opdrættet i akvakulturanlægget blev registreret i 1992. *Vibrio* sp. og *Vibrio angullarum* serotype 04,06 og 08 samt ikke typbare stammer blev isoleret. Desuden registreredes høje prevalenser af lordose og scoliose, infektioner med en encellet dinoflagellatlignende parasit samt mykoser *(Saprolegnia* sp).

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