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Vertebral Deformity Susceptibilities of Marine Fishes Exposed to Herbicide

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We often measure LC_{50} values (median lethal concentrations) of chemicals to evaluate their acute toxicities on fish. Some chemicals, however, pesticides, e.g., cause vertebral deformities at less than their LC_{50}'s (Baba *et al.* 1974, 1977a, b). We therefore sometimes cannot evaluate the acute toxicities of chemicals on vertebral deformity at less than their LC_{50}'s. Once a fish has a vertebral deformity, it can be assumed that the fish will die or will be more susceptible to predation by predators. Thus, we need to evaluate the acute toxicities of chemicals on fish vertebral deformity as well.

Many substances, e.g., trifluralin (Couch *et al.* 1979; Wells and Cowan 1982), malathion (MacCann and Jasper 1972; Weis and Weis 1976) certain heavy metals (Koyama and Itazawa 1977; Bengtsson 1975) and chlorinated hydrocarbons (Bengtsson 1975) have been known to cause vertebral deformities in freshwater fish. Furthermore, some marine fish exhibit higher susceptibilities to certain pesticides (Hirose and Kitsukawa 1976; Baba *et al.* 1974) and certain chemicals (Dawson *et al.* 19750977). However, there is little information on vertebral deformities caused by chemicals in marine fish. Thus there is a need to evaluate the acute toxicity of chemicals on vertebral deformity of marine fish.

At first, it is necessary to determine which marine fish are suitable for evaluating the acute toxicities of chemicals on vertebral deformities. In the present study, 10 marine fish species were examined for their susceptibility to vertebral deformity by exposing them to acute concentrations of chemicals to determine whether vertebral deformity resulted and which marine fish are suitable for the evaluation of acute toxicities of chemicals on vertebral deformities. In the present study, vertebral deformities were defined as a fracture or dislocation caused by the herbicide trifluralin, which was sometimes detected in seawater and sediment (Readman *et al.* 1993). This herbicide was selected for the present study because it has been documented as a chemical causing vertebral deformities (Couch *et al.* 1979; Wells and Cowan 1982).

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MATERIALS AND METHODS

In the present study, the vertebral deformity susceptibilities of 10 marine fish species were examined. Larvae of each fish species were used because of their higher susceptibilities to the toxicity of chemicals than young or adult fish. These marine fish species were Japanese flounder (*Paralichthys olivaceus*), black sea bream (*Acanthopagrus shlegeli*), red sea bream (*Pagrus major*), herring (*Clupea pallasi*), jacopever (*Sebastes schlegeli*), yellowtail (*Seriola quinqueradiata*), longchin goby (*Chasmichthys dolichognathus*), girella (*Girella punctata*), mullet (*Mugil cephalus*) and grunt (*Parapristipoma trilineatum*). Japanese flounder, black sea bream and red sea bream were obtained from the Kanagawa Prefectural Aquacultural Center located in central Japan. Herring and jacopever were obtained from the Japanese Association of Sea Farming, Miyako Branch located in northeastern part of Japan, Yellowtail, longchin goby, girella, mullet and grunt were collected at the shore near our laboratory located in central Japan. The mean standard lengths and body weights are shown in Table 1. The fish were acclimated to the experimental water temperature in 60-L glass aquaria filled with filtered seawater for 1 wk. During acclimation, seawater was aerated to keep the oxygen saturation level above 60%. About 10-L of seawater in the aquaria were changed to remove feces and remaining food every day. Dissolved oxygen concentration and salinity of the seawater were measured at the start and end of acclimation and were similar to the test solution.

Table 1. Water temperatures, the ranges of trifluralin concentration and the sizes of fish used.

Species	Water temp. mean (℃)	Standard length mean (cm)	Body weight mean (g)	Trifluralin conc. range (mg/L)
Yellowtail	24.2	3.1	0.45	0.005-0.071
Japanese flounder	24.5	4.0	1.02	0.02 -0.076
Black sea bream	24.5	3.6	1.76	0.007-0.056
Longchin goby	24.3	3.7	1.05	0.012-0.23
Girella	24.4	4.4	2.69	0.023-0.21
Red sea bream (small)	25.8	1.9	0.20	0.023-0.21
(medium)	25.6	3.0	0.72	0.008-0.136
(large)	24.7	3.4	1.11	0.006-0.138
Mullet	24.8	4.2	1.63	0.003-0.049
Grunt	24.4	5.7	3.15	0.01 -0.131
Herring	16.4	4.5	0.67	0.005-0.159
Jacopever	19.2	2.5	0.33	0.012-0.074

Being warm water fish, most of the experimental water temperatures were about 25 °C , except for herring and jacopever. The experimental water temperatures for herring and jacopever were 16°C and 19 °C respectively, as they are cold water fish. Glass aquaria were filled with test solutions and the aquaria were placed in a water bath to maintain a constant temperature.

Dissolved oxygen concentrations and salinities of the test solutions were measured every day.

After acclimation, 6 to 10 fish per aquarium were exposed to the test solution containing trifluralin for 96 hr. Because there were not sufficient number of fish, we used six yellowtail, girella, small red sea bream, grunt and jacopever per concentration, and seven Japanese flounder, black sea bream, large red sea bream and herring per concentration, and eight longchin goby and mullet per concentration, and ten medium sized red sea bream per concentration. While the volume of most of the test solutions were 20-L, 5-L were used for the small red sea bream and jacopever because of their smaller size and number.

Trifluralin(α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl- *p*-toluidine) was obtained commercially from Sionogi Chem. Ind.Ltd. Trifluralin was provided as an emulsifiable-concentrate and contained solvent and emulsifier 55%. The stock solution of 1000 mg/L of trifluralin was prepared. The test solutions were prepared by adding the stock solution of trifluralin to filtered seawater. From five to seven concentrations of trifluralin solutions were prepared for each fish species. The ranges of trifluralin concentrations are shown in Table 1. Because of natural decreases in dissolved oxygen and trifluralin concentrations, the test solutions were renewed every day. After extraction with hexane from the test solutions, the hexane extractions were analyzed by gas chromatography (Shims GC-5A) equipped with a flame ionized detector using a 3m x 3mm i.d. glass column packed with 5%OV-1. The details of analysis method were similar to those of Spacie and Hamelink (1979) except for condition of GC. The concentrations of trilfluralin were measured four times, immediately before and after renewing the test solutions on the second and third day. The trifluralin concentrations were shown as the mean.

During and after exposure, the dead and surviving fish were fixed with 5% formaldehyde-seawater and then lateral and dorsal views were X-rayed to examine the vertebral deformities. After exposure for 96 hr, LC_{50} of trifluralin was calculated using probit or graphic methods (APHA 1989) to compare with the concentrations causing vertebral deformities. The difference between the means was tested by the Student's t-test.

RESULTS AND DISCUSSION

The temperatures of the test solutions did not fluctuate 1 °C from the designated water temperatures. Salinities of the test solutions ranged from 29.9-33.8%0 Dissolved oxygen concentrations were above 4.1mg/l and the degrees of saturation were above 60%.

Some fish exposed to trifluralin exhibited the typical deformities of the vertebral column and these deformities seem to be fractures or dislocations. There were no vertebral deformities in the control fish. In girella, mullet and herring, most

of the fish with vertebral deformities showed convulsion, and had lateral stripes on their bodies caused by hemorrhage as well.

From the present experiments, we determined the 96-hr LC_{50} values (median lethal concentration) and no mortality concentrations of trifluralin to evaluate the acute lethal toxicity and to compare with the effective concentrations on vertebral deformities. Because all of the yellowtail and herring died at 0.005mg/L which was the lowest concentration, they exhibited the lowest 96-hr LC_{50}'s, <0.005mg/L. Girella and longchin goby exhibited the highest 96-hr LC_{50}'s, 0.11 and 0.12mg/L respectively. Yellowtail and herring exhibited the lowest no mortality concentrations, <0.005mg/L, and girella exhibited the highest no mortality concentration, 0.061 mg/L.

The no observed deformity concentration (NODC) and the lowest observed deformity concentration (LODC) of trifluralin were also determined to evaluate the effect on vertebral deformities (Table 2). NODC is the highest concentration at which the deformities were not observed. LODC is the lowest concentration at which the deformities were observed. Yellowtail and jacopever did not have vertebral deformities even with exposure to trifluralin at the highest concentrations, 0.071 and 0.074mg/L respectively. Therefore, they exhibited the highest LODCs, >0.071 and >0.074mg/L. Mullet and red sea bream (large size) exhibited the lowest LODCs, 0.005 and <0.006mg/L respectively. While mullet exhibited the lowest NODC (0.0003mg/L), yellowtail and jacopever exhibited the highest NODCs (>0.071 and 0.074mg/L). NODCs and LODCs of yellowtail and jacopever could not be compared with their 96-hr LC_{50}'s because of no deformity. While most of NODCs were less than 50% of their 96-hr LC_{50}'s, NODC of herring was 1.8 times higher than their 96-hr LC_{50}. While most of LODCs were less than 73% of their 96-hr LC_{50}'s, LODC of herring was 2.6 times higher than their 96-hr LC_{50}.

At first, a kind of deformity rate (deformity rate A), which was the deformity rate observed below no mortality concentration, was calculated. This deformity rate was calculated from the number of live fish below the no mortality concentration. However, from that calculation the deformity rates of red sea bream (medium sized), grunt and herring became zero, while they had vertebral deformities above LODC. Thus, we determined another kind of deformity rate (deformity rate B) which was the deformity rate observed above LODC and calculated from the number of live and dead fish which had vertebral deformities above LODC. Deformity rates A and B ranged from 0 to 50% and from 14 to 82%, respectively (Table 2). Without vertebral deformities with exposure to trifluralin above LODC and below the no mortality concentration, yellowtail and jacopever's deformity rates A and B could not be determined. While medium sized red sea bream and herring's deformity rates B could be determined, their deformity rates A could not be determined because of impossibility of determining no mortality concentration. Red sea bream (small size) and girella exhibited the highest deformity rates A, 50 and 48%, and girella exhibited the highest deformity rate B, 82%.

Table 2. Acute effective concentrations of trifluralin on vertebral deformity and 96-hr LC_{50}

Species	NODC	LODC	Deformity rate A*	Deformity rate B**	No mortality conc.	96-hr LC_{50}
	(mg/L)	(mg/L)	(%)	(%)	(mg/L)	(mg/L)
Yellowtail	>0.071	>0.071	ND***	ND	< 0.005	< 0.005
Japanese flounder	0.02	0.03	11	33	0.03	0.056
Black sea bream	0.007	0.019	38	75	0.024	>0.056
Longchin goby	0.012	0.023	10	33	0.042	0.12
Girella	0.023	0.031	48	82	0.061	0.11
Red sea bream (small)	< 0.013	< 0.013	50	50	0.013	0.026
(medium)	0.008	0.016	0	50	< 0.008	0.022
(large)	< 0.006	< 0.006	7	26	0.006	0.021
Mullet	0.003	0.005	25	55	0.016	0.032
Grunt	0.012	0.019	0	38	0.011	0.033
Herring	0.009	0.013	0	14	< 0.005	< 0.005
Jacopever	>0.074	>0.074	ND	ND	< 0.012	>0.074

*: Deformity rate below no mortality conc, **:Deformity rate above LODC, ***: Not determined

Figure 1 shows the relationship between the number of vertebrae and deformity rates A and B. The fish species having vertebrae numbering less than 27 tended to exhibit higher deformity rates compared with fish species having vertebrae numbering more than 30. The means and standard deviations of deformity rates A and B of fish species having vertebrae numbering less than 27 were 24±2 1.9% (n=7) and 53.7±19.6% (n=7). The means and standard deviations of deformity rates A and B of fish species having vertebrae numbering more than 30 were 7.0±6.1% (n=3) and 26.7±11.0% (n=3). Although there was no significant difference between the deformity rate A of the fish having vertebrae numbering more than 30 and less than 27, there was significant difference (P<5%) between the deformity rate B.

Because the vertebral number varied from 24 to 54 among the fish species used in the experiment, the positions of vertebral deformities could not be compared among fish species by the order of vertebrae. Thus, the locations of vertebral deformities were compared in relation to their position along the length of vertebral column and are shown in Figure 2. Mullet and herring had vertebral deformities at various positions from 10 to 100% of the total vertebral column. Japanese flounder, longchin goby, grunt, red and black sea bream and girella had deformities from 30 to 60% of the total vertebral column.

In the present study, the lowest observed deformity concentrations (LODC) of trifluralin were lower than the 96-hr LC_{50}'s except for yellowtail, herring and jacopever (Table 2). As shown by Hirose and Kitsukawa (1976) and Baba et *al.* (1974, 1977a, 1977b) girella, longchin goby and yellowtail, exposed to some organic phosphorus or carbamate insecticides, had vertebral deformities at concentrations lower than LC_{50}. These results suggest that the LC50 is not

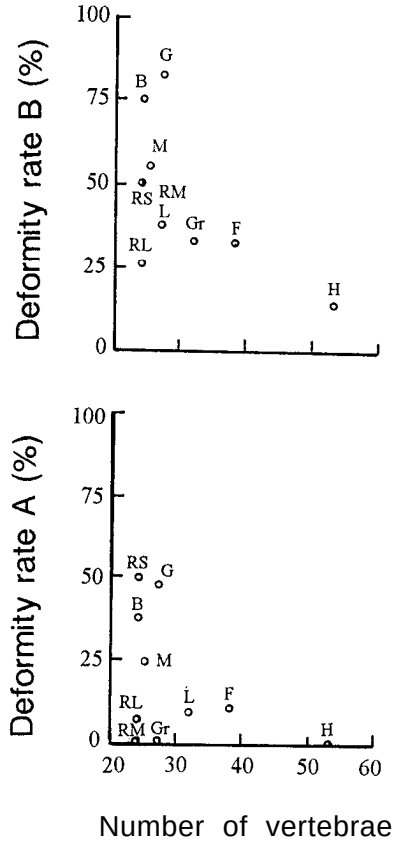

Figure 1. The relationship between number of vertebrae and deformity rates.

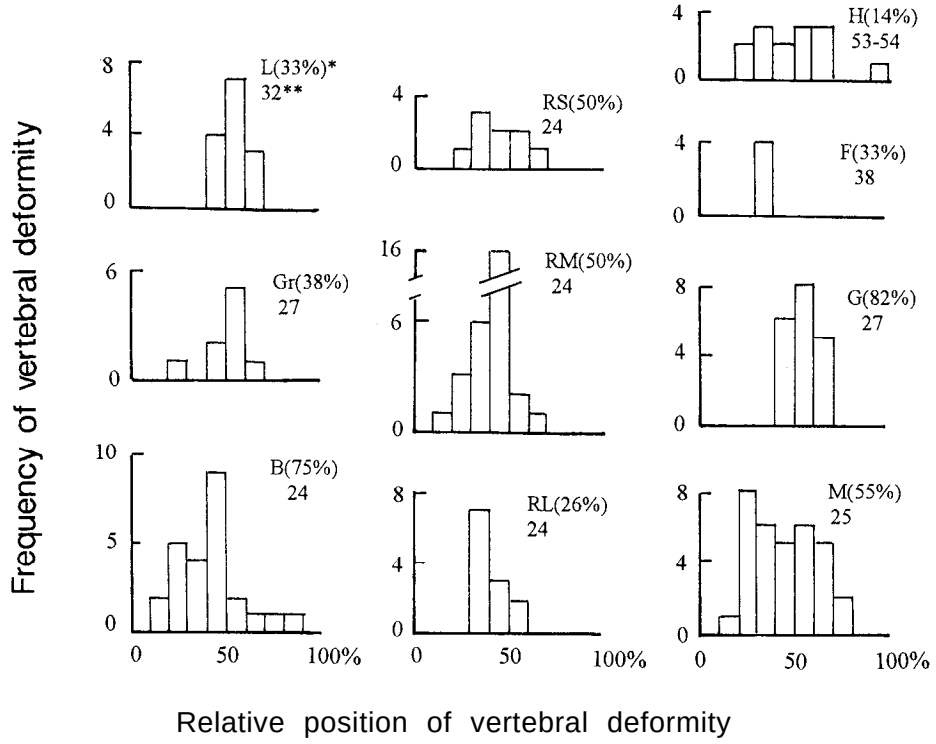

Figure 2. The relationship between relative positions of vertebral deformities and frequency of vertebral deformities.
*: deformity rate B, * *: the number of vertebrae
H: herring, F: Japanese flounder, L: longchin goby, Gr: grunt,B: black sea bream, G: girella, M:mullet, R: red sea bream(S: small size, M: medium size, L: large size)

a satisfactory indicator for evaluating the acute toxicities of chemicals on vertebral deformity of marine fish. Therefore, the LODC and NODC of chemicals to marine fish should be determined separately from the LC50. However, nobody has examined which fish species are suitable for evaluating the acute toxicity of chemicals on vertebral deformity. Fish species suitable for evaluating the toxicity of chemicals have to exhibit higher deformity rates at lower concentrations of chemicals. Girella, red and black sea bream and mullet exhibited higher deformity rates. Mullet, red and black sea bream and grunt exhibited lower LODCs and mullet, red and black sea bream and herring exhibited lower NODCs. Because girella did not exhibited lower LODC and NODC, and because grunt and herring did not exhibit higher deformity rates, they were not suitable fish species for evaluating the acute toxicity of chemicals on vertebral deformity. Mullet, red and black sea bream exhibited higher deformity rates and lower LODCs and NODCs, and they were suitable fish species for evaluating the toxicity.

While the fish species having vertebrae numbering less than 27 exhibited significantly higher deformity rates than the fish species having vertebrae numbering more than 30 (Figure 1), these results suggest that the number of vertebrae is one of the important factors causing vertebral deformity. As Aleev (1963) pointed out, fish having a shorter trunk and fewer myotomes are less flexible than fish having a larger number of vertebrae. The number of myotomes correspond with the number of vertebrae. Mullet, red and black sea bream having smaller number of vertebrae seem to be less flexible and are likely to have vertebral deformity at higher deformity rates.

As shown in Figure 2, mullet and herring which have intermediate body forms between anguilliform and fusiform, had vertebral deformities in various positions of the vertebral column. On the other hand, Japanese flounder, which have depressiform, longchin goby and grunt, which have tetraodontiform, girella, red and black sea bream, which have fusiform, had vertebral deformities in the central part of the vertebral column, Especially, girella, red and black sea bream had vertebral deformities from 40 to 60% in relation to their position along the length of vertebral column and exhibited higher deformity rates as well. Hirose and Kitsukawa (1976) and Baba *et al.* (1974, 1977a, 1977b) reported that vertebral deformities were caused in the central part of girella and yellowtail exposed to diazinone or other pesticides. According to their results and the results of the present study, the fish species having a smaller number of vertebrae, especially less than 27, and having fusiform shape seemed to have vertebral deformities at a higher rate and in the central part of their vertebral column. Thus red and black sea bream and mullet seem to have higher susceptibility of vertebral deformity.

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