

Is *Vibrio splendidus* pathogenic to Atlantic cod larvae?

Nina Sandlund^{1*}, Siri Frafjord Ørstavik^{1*}, Ingrid Uglenes Fiksdal¹, Øivind Bergh^{1,2}

¹ Institute of Marine Research, P.O. Box 1870 Nordnes, NO-5817 Bergen, Norway

² University of Bergen, Institute of Biology, P.O.Box 7803, NO-5020 Bergen

*Contributed equally

nina.sandlund@imr.no

Background:

Vibrio splendidus has often been associated with high mortalities during larval stages of both marine fish and bivalves, and was recently demonstrated to cause mortality to cod, *Gadus morhua* larvae, when administered through rotifers (Reid et al. 2009). In this experiment, different *V. splendidus*-like strains were virulence tested *in vivo* using the yolk sac larvae bath challenge model by Sandlund & Bergh (2008).

Materials and Methods:

Cod eggs were randomly sampled and individually transferred to a 24-well multidish system where each well contained 2 ml of aerated sterile seawater. The eggs were exposed to bacteria at the day of arrival (10 days post fertilisation) and they hatched within 96 hours. Each challenge group consisted of 72 individuals. Eggs and larvae were exposed to either a high or a low challenge dose, approximately 10^6 and 10^4 CFU ml⁻¹, respectively. Unchallenged larvae were used as a negative control group and larvae challenged with the pathogenic bacterium, *Vibrio anguillarum* strain HI 21413 was used as a positive control. Samplings for immunohistochemistry and mortality readings were done daily. Immunostaining of larval sections was performed according to Sandlund et al. (2006) using the same polyclonal antiserum against *V. splendidus*, strain LT 06

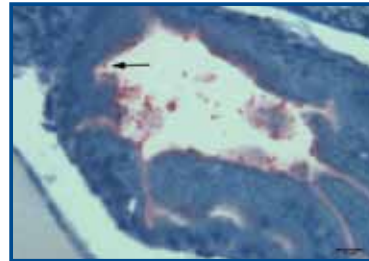


Figure 1. Intestine of larva challenged with HI 22109, 4 dph. Free bacterial cells are present in the intestinal lumen and some are attached to the brush border (arrow). Scale bar 10 µm.

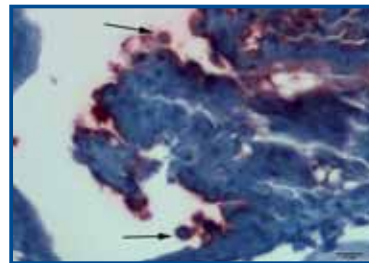


Figure 2. Intestine of larva challenged with HI 22099, 5 dph. Shredded and necrotic cells are visible in the intestinal lumen. Scale bar 10 µm.

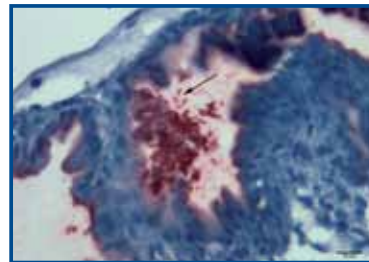


Figure 3. Intestine of larva challenged with LT 06, 7 dph. Positively stained bacteria are visible, both in clusters and as single cells (arrow). Scale bar 10 µm.

Bacterial strain	Origin	References
HI 22094	Diseased cod, <i>Gadus morhua</i> , larvae	Sandlund unpubl. results
HI 22095	Diseased cod larvae	Sandlund unpubl. results
HI 22099	Diseased cod larvae	Sandlund unpubl. results
HI 22107	Diseased cod larvae	Sandlund unpubl. results
HI 22109	Diseased cod larvae	Sandlund unpubl. results
LT 06	Diseased great scallop, <i>Pecten maximus</i> , larvae	Torkildsen et al. 2005
DMC-1	Diseased turbot, <i>Scophthalmus maximus</i> , larvae	Thomson et al. 2005
HI 21413	Diseased cod larvae	Sandlund & Bergh (2008)

References:

- R. Thomson, H. L. Macpherson, A. Riaza and T. H. Birkbeck 2005. *Vibrio splendidus* biotype 1 as a cause of mortalities in hatchery-reared larval turbot, *Scophthalmus maximus* (L.). Journal of Applied Microbiology 99:243-250
- L. Torkildsen, C. Lambert, A. Nylund, T. Magnesen and Ø. Bergh 2005. Bacteria associated with early life stages of the great scallop, *Pecten maximus*: impact on larval survival. Aquaculture International 13:575-592
- N. Sandlund, L. Torkildsen, T. Magnesen, S. Mortensen and Ø. Bergh 2006. Immunohistochemistry of great scallop *Pecten maximus* larvae experimentally challenged with pathogenic bacteria. Diseases of Aquatic Organisms 69:163-173
- N. Sandlund and Ø. Bergh 2008. Screening and characterisation of potentially pathogenic bacteria associated with Atlantic cod *Gadus morhua* larvae: bath challenge trials using a multidish system. Diseases of Aquatic Organisms 81:203-217
- H. I. Reid, J. W. Treasurer, B. Adam, T. H. Birkbeck 2009. Analysis of bacterial populations in the gut of developing cod larvae and identification of *Vibrio logei*, *Vibrio anguillarum* and *Vibrio splendidus* as pathogens of cod larvae. Aquaculture 288:36-43

Conclusions:

- The statistical analysis (2 x 2 contingency table; p < 0.0055 Bonferroni correction) revealed no significant difference when comparing mortality observed in the negative control group and the larval groups challenged with the various *V. splendidus* strains. The mortality rates of the positive and negative control groups were significantly different.
- The immunohistochemical examinations revealed large quantities of bacteria in the gastrointestinal tract, both in the lumen and attached to the brush border. Necrotic cells and tissue were also observed in challenged groups.

