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(Accepted 18 November 1993)

Journal of TFRI TAIWAN 1993 Fisheries Research

Effects of Temperature on the Toxicity of Masoten to Larval Black Sea Bream, *Acanthopagrus schlegeli*

Abstract

Heavy mortalities of black sea bream have occurred from time to time during or after a routine organophosphate (mainly Masoten) treatment against ectoparasites. The purpose of this study was to determine the effects of temperature on the toxicity of Masoten to black sea bream to avoid mass fish kills due to overdosage. A total of 810 larval black sea bream, *Acanthopagrus schlegeli*, with a mean weight of 0.78 g were divided into 9 experimental groups and each treatment was triplicated. Each experimental group was randomly assigned to a 3 temperatures (22, 26 and 30°C) × 9 Masoten concentrations (0, 0.12, 0.21, 0.36, 0.62, 1.07, 1.85, 3.2 and 5.54 ppm) factorial design. Fish surviving 24–h and 48–h after exposure were studied. The results showed that the toxicity of Masoten to the larval fish increased significantly ($p<0.0001$) with increasing water temperature. The 24–h TLm of the larvae to Masoten was 2.34 ppm at a water temperature of 22°C, 1.43 ppm at 26°C and 0.57 ppm at 30°C. Their 48–h TLm were 1.31 ppm (22°C), 0.80 ppm (26°C) and 0.43 ppm (30°C). Both 24–h and 48–h TLm increased 3～4 fold when the water temperature was increased from 22 to 30°C.

Key words: Temperature, Toxicity, Masoten, Black Sea Bream

Ectoparasites, such as fish louse (*Argulus* spp.), anchor worm (*Lernea cyprinacea*) and skin or gill flukes (*Dactylogyrus* spp.), when present in large numbers, cause serious health problems to the farming of the black sea bream in Taiwan. Bathing the infested fish in organophosphate solution has been the most common practice of fish farmers in treating the diseases. Four of the most important phosphorothioates used are Dipterex (*O*, *O*–dimethyl 2,2,2–trichloro–1–hydroxyethylphosphonate), Parathion (*O*, *O*–dimethyl *O*–*p*–nitrophenyl phosphorothioate), Neguvon (2,2,2–Trichloro–1–Hydroxyethylphosphonic acid dimethyl easter) and Masoten (Dimethylester of (2,2,2–Trichloro–1–Hydroethyl)–Phosphonic acid). Dipterex and parathion are insecticides which were originally designed for plant use and are more toxic to fish than Masoten, which is manufactured specially for animal use.

The active component in Neguvon and Masoten is trichlorfon, a white crystalline powder without objectionable odor which degrades in water to dichlorvos (DDVP). DDVP is more toxic to fish than trichlorfon[1]. Water temperature increment is known to

Chang, C. F., M. S. Su and I C. Liao (1993) Effects of temperature on the toxicity of Masoten to larval black sea bream, *Acanthopagrus schlegeli*. J. Taiwan Fish. Res., 1(2): 31–37.

enhance the degradation of trichlorfon and thus the formation of DDVP[2]. The recommended dose of Masoten is 0.2～0.4 ppm for 24-h. Several cases of mass mortality of fish, however, have occurred during or after treatment in fish farms in Taiwan. The mortality could be linked to the increased toxicity of organophosphates at high water temperature. In Norway, salmon infested by salmon louse (*Lepeophtheirus salmonis*) are commonly bathed in 300 ppm Neguvon for 15 ～ 60 min. The treatment time is decided by water temperature[3]. The purpose of this study was to determine the effects of temperature on the toxicity of Masoten to black sea bream. This data is badly needed in preventing mass fish kills due to overdosage during treatment.

Materials and Methods

Experimental fish

Black sea bream, *Acanthopagrus schlegeli*, larvae from a naturally spawning female were obtained from the hatchery and nursery facility of the Tungkang Marine Laboratory, Tungkang, Taiwan. They were acclimated for 10 days at 25°C and 30 ppt salinity in a recirculating system. The fish were not fed one day before the experiment. A total of 810 fish larvae with a mean weight of 0.78 ± 0.04 g and body length of 1.5 ～ 2.5 cm were divided into 27 groups. Each treatment had 3 replicates. Each fish group was housed in a 1000 ml glass beaker containing 800 ml fresh seawater. Beakers were placed in one of the three incubators. The temperatures of the incubators (± 0.5°C) were 22, 26 and 30°C respectively. The toxicity test began 8 h after incubation.

Toxicity test

Procedures of the toxicity test followed the method of Stephan[4], with a slight modification. Masoten (Bayer Chemie, Leverkusen, Germany), containing 80% of Dimethylester of (2,2,2-Trichloro-1-Hydroethyl)-Phosphonic acid, was dispersed in seawater to make a 1000 ppm stock solution. The stock solution was added to the beakers to make graded concentrations of Masoten (0, 0.12, 0.21, 0.36, 0.62, 1.07, 1.85, 3.2 and 5.54 ppm). These test concentrations were established in a preliminary study which indicated an incipient lethal concentration of 5.54 ppm and an incipient sublethal concentration of 0.12 ppm. Aeration was provided in each beaker. Dissolved oxygen and pH were monitored once every 12 h. Dissolved oxygen concentration ranged between 6.5 ～ 6.8 ppm and pH ranged between 8.0～8.2 throughout the experiment. These two parameters were not detrimental to the fish. Fish mortality was determined once every 12 h by the response of the fish to a gentle touch in the opercle with a glass stick. Those fish that failed to show any movement were declared dead and were removed. Each toxicity test lasted for 48 h. All the surviving fish were preserved in 10% formalin fixative. Routine histological examinations of gill, liver, muscle and kidney of the preserved fish were conducted to determine the pathological effects of the pesticide on the fish.

Statistical analysis

Medium tolerance limit (TLm) was calculated by the method of Van Der Waerden. Temperature effects were examined using ANOVA of the TLm data. Differences between treatments were determined using the Duncan's New Multiple Range Test (P=0.05).

Results

The 24- and 48-h mortality rates of larval black sea bream exposed to Masoten at 3 temperatures were shown in Figs. 1 and 2, respectively. Exposure to high Masoten concentration significantly increased fish mortality ($P<0.0001$). The toxicity effects of Masoten increased with increasing water temperature. Fish during the early stages of exposure appeared to be unstable and swam restlessly, shuddered, cramped and died off after prolonged exposure. Total mortality

occurred when the larvae were exposed to concentrations higher than 1.07 ppm at 30°C; 3.2 ppm at 26°C and 5.54 ppm at 22°C for 24 h (Fig. 1). Similarly, total mortality was observed 48 h after the fish were exposed to 1.07 ppm (30°C), 1.85 ppm (26°C) and 3.2 ppm (22°C) (Fig. 2).

The 24-h TLm of the larvae to Masoten was 2.34 ppm at a water temperature of 22°C (Fig. 3), 1.43 ppm at 26°C and 0.57 ppm at 30°C. Their 48-h TLm were 1.31 ppm (22°C), 0.80 ppm (26°C) and 0.43 ppm (30°C). The toxicity of Masoten to larval black sea bream, therefore, was approximately 3 ~ 4 fold when the water temperature increased from 22°C to 30°C.

Regression analysis indicated that TLm of the fish to Masoten decreased linearly with increased water temperature (Fig. 3) Toxicity of Masoten to black sea bream larvae increased significantly with increased water temperature.

Histological examinations of the fish after 48-h exposure did not result in any noticeable pathological changes in the tissues examined.

Discussion

The organophosphate Masoten is easily soluble in water. In aqueous solutions its active compound, trichlorfon, slowly transforms to DDVP which is a potent cholineserase inhibitor[5]. DDVP acts as a nerve poison by blocking synaptic transmission in the cholinergic parts of the nervous system of invertebrates[6]. It also inhibits the acetylcolinesterase activity of the cholinergic nervous system in brain and muscle of the rainbow trout and Atlantic salmon[7]. The early symptoms of the toxic effects in man, which usually occur between 2 and 8 h after exposure, include anorexia, abdominal cramps, anxiety and tremulousness[8]; Later symptoms include muscular twitching and convulsions and respiratory difficulty which may result in death in as little as one

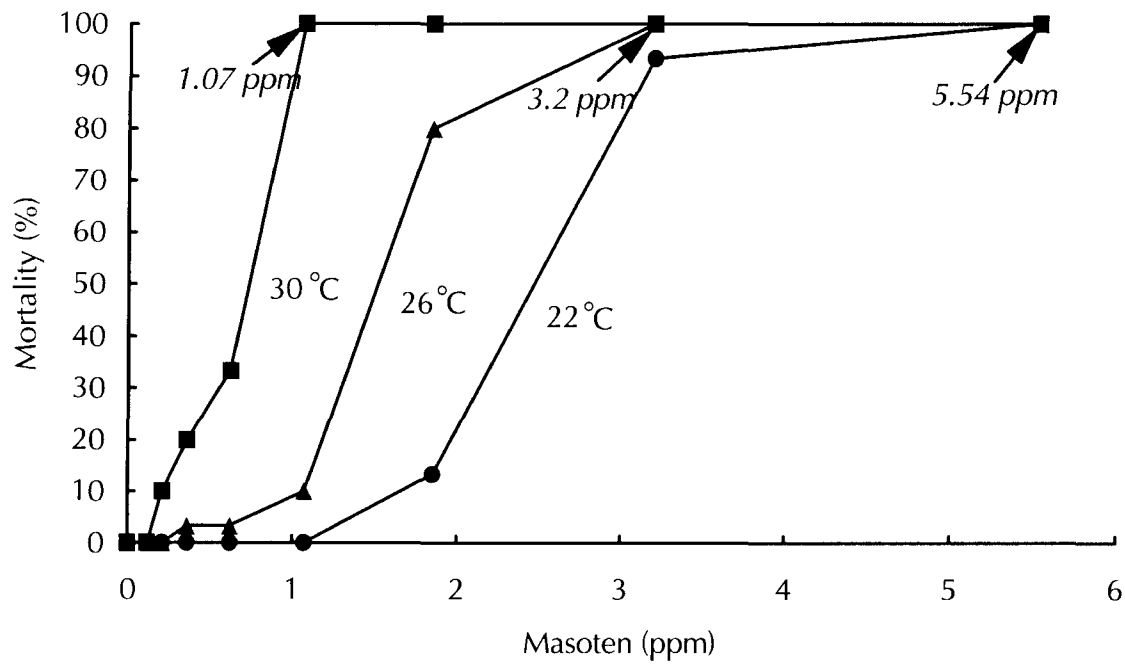

Fig. 1. The 24-h mortality rates of larval back sea bream, *Acanthopagrus schlegeli*, exposed to various concentrations of Masoten at 22, 26 and 30°C.

hour after the onset of symptoms. The present study shows that the fish appeared to be unstable and swam restlessly after being exposed to 3.2 ppm at 26°C for 5 h. The affected fish then shuddered, cramped and died off after prolonged exposure.

Samuelsen[2,9] reported that the transformation rate of trichlorfon (Neguvov) to DDVP in seawater increased linearly with water temperature when temperature was increased from 4.5°C to 13.5°C. In the present study, the Masoten concentration causing total mortality of the fish after 24–h exposure at 22°C was approximately five times that at 30°C. Similarly, the concentration difference of the 24–h TLm between 22°C to 30°C was four times. The half life of Trichlorfon and DDVP decreased when pH was increased from 7.5 to 8.0 under high aeration. The decrease of the half life is more significant at 13.5°C than at 4.5°C[2]. It is, therefore, very possible that the concentration of Masoten (or DDVP) would be reduced rapidly at high water temperature tested (30° C) in the present study. The Masoten concentration at 22°C causing total mortality within 48–h exposure was approximately 3 fold that at 30°C. Similarly, the concentration difference of the 48–h TLm between 22°C to 30°C was 2.94 fold.

Suchnick et al.[10] reported that 0.25 ppm Masoten could be used against anchorworms, lice, and gill flukes on goldfish and bait fish fishery, but treatment time was not suggested. Cusack and Johnson[11] demonstrated that marine zooplankton population declined significantly when DDVP concentration in the culture water was 1.0 ppm. Generally, the safety concentration of the drug is one tenth of its TLm[12]. Accordingly, the 24–h safety concentration of Masoten to the black sea bream larvae should be 0.234 ppm at 22°C, 0.143 ppm at 26°C, and 0.057 ppm at 30°C. Their 48–h safe concentration are 0.131 ppm (22°C), 0.08 ppm (26°C), and 0.043 ppm (30°C), respectively. Lin and Ting[13] reported that the safety concentration of Masoten to milkfish (*Chanos chanos*) is 3 ppm (24–h) and 2.9 ppm (48–hr) at 30 ± 1°C. Lin et al.[14,15] also reported that the 48–h safety

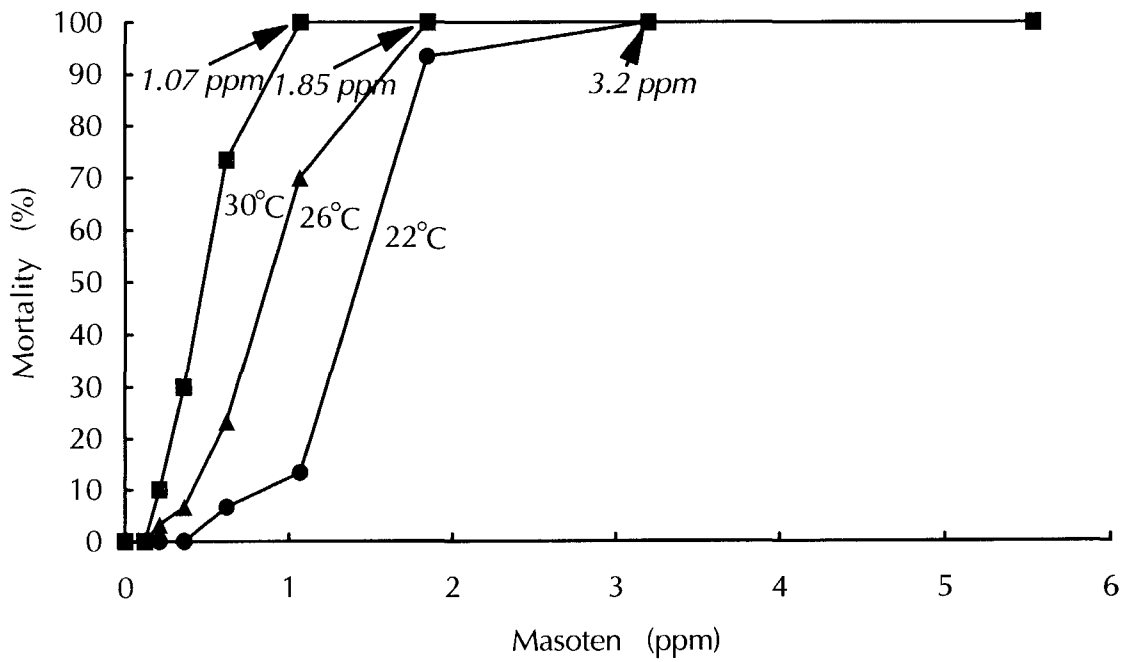

Fig. 2. The 48–h mortality rates of larval black sea bream, *Acanthopagrus schlegeli*, exposed to various concentrations of Masoten at 22, 26 and 30°C.

concentration for seabass (*Lateolabrax japonicus*) is 0.3 ppm at 27～34.5°C, and 0.135 ppm at 17.5～23°C for grouper (*Epiephelus salmoides*). Milkfish is thus more tolerant to Masoten than seabass, grouper and black sea bream. The black sea bream, on the other hand, showed the highest sensitivity to Masoten. In Taiwan, the generally recommended dose of Masoten for both marine and freshwater fish was 0.2～0.4 ppm for 24-h. This suggested dosage will cause mortality of the black sea bream larvae when water temperature is higher than 26°C. It is strongly recommended that when Masoten is used in a tropical climate to control parasites, predators or zooplankton, water temperature along with pH and aeration, are the key factors which must be considered in order to avoid mass fish kills due to overdosage. A concentration lower than 0.2 ～ 0.4 ppm is preferred when water temperature is higher than 26°C.

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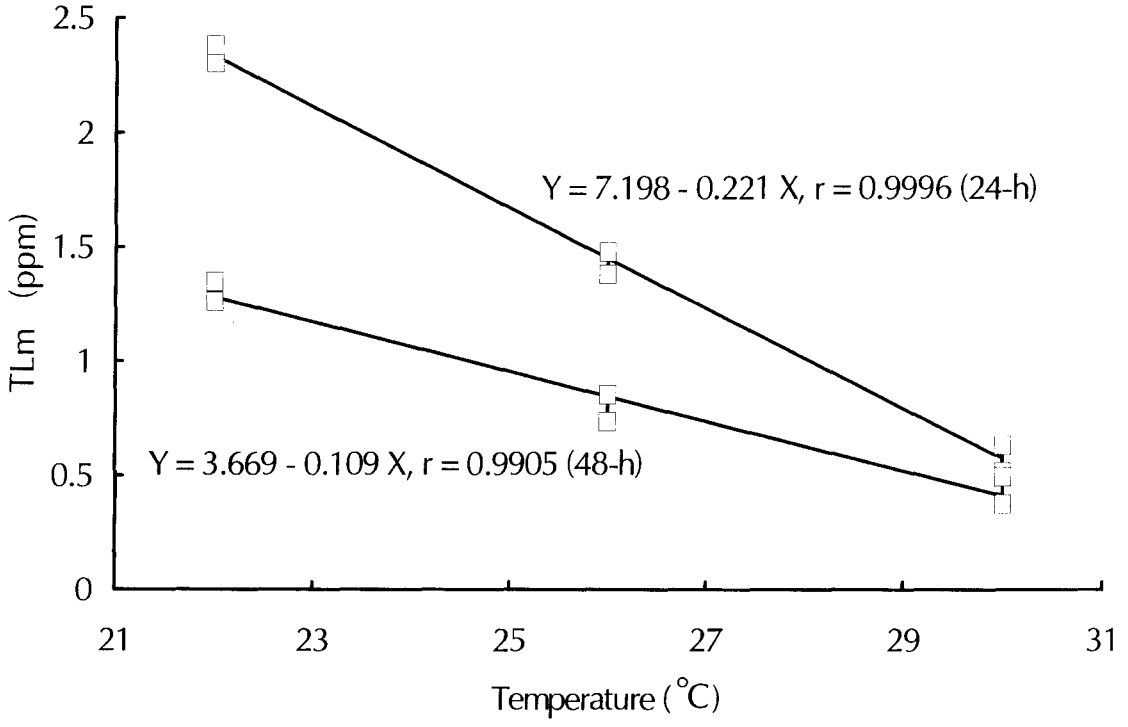

Fig. 3. The effect of water temperature on 24 and 48–h medium tolerance limit (TLm) of black sea bream, *Acanthopagrus schlegeli*, exposed to Masoten. Vertical bar indicates the 95% limit of dependence.

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(1993 年 11 月 18 日 接受)

Journal of TFRI TAIWAN 1993 Fisheries Research

不同溫度下馬速展對黑鯛幼苗的毒性研究

摘 要

馬速展 (Masoten) 在治療黑鯛體外寄生蟲感染症時，雖施用相同之安全劑量，但有時還會引起大量死亡。本試驗之目的在於探討不同溫度下馬速展對黑鯛幼苗之毒性，以建立本藥物使用之安全方法。將 810 尾黑鯛幼苗，平均體重爲 0.78 g，分成三種溫度 (22, 26, 30°C)×九種藥物濃度 (0, 0.12, 0.21, 0.36, 0.62, 1.07, 1.85, 3.2, 5.54 ppm) 之27試驗組，每組三重覆。試驗結果顯示，24小時之半數致死濃度 22°C 爲 2.34 ppm，26°C 爲 1.43 ppm，30°C 爲 0.57 ppm；48小時之半數致死濃度22°C爲 1.31 ppm，26°C爲 0.80 ppm，30°C爲 0.43 ppm。溫度越高馬速展對黑鯛幼苗的毒性越強 ($P<0.0001$)，當水溫由22°C上升至30°C時，馬速展對黑鯛幼苗的毒性增強 3～4 倍。

關鍵字：溫度，毒性，馬速展，黑鯛

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