

Differences between *A. simplex s.s.* and *A. pegreffii*: *in vitro* infectivity and freezing tolerance

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Freezing of fish is one of the most efficient technological treatments to kill *Anisakis* larvae in the third stage (L3), but, although data are available for *Anisakis s.l.* there has been no systematic studies of the effect on different *Anisakis* species. Previous results from our group have shown in experimentally infected mince fish that viability of *Anisakis s.s.* larvae is affected by freezing rate, final freezing temperature & storage time, so that at fast freezing rates all L3 can be non viable even at -10 °C. The aim of this work was to compare the ability of penetration in agar of *A. simplex s.s.* vs *A. pegreffii* and to study the effects of freezing rate and final freezing temperature on the mobility of *A. pegreffii* in order to compare the possible differences in infectivity of this species with the previously reported *A. simplex s.s.*

Live *A. simplex s.s.* and *A. pegreffii* L3 were obtained from heavily parasitized fish viscera. They were washed with 0.85% NaCl and stored at 4 °C until use. A total of 100 larvae were placed in flasks (10 L3/flask) containing 0.75% agar and a layer of simulated gastric juice. They were allowed to penetrate into agar in an incubator at 37 °C in 5% CO₂. The number of larvae penetrated into agar was monitored at 1, 3, 6, 20, 24, and 92h. Minced hake muscle was infected with L3 *A. pegreffii* (10 L3/ 75g mince) and subjected to three freezing rates until final temperatures of -10 and -15 °C were reached. The time temperature profiles were recorded per each experimental unit. After thawing, viability of larvae was monitored. Species identification was performed for all the batches studied.

The *in vitro* infectivity measured by the agar penetration test at 37 °C showed that *A. pegreffii* had slightly lower penetration ability than *A. simplex s.s.* The percentage of survival of *A. pegreffii* decreased with increasing freezing rates as it was found previously for *A. simplex s.s.* but the viability at a given freezing rate or final time was lower for *A. pegreffii*.

These experiments show that *Anisakis pegreffii* is less resistant than *Anisakis simplex s.s.* in terms of penetration ability in agar and freezing tolerance.

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Key words: *Anisakis simplex s.s.*, *A. pegreffii*, freezing, viability, agar penetration test