The effect of Trichlorfon on the control of
*Lernanthropus kroyeri* (van Beneden, 1851)
(Lernanthropidae) infestations in Cultured Sea Bass, *Dicentrarchus labrax* (Linnaeus, 1758)

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**Abstract**

Sea bass, *Dicentrarchus labrax* naturally infected with *L. kroyeri* were exposed to dosage of trichlorfon for 7 days: 0 (as control), 50 mg kg⁻¹. On days 0, 7, 14, 21, 28 and 35 of the experiment, fish were randomly sampled and weighed, and then parasites on the gills were counted. Comparisons were made to untreated control fish. Sea water temperature and salinity in the pens were measured as 16.0-16.5 ºC and 35 ppt, respectively. When the study began, all of the examined fish were infected with *L. kroyeri*. There were no statistical differences (*P > 0.05*) in terms of mean number of *L. kroyeri* within each group, including the control. According to the IRR coefficients, 50 mg dose of trichlorfon reduce the males at 98.3%, females at 97.1% and the total 97.1% when compared to the control group. No mortality was found and adverse drug reactions or palatability problems were not associated with the treatments during the study.

**Introduction**

*Lernanthropus* is the most common genus of parasitic copepods. So far, more than 100 species isolated from gills of different marine teleosts have been described. Some species of *Lernanthropus* are strictly host specific, but many are parasitic on several species of fish within one or several genera (Kabata, 1979; Oliver and van Niekerk, 1995; Timi and Etchegoin, 1996; Lugue and Paraguas, 2003; Sharp et al., 2003). *Lernanthropus kroyeri* attaches with their third pair of legs to the gill filaments of farmed sea bass (*Dicentrarchus labrax* L.) in Mediterranean region. They elicit severe pathological effects with their cephalic extremities (Manera and Dezfuli, 2003; Toksen, 2007). The principal chemotherapeutic agents include dichlorvos, azamethiphos, hydrogen peroxide, cypermethrin and deltamethrin, currently are administered as bath treatments to control copepod parasites under commercial conditions in various countries. Emamectin benzoate, diflubenzuron and teflubenzuron are administered orally against to different copepod parasites (Roth, 2000). Emamectin benzoate and teflubenzuron were administered successfully against to *L. kroyeri* in a Turkish sea bass mariculture enterprise (Toksen et al., 2006; Toksen et al., 2009).

The purpose of the present study was to

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evaluate the efficacy of trichlorfon towards infestations of *Lernanthropus kroyeri* under commercial conditions.

**Materials and methods**
The trials were conducted in March-May of 2006 at a commercial sea bass farm in Izmir, Turkey. Four pens, each with a volume of 50 m³ (1 control and 3 treatments), were stocked with 1200 sea bass weighing 80-100 g (mean weight: 92 ± 1 g SD) (each pen included 240 fish). Parasite enumeration was performed by removing 20 fish from each group for each counting. During the study, water temperatures were recorded at 2 m depth, daily. Salinity was assumed to remain stable throughout the trial period.

Sea bass naturally infected with *L. kroyeri* were exposed to dosage of trichlorfon for 7 days: 0 (as control), 50 mg kg⁻¹. A commercial sea bass pellet was coated with trichlorfon. All of the fish except the fish of control group were fed this medicated feed for a period of 7 consecutive days (days 0-6) at a rate of 0.8%-1% biomass. On days 0, 7, 14, 21, 28 and 35 of the experiment, fish were sacrificed and weighed, and then parasites on the gills were counted. All attached parasites were removed and fixed in 5% formalin. Fish was examined individually using a low power microscope and the mean quantities of parasites were calculated.

In this study negative binomial regression was used since the numbers of parasite are the dependent variable. All the models in the study were subjected to alpha test to see if the NBR is the correct model for the analysis.

**Results**
During the treatments, abnormal behaviour wasn’t observed and there wasn’t evidence of reduced appetite or palatability problems associated with the medicated feed in the treated cages. Sea water temperature and salinity in the pens were measured as 16.0-16.5 °C and 35 ppt, respectively.

When the study began, all of the examined fish were infected with *L. kroyeri*. There were no statistical differences (P > 0.05) in terms of mean number of *L. kroyeri* within each group, including the control. Afterward, trichlorfon (50 mg) was applied to the groups.

The NBR models were used for analyzing the impact of trichlorfon on the number of male, female and total parasites, each representing dependent variables, are presented in Table 1.

Independent variables in the NBR models are:

- **Trichlorfon (Dose: 50 mg/kg):** The variable representing the dose of trichlorfon, zero for the control group and 10 for the experiment group.
- **Week:** The variable representing the time periods, 1-6 weeks, in which the applications were done.

In Table 1, NBR estimations for the models measuring the effect of trichlorfon on the number of *L. kroyeri* are presented. NBR was used for estimating this relationship as suggested by the Alfa test. All of the models point out that 50 mg dose of trichlorfon has
a decreasing effect on the number of males, females and the total number of parasites since all the parameters in the models were found statistically significant. According to the IRR coefficients, 50 mg dose of trichlorfon reduce the males at 98.3%, females at 97.1% and the total 97.1 when compared to the control group.

**Discussion**

*Lernanthropus kroyeri* (van Beneden, 1851) is a serious copepod pathogen of farmed sea bass (*Dicentrarchus labrax* L.) in Mediterranean region. Sea bass infected with parasite show pale, necrotic gills with a rich mucus production and prominent haemorrhages. Histologically infected gills show erosion, desquamation, vacuolar degeneration of the secondary lamellae and fusion in the distal ends of the secondary lamellae. So, sea bass infected with *L. kroyeri* need to be treated effectively.

Even though some successful results have been reported with bath treatments, immersion treatments of billions of fish are impractical and stressful to the fish. As a result of these limitations, treatments are being developed that can be administered in feed. Some studies carried out with emamectin benzoate (Toksen et al., 2006) and teflubenzuron (Toksen et al., 2009) were effective against larval and mature stages of *L. kroyeri*.

The other groups which use against to copepod parasites are organophosphates. Organophosphates are acetylcholinesterase inhibitors used as insecticides. Organophosphates are used in shrimp hatcheries to control monogenetic trematode infections in shrimps. But the effects on non-target crustaceans are a major concern for all the organophosphates (Gesamp, 1997). LC50 (96 h) values for azinphos-ethyl, chlorpyrifos, diazinon, dichlorvos, malathion and trichlorfon imply that these substances are all highly acute toxic to crustaceans (Baticados and Tendencia, 1991; McHenery et al., 1991;
Dichlorvos is highly acute toxic to fish (McHenery et al., 1991) and trichlorfon and diazinon are slightly to highly acute toxic to fish (Brecken-Folse et al., 1994; Howe et al., 1994; Richardson, 1992).

Trichlorfon was effectively applied orally to sea bass against *Caligus minimus* (Copepoda) (Toksen, 1999). Trichlorfon and dichlorvos were the first chemicals widely used to control sea lice (Brandal and Egidius, 1977; Grave et al., 1991). These bath treatment effectively remove both the pre-adult and adult stages of sea lice, but not the chalimus larvae from salmon (Brandal and Egidius, 1979). Experimentally, Brandal and Egidius (1977) tested the trichlorfon orally and observed blindness in salmon. In this study, trichlorfon was applied for 7 days: 0 (as control), 50 mg kg\(^{-1}\) to commercial sea bass. During the season of rearing, from applying days to harvest, no blindness was observed on the fish.

According to Silva et al., (1993) and Sturm et al. (1999), trichlorfon cause a decrease in growth of the fish weight, appetite loss and motility diminution. But Guimares and Calil (2008) stated that trichlorfon bath applications would not cause any significant difference in length or weight growth in *Oreochromis niloticus*. Same results were related by Ruddle and Zhong (1988) and Ludwig (1993), suggesting that no adverse effects existed in the use of Trichlorfon in aquaculture.

It could be concluded that the trichlorfon treatments against parasitosis would not cause adverse effects in sea bass. These trials demonstrated that trichlorfon an effective treatment for infestations of *L. kroyeri* at dose 50 mg kg\(^{-1}\). The application of such organophosphates must not be used indiscriminately, since other important effects occur, for example the inhibition of the acetylcholinesterase enzyme. So, further investigations are still needed to determine the exact amounts, treatment durations, and efficacy of the chemicals used to treat *L. kroyeri* in sea bass in consideration of EU legislation on trichlorfon use.

Acknowledgments

The authors wish to thank Dost Sea Products in Izmir, Turkey for supporting the present study.

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