In vitro treatments of deltamethrin against the isopod parasite *Anilocra physodes*, a pathogen of seabass *Dicentrarchus labrax* L.

F. Athanassopoulou¹, D. Bouboulis², and B. Martinsen³.


Abstract
The in vitro efficacy of the pyrethroid deltamethrin against the isopod parasite *Anilocra physodes* was determined under laboratory conditions. The following concentrations of the drug were tested on adult and pre-adult parasites by means of bath treatments of half an hour duration: 10mg/L, 1mg/L, 3mg/L, 0.3mg/L, 0.1mg/L, 0.01mg/L, 0.05mg/L, 10µg/L, 5µg/L, 0.15µg/L, 0.1µg/L. Results were assessed after 30 minutes, 2, 12, 24 and 48 hrs. The best concentration was proved to be 0.05mg/L where all parasites died at 2 hours and remained dead for 48 hrs.

Introduction
Recently, copepod and isopod infestations have been reported in cultured marine fish in the Mediterranean region (Athanassopoulou et al., in press). High mortalities have been recorded due to the isopod parasite *Anilocra physodes* and to a lesser extent, the copepods *Caligus* spp and *Lernanthropus kroyeri* in juvenile cage-reared sea bass and sea bream *Sparus aurata* (Theohari, Ragias & Bai, 1997). However, it is *Anilocra physodes* and *Ceratothea oestroides* that have become the major parasite problem for young sea bass reared in cages in the Aegean Sea (Sarusic, 1999).

The parasite is found in the mouth, the skin and branchial cavity of a number of marine fish. Adult parasites most commonly are seen in pairs in the buccal cavity the male being smaller than female. After copulation, pullus larvae are hatched in the marsipium of the female parasites and released to the environment where they undergo several metamorphosis stages. It is the second stage (pulli II) that is believed to attack the cultured fish and the stage that causes most problems (Lindsay and Moran, 1976). These pulli II show low specificity in terms of attachment location on the host in contrast to the adult stages that normally attach to the buccal epithelium and cause less damage. In the latter case lesions are caused by the copulation activity or due to their size (atrophy of the tongue and dysplasia of teeth) resulting in poor growth of the hosts.

In the present paper, we present for the first time, preliminary data on the efficacy of the chemical Deltamethrin against *Anilocra physodes*, under laboratory conditions.
Materials and Methods
Deltamethrin (pyrethroid) was used in the form of a 1.0% w/v solution (ALPHAMAX, Hoechst Marion Roussel). From the initial solution, required amounts were diluted in fresh seawater in order to obtain the doses referred to in Tables 1 - 3. Each dose was tested in three replicas and all solutions used in the present study were prepared within thirty minutes of use. Parasites were collected carefully from infected sea bass in cages, placed in containers with seawater and transported immediately to the laboratory where they were checked for viability, counted and transferred into 1L vials filled with fresh seawater at the same temperature. Each vial contained fifteen parasites which were of adult stage. The parasites remained under aeration and constant temperature (20-22°C) for 12 hours prior to experimentation. In total, three experiments were carried out: One at temperature 18°C the rest at temperature of 23°C. pH and oxygen levels were constant (7.74 and 5.2 mg/L respectively).

In the first experiment, the following concentrations were tested: 10, 3, 1, 0.3, 0.1, 0.01 mg/L. Parasites were submerged in fresh chemical dilutions of the appropriate concentration for thirty minutes whilst one vial was used as control (the parasites were submerged into fresh seawater of the same temperature). After thirty minutes the parasites in all vials (including the control) were returned to fresh seawater. The viability of the parasites was examined at half an hour, at 2, 12, 24 and 48 hours. Criteria for viability were the reaction after touch with a needle and the respiratory function. In the second experiment, the following concentrations were tested: 0.1, 0.05, 0.15 µg/L for thirty minutes, whereas in the third concentrations of: 10, 5 and 0.05 mg/L were used. Except for temperature, the rest of conditions in these two experiments were identical to the first experiment. Statistical analysis was performed with the ANOVA test of variance using a SPSS program.

Results and discussion.
The results (Tables 1, 2 & 3) showed that the minimal in vitro dose that kills the parasites in 12 hours is between 0.01-0.05 mg/L. The minimal dose that kills the parasites in 2 hours

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<tr>
<th>Deltamethrin conc. (mg/l)</th>
<th>Time after treatment / outcome*</th>
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<td>0.01</td>
<td>12d, 3m**</td>
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<td>Control</td>
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Table 1. Mortality of parasites following 30 minutes exposure to alphamax (n=15, 3 replicates). *d = dead, a = alive, m = moribund. **statistically significant difference (P<0.05). Temp = 18°C
was found to be 0.05 mg/L. In both cases, the parasites shown no signs of recovery after 48 hours.

Despite the constant increase of cultured tonnage of fish in Mediterranean and the associated increase of copepod and isopod infestations, there is no effective treatment of these parasites as only a few drugs are available and licensed for use in Mediterranean marine fish. Furthermore, the doses of these drugs have only been determined for cold water species –mainly salmonids- therefore there is no data on efficacy or toxicity in Mediterranean fish.

To date the main drugs that have been used against isopods of the family Cymothoidae contain organophosphate substances such as trichlorphon (100ppm for 5min) , methyl isoxathion (1ppm for 1hr) and dichlorvos (1.5 - 2ppm depending on temperature) [Hatai and Yasumoto, 1980, Hatai and Yasumoto, 1982]. Pyrethrins and in particular cypromethin & deltamethrin are a new drug category that have shown good results against adult and pre-adult L. salmonis in salmon but their action against calimus stages is variable. The usual dose in salmon is 5ppb for 30min and their effect is detectable after 7 days of treatment. The dose of deltamethrin in the present study was proved very effective in shorter exposure time and a ta similar dose to that used for salmon against L.salmonis. The in vitro experiments were thus encouraging, but in order to assess the toxicity of this drug in Mediterranean fish it was necessary to carry out experimental treatments in infected sea bass; this will be published soon.

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Table 2. mortality of parasites following 30 minutes exposure to alphamax (n=15, 3 replicates). *d = dead, a = alive, m = moribund. Temp = 23°C

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<td>0.05 mg/l</td>
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<td>3</td>
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Table 3. mortality of parasites following 30 minutes exposure to alphamax (n=15, 3 replicates). *d = dead, a = alive, m = moribund. Temp = 23°C
References


