CHAPTER IV

RESULTS AND DISCUSSION

Acute toxicity tests of TBTO on juvenile sea bass (*Lates calcalifer*)

Average percent mortality of sea bass (*L. calcalifer*) exposed to TBTO over 96-h period is presented in Table 4.1. During the bioassay, sea bass died within 24 hours after exposed to TBTO. All doses used in the experiment were able to kill the sea bass within 24 h. On the other hand, mortal fish was not observed in the control at all. The median lethal concentration (LC50) values for the toxicity test on *L. calcalifer* are shown in Table 4.2. The interpretations of 24-h, 48-h, 72-h, and 96-h values by Probit analysis were summarized in Appendix B. The average survival rate through 96-h period is shown in Figure 4.1.

The acute toxicity of TBTO for *L. calcalifer* from this study indicates that 96-h LC50 value of 0.987 μg/L was lower than that reported for other fish species summarized in Table 2.5. The lowest 96-h LC 50 observed in fish was 1.41 μg/L for juveniles *Salmo gairdneri* (Martin *et al.*, 1989).

All the sea bass died during the bioassay displayed the same series of progressive signs ; darkened pigmentation, air gulping, accelerated ventilation with rapid mouth and opercular movements which become arhythmic and convulsive, gill hemorrhage, loss of stability, and then sank to the bottom of the test chambers. Fish in lower TBTO-concentration (0.6 and 0.8 μg/L) stayed near the bottom of the aquarium with less observed motility. The survival fish had darkened pigmentation at the end of bioassay, but they returned to normal pigmentation within 24 hours after being placed in clean seawater and apparently recovered from TBTO intoxication.

Sensitivity of fish when exposed to TBTO varies extensively. Generally, small fish are less tolerant of toxicants than larger fish, because they have a higher metabolic rate reflecting a faster assimilation of waterborne toxicants (Martin *et al*, 1989). Moreover, the solubility of TBT at different temperature is also an important factor.

Inaba *et al.* (1995) found that the fifty percent solubility of TBT was dropped when the temperature is decreased from 25 to 10 °C. Hence, there were more TBT^+ available in the solution at higher temperature. Within the same species, similar size of *Ictalurus punctatus*, the study of Brooke *et al.* (1986), reported the 96-h LC50 of 5.5 μg/L of TBT in flow through test, whereas the 96-h LC50 in static test was 12.0 μg/L (Rexrode, 1986). Among the estuarine species : *Menidia menidia*, *Menidia beryllina*, *Fundulus heteroclitus*, *Cyprinodon variegatus*, and *Brevoortia tyrannus*, the 96-h LC50 values of TBT were 8.9, 3.0, 23.4, 25.9, and 4.5 μg/L, respectively. Tsuda (1990) concluded that there were differences between freshwater and seawater fish in their ability to excrete chemical pollutants contaminated in their environment. These differences among types of fish are probably due to the differences in their ability to tolerate the specific chemical potency as well as the physiological mechanisms of the fish to regulate their osmotic pressure.

However, a direct comparison of acute values in fish is rather difficult. Normally, LC50 values of any bioassay tests would vary from test to test depending upon many different variable factors. These may be caused by differences in the range of concentrations tested and/or variability in the TBTO sensitivity of the different tested species . Sensitivities of hatchery fish, which generally come from inbred stock, to toxicants may be different from wild fish. It cannot be overlooked that the adsorption properties of TBTO for all surfaces could be interfered by lowering of the active toxicant concentration in the solutions. Differences in water quality used in the experiments such as salinity, pH, temperature, and another important factor may be important factors resulting in difficulties for a direct comparison between laboratories..

Several modes of action of TBT toxicity have been reported. Some of which were inhibition in mitochondrial oxidative phosphorylation, neurotoxicity, and an action on gill epithelial tissues (including the gill function). Pinkney *et al.* (1989) studied the effects of TBT on fish gills, it was suggested that different mechanisms of toxicity may dominate under different exposure conditions. At extremely high concentrations, extensive gill damage was found along with signs of oxygen deficiency

Table 4.1 Percent mortality of *L. calcalifer* at various TBTO concentrations over 96 hours

TBTO concentration (µg/L)	Number of fish in experiment	Number of Replication	Percent mortality											
			24-hour			48-hour			72-hour			96-hour		
			No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)	No. of dead	death (%)	Mean death (%)
Control	10	1	0	0		0	0		0	0		0	0	
	10	2	0	0	0	0	0	0	0	0	0	0	0	0
	10	3	0	0		0	0		0	0		0	0	
0.6	10	1	1	10		1	10		2	20		2	20	
	10	2	0	0	6.67	1	10	13.33	1	10	16.67	1	10	16.67
	10	3	1	10		2	20		2	20		2	20	
0.8	10	1	1	10		3	30		3	30		3	30	
	10	2	2	20	13.33	3	30	26.67	3	30	30	4	40	33.33
	10	3	1	10		2	20		3	30		3	30	
1.0	10	1	1	10		3	30		3	30		4	40	
	10	2	3	30	20	4	40	33.33	4	40	36.67	4	40	40
	10	3	2	20		3	30		4	40		4	40	
1.2	10	1	2	20		5	50		5	50		5	50	
	10	2	3	30	30	5	50	50	6	60	60	6	60	63.33
	10	3	2	20		3	30		5	50		6	60	
1.4	10	1	3	30		5	50		7	70		8	80	
	10	2	4	40	43.33	5	50	63.33	6	60	80	6	60	86.67
	10	3	2	20		5	50		7	70		8	80	

such as air-gulping and accelerated ventilation. At concentrations in the range of 96-h LC50, gill damage was less severe (Chliamovitch&Khun, 1977). The physical series of toxic signs indicated either a neurotoxic mechanism or a general metabolic poisoning from inhibition of oxidative phosphorylation.

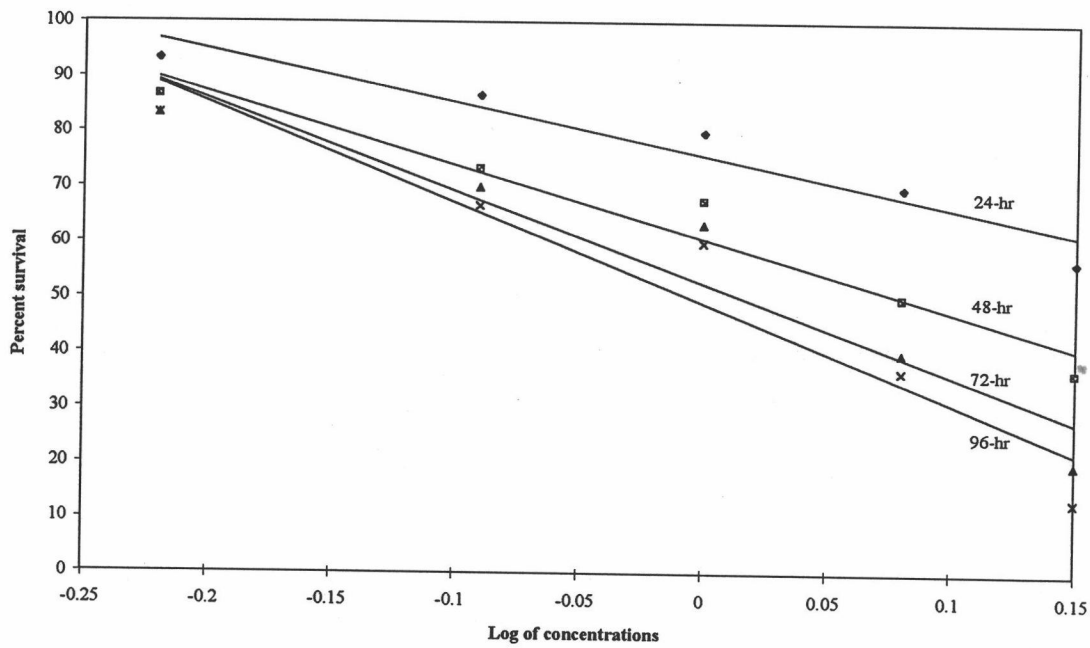

Figure 4.1 Percent survival of *L. calcalifer* during acute toxicity test

Table 4.2 Calculated LC50 of TBTO to *L. calcalifer*, using Probit analysis from data in Table 4.1.

Time of exposure (hour)	LC50 (µg/L)	95% confidence limits (µg/L)	R^2
24	1.62712	1.33239-2.91418	0.9847
48	1.19340	1.04851-1.48923	0.9783
72	1.03842	0.93315-1.17644	0.9263
96	0.98785	0.89254-1.09775	0.9146

From the acute toxicity tests of the present study, it is evident that the toxicity of TBTO to *L. calcalifer* was a function of both dosage and duration of exposure. When considering LC50 values for the natural aquatic environment, more variable factors are involved resulting in a wider confidence interval around the true LC50 value. As the natural aquatic environment drastically differ from the laboratory condition, therefore knowledge of LC50 values determination is an important knowledge used for setting a regulation policy for employing this chemical substance. However, it is premature at this stage for direct implement of the policy on the basis of acute toxicity data obtained from the present study unless descriptive *in situ* (on site) or *ex situ* (off site) testing have been completed..

Water quality

As can be seen from Table 4.3, the water quality during the acute toxicity tests of TBTO were quite consistent and within a normal requirement for *L. calcalifer* and other aquatic animals (APHA, 1992 and Parker, 1994). All water quality parameters among treatments were in the same range throughout the tests.

It was clear that the mortality of juvenile sea bass in the acute toxicity tests was not affected from water quality.

Table 4.3 Water quality during the acute toxicity tests of TBTO on *L. calcalifer*

Parameters	Range
Temperature (°C)	26-27
Dissolved oxygen (mg/L)	6-8
pH	7.8-8.1
Conductivity ($\mu mhoscm^{-1}$)	24,000-26,000
NH_3 (mg/L)	< 0.5
Hardness (mg/L as $CaCO_3$)	~ 2300
Alkalinity (mg/L as $CaCO_3$)	~ 180
Salinity (ppt)	20

Sublethal toxicity of TBTO on sea bass (*Lates calcalifer*)

Water quality

Since all parameters of water quality were in the normal range throughout this investigation, it is suggested that water quality should not be involved in significant differences in oxygen consumption and growth of *L. calcalifer* among experimental treatments. Water quality monitoring during 8-week sublethal test period is shown in Table 4.4.

Table 4.4 Water quality during the sublethal toxicity tests of TBTO on *L. calcalifer*

Parameters	Range
Temperature (°C)	25-27
Dissolved oxygen (mg/L)	6-9
pH	7.8-8.3
Conductivity ($\mu mhoscm^{-1}$)	29,000-34,000
Hardness (mg/L as $CaCO_3$)	2400-2600
Alkalinity (mg/L as $CaCO_3$)	190-230
Salinity (ppt)	19-21

Effects of TBTO on oxygen consumption of *L. calcalifer*

1. At short-term exposure

The rates of oxygen consumption of *L. calcalifer* exposed to various concentrations of TBTO after 1 hour are shown in Table 4.5, and Figure.4.2.

After 1 h of exposure to TBTO, a significant effect of TBTO on the oxygen consumption of fish comparing to the controls was observed. This is supported by the analysis of variance shown in Appendix E. Oxygen consumption rate of fish in the controls (seawater and acetone) significantly lower than those exposed to TBTO

concentrations. There was no statistical difference on oxygen consumption of the fish among different TBTO treatments.

L. calcalifer exposed to TBTO showed signs of stress, and had darkened pigment when first introduced to the chemicals. TBTO-exposed fish was very active and had a rapid rate of the operculum movement, comparing to the controls. After 1 h of acclimation, TBTO-exposed fish showed decreased activity , but still had rapid movement of operculum. Fish were measured for an oxygen uptake for 1 h before being returned to normal seawater. The respiratory distress based on the oxygen consumption gradually disappeared.

Therefore it may be concluded that the TBTO levels of 0.03 , 0.05, 0.10, and 1.00 μg/L were sufficient to enhanced significantly the oxygen consumption rate of juvenile sea bass in short-term exposure.

Table 4.5 Mean oxygen consumption rates of *L. calcalifer* at short-term exposure to various concentrations of TBTO

Concentratin of TBTO (μg/L)	Oxygen consumption, mg/g/h (mean ± S.D.)
Control (seawater)	$0.4456^{a} \pm 0.0700$
Control (acetone)	$0.4460^{a} \pm 0.0368$
0.03	$0.6443^{b} \pm 0.1227$
0.05	$0.6282^{b} \pm 0.2046$
0.10	$0.6664^{b} \pm 0.2084$
1.00	$0.6305^{b} \pm 0.1530$

The same superscript indicate no significant difference in the column at P= 0.05

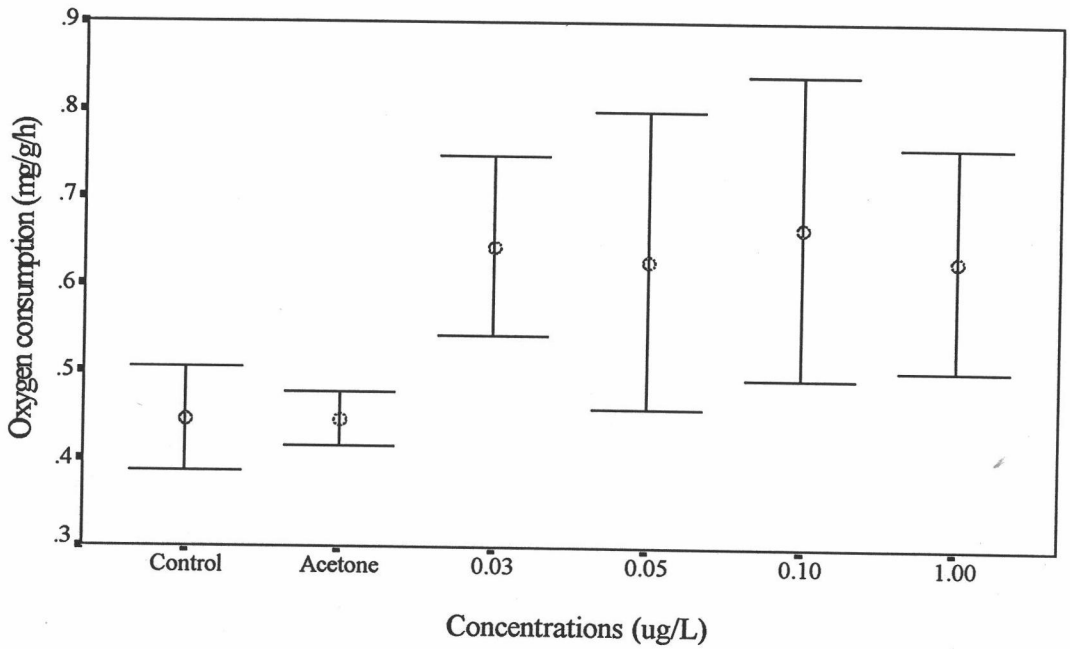

Figure 4.2 Influence of different TBTO concentrations on the oxygen consumption rate of *L. calcalifer* at short-term exposure

2. At continuous exposure for 8 weeks

Mean oxygen consumption rates of sea bass during continuous exposure is shown in Table 4.6. Oxygen consumption rates of *L. calcalifer* at all treatments decreased from the beginning of the test (Week 0) to the end (Week 8) of the experiment (Figure 4.3).

Analysis of variance indicated that there were significant effect of TBTO exposure time on the oxygen consumption of *L. calcalifer*. The statistical result is shown in Appendix E.

In general, large fish consume more oxygen than small fish. However, on a unit-weight or -mass basis (specific oxygen consumption), small fish consume more oxygen than large fish. The results from this study were following this basis. The oxygen consumption rate of *L. calcalifer* decreased with the increase in the fish size.

Significant differences in oxygen consumption of *L. calcalifer* among various TBTO treatments were only found statistically at week 0 (at the first day), but no significant was found thereafter. At the second week, oxygen consumption at 0.05

μg/L and 0.10 μg/L TBTO concentration were higher than that of the control but not statistically significant difference. It is suggested that *L. calcalifer* in TBTO treatments was in the adaptation phase to a new environment. From following consecutive weeks, the fish became more resistant to TBTO resulting in the adjustment in the rates of oxygen uptake. It should be also noted that TBTO can enhanced the oxygen consumption rate of *L. calcalifer* at the beginning of the test, following by a phase of early compensation and physical adaptation which occurred even in the continuous presence of TBTO, and then appeared to be intimately associated with the process of acclimation.

Table 4.6 Mean oxygen consumption rates of *L. calcalifer* during continuous exposure for 8 weeks

Concentration of TBTO (μg/L)	Oxygen consumption, $mgO_2/g/h$, mean (± S.D.)				
	Exposure period				
	Week 0	Week 2	Week 4	Week 6	Week 8
Control	0.4456[a] (± 0.0700)	0.3736 (± 0.0964)	0.2429 (± 0.0535)	0.2279 (± 0.0415)	0.1762 (± 0.0641)
0.03	0.6443[b] (± 0.1227)	0.3412 (± 0.1043)	0.2245 (± 0.1087)	0.2282 (± 0.0417)	0.1721 (± 0.0183)
0.05	0.6282[b] (± 0.2046)	0.4274 (± 0.1470)	0.2317 (± 0.0633)	0.2568 (± 0.0679)	0.1569 (± 0.0402)
0.10	0.6664[b] (± 0.2084)	0.4296 (± 0.1639)	0.2194 (± 0.1003)	0.2249 (± 0.0786)	0.2119 (± 0.0942)

The different superscript indicates significant difference in the column at $P \leq 0.05$

Oxygen consumption was measured every other weeks, and showed signs of recovery at the fourth week. From Week 4 to Week 8, the oxygen uptake of *L. calcalifer* in all TBTO treatments were approximately the same as that of the control. The recovery oxygen consumption rate at such period may be due to an ability of fish to adapt to lower levels of toxicants by the biochemical mechanisms. The depressions of TBTO on respiration of *L. calcalifer* in this test period may be negligible resulting in adaptive adjustments ability of the fish to continue their normal oxygen uptake. An alternative possibility is that the fish had enough time to detoxify the toxicants.

All fish in TBTO treatments showed signs of respiratory distress on the first day by surface, rapid deep breath and high rate of operculum movement. Breathing patterns gradually returned to normal after several days. There were no symtoms resulted from toxicity of TBTO thereafter. However, residual TBTO level in the tissue was not quantified. Because time limitation, the effects of TBTO on various tissues of *L. calcalifer* was not determined histologically.

In some toxicant, Mathiessen and Brafield (1973) showed that zinc-exposed sticklebacks (*Gasterosteus aculeatus*) recovered from respiratory distress in 9 days. An ability of damaged gills and signs of respiratory distress were recoverd by rapid lost of toxicants once they have been returned to the normal water. It was suggested that zinc enhanced the activity of chloride cells functioned on detoxification of such substance. It was also emphasized that euryhaline fish can immediately eliminate ion influxes.

There are extremely limited data which concerned about the effects of TBT on oxygen consumption of fish. However, there were some research carried out in the TBT effects on oxygen consumption in other aquatic animals. Widdows and Page (1993) treated mussels (*Mytilus edulis*) to TBT at concentrations of 0.5-10.0 μg/L for one month. The respiration of mussels was enhanced with increasing TBT concentration resulting in a peak at approximately 10 μg TBT/g dry weight tissues and declining at higher concentrations, ultimately of the cessation of pumping rate. The maximum rate represented a two-fold increase above the control rate of respiration and probably reflected the maximum potential increase in the metabolic rate

of mussels. It was suggested that the enhanced respiration rate reflected the primary mechanism of TBT toxicity, which is able to bind and inactivate membrane bound enzymes (specifically the proton translocating, ATPases in the mitochondrial membrane, resulting in the uncoupling ATP synthesis from oxygen uptake). In contrast, Sujatha *et al.* (1996) found that *Villorita cyprinoides* expose to TBTO at sublethal concentrations of 0.006, 0.008, and 0.010 mg/L showed the depletion in oxygen consumption was > 90% as compared with the control. They suggested that the decreased in the oxygen consumption has been attributed to valve closure, decreased ciliary acitivity and direct impairment of the metabolic activity. They also suggested the drastic suppression in the rates of oxygen consumption may be due to the considerable damage inflicted upon the gill tissue and to the formation of mucus layers over it, which result in a decreased flow of water onto the gill surface and lead to reduced oxygen intake.

From the studies mentioned above, it can be seen that TBT can cause either enhanced or decreased the oxygen uptake of aquatic animals. Many toxicants have been reported to stimulate oxygen consumption to sublethal concentration, and inhibit the oxygen uptake at lethal concentrations (Murty, 1986). These general correlations were difficult to be concluded unambiguously.

Therefore, the modes of action of TBT on oxygen consumption of *L. calcalifer* need to be clarified in both lethal and sublethal levels.

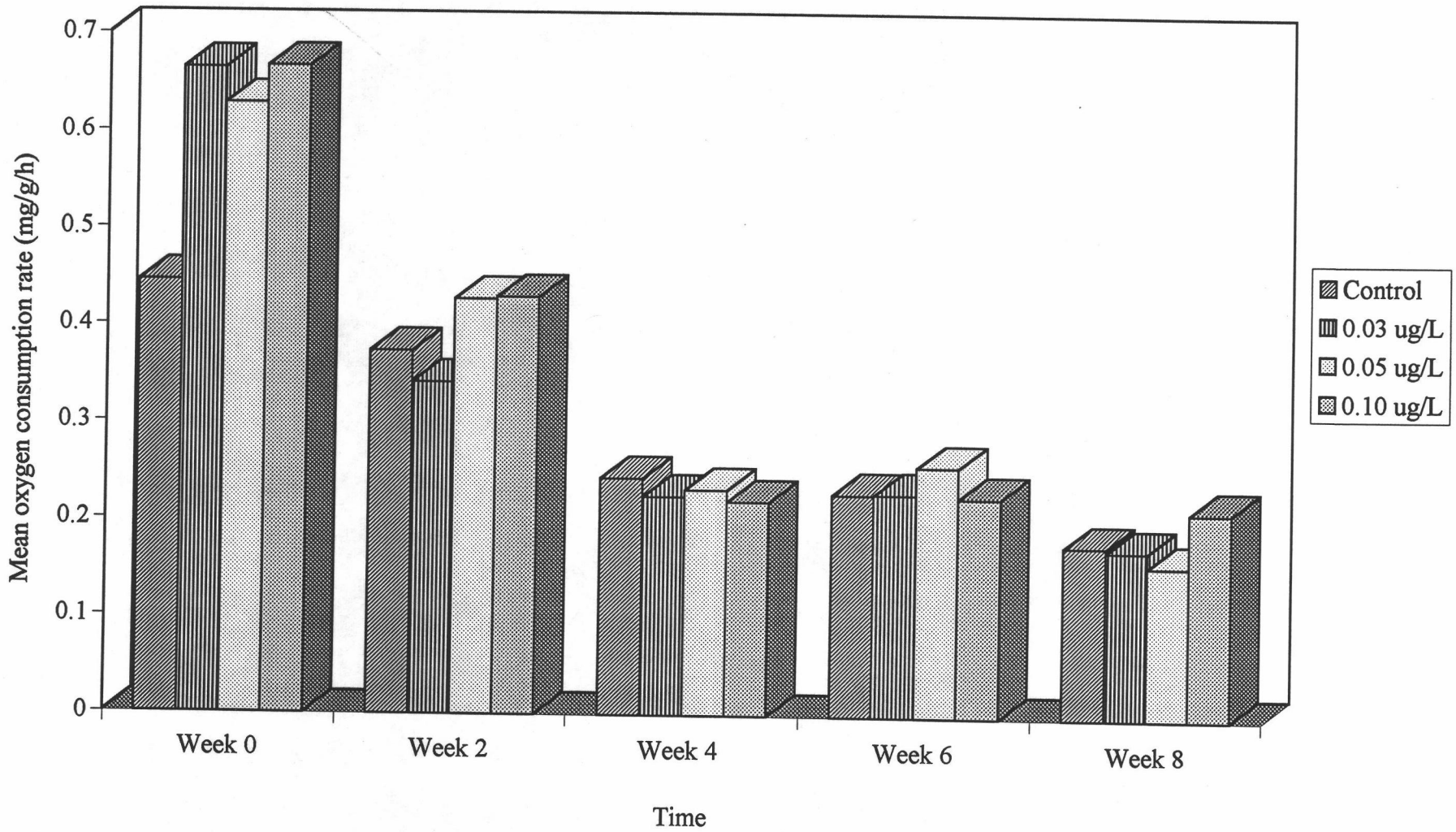

Figure 4.3 Mean oxygen consumption rate of *L. calcalifer* in 8-week test period

Effects of TBTO on growth of *L. calcalifer*

Growth

Length and weight of juvenile sea bass (*L. calcalifer*) were used to represent the growth of experimental animals. Table 4.7 and Table 4.8 show the mean of length and weight for each treatment during 8 exposure weeks to TBTO. An analysis of variance was performed separately for each parameters. The data were tested at an $\alpha = 0.05$ for growth differences among concentrations.

L. calcalifer that exposed to concentration of TBTO levels had the lower growth than that of the control. There was no significant difference in growth among the TBTO-treatment, and triplicate treatments.

Significant reduction in lengths were found in week 8 at 0.03 and 0.10 μg/L TBTO concentration. The lengths of the fish exposed to TBTO at 0.03 and 0.10 μg/L were 5.04 %, and 6.98 % lower than that of the control fish. When the fish were exposed to a higher concentration of TBTO (at 0.05 μg/L) the average length of treated fish was 3.61 %lower than that of the control, however, this difference was not statistically significant.

Significant reduction in weight was found in week 8 at 0.10 μg/L. The weight of the fish in this concentration is approximately 18.36 % lower than that for the control. The weights of *L. calcalifer* at two lower concentrations (0.03 and 0.05 μg/L) were 10.60 %, and 9.70 % lower than that for the control fish, but these differences were not again statistically significant.

The relationship between length and weight after 8 weeks exposure time is presented for each concentrations in Figure 4.4. There was no TBTO effect on lenght-weight relationship among experimental treatments.

Relative growth rate

After 8 weeks, fish length and weight (indicators of growth rates) in the control treatment increased by 60.22 %, and 337.08 %, respectively. Fish lengths in the 0.03, 0.05, 0.10 μg/L TBTO-treatment increased by 55.80 %, 52.62 %, and 55.29 %,

whereas the weights of such treated fish increased by 276.52%, 236.94 %, and 278.80 %, respectively. Although there is no statistically significant difference between the growth rates of *L. calcalifer* at each treatment, the average growth rate of *L. calcalifer* in the control seems to be slightly better than the TBTO treatments as can be seen in Table 4.9, and Table 4.10.

It could be observed from the first to the second week that fish growth rates were relatively high. These can be resulted from the high density of *L. calcalifer* in acclimation tank before testing. When tranferred to a new environment (the testing aquarium) fish had more space and had higher opportunity to eat, so they could grow rapidly in such period. Later, fish would adapt themselves to stay in that condition and their adaptation should take a period of time.

From data of this study, it was suggested that TBTO may affect growth parameters (the lenght and the weight) in the sea bass (*L. calcalifer*) in an 8-week testing period. The average length and weight of *L. calcalifer* in the control experiment were slightly greater than other TBTO treatments. However, statistically significant reduction of fish length occured at 0.03 and 0.10 μg/L treatment. However, significant reduction in weights was found only at 0.10 μg/L treatment. Similar results were reported by Brooke *et al.* (1986), who investigated the 33-d chronic exposure of fathead minnows (*Pimephales promelas*) with TBTO in early life-stage at concentrations of <0.02, 0.08, 0.15, 0.45, 0.90, and 2.20 μg/L at 24°C. Mean weights of the fish exposed to 0.45 μg/L and above at the end of the experiment were significantly less than the control fish ($P \leq 0.01$). Mean standard length at the end of the experiment was also significantly reduced at TBTO concentration of 0.05 μg/L ($P \leq 0.05$). No toxicity effect was observed at the concentration of ≤ 0.03 μg/L. It was calculated that the acute/chronic toxicity ratio was > 32.5. An apparent difference between their results and this study may be resulted from different fish species used in the experiments. While *Pimephales promelas* is a freshwater fish, *L. calcalifer* reared in this experiment is defined as estuarine fish species. Moreover, the toxicity levels of TBTO may be due to the differences in their physico-chemical properties in different tested temperature and water parameters between the two experiments.

More importantly, differences in detoxification ability of these two species may be a result of the disagreement of Brooke *et al.* (1986) and the present study. Hall *et al.* (1988) conducted chronic tributyltin(TBT) toxicity test with juvenile Atlantic salmon (*Salmo salar*) and larval inland silverside (*Menidia beryllina*) for 24 days. The fish were exposed to 0, 93, and 490 ng TBT/L at temperature and salinity of 20°C and 8-10 ppt, respectively. It was emphasized that averge weights of the control were significantly higher than TBT-exposed fish in both species. Pinkney *et al.* (1988) treated 13- and 16- day-old striped bass larvae (*Morone saxatilis*) to varying concentrations of TBT from methacrylate-painted panels for 6 to 7 days. At the lower exposure concentrations of 0.067 μg/L, growth parameters (weight) changed in the 13-day-old larvae only. No significant changes occured in the growth parameters of 16-day-old larvae exposed to 0.444 μg/L or less. The authors did not know whether this apparent different ages was a true effect or simply the result of differences between the various batch of larvae.

In other organotin compound studies on fish, Javinen *et al.* (1988) treated fathead minnows larvae (*Pimphales promelas*) with 2-30 μg/L of triphenyltin hydroxide (TPTH) on continuous exposure in a 30-day experiment. A significant reduction in growth occurred at 0.23 μg/L. The weight of the fish exposed to this concentration of TPTH was 15% lower than that of the control. It was shown that adverse effect on fathead minnow growth increased positively with exposure concentration and duration of TPTH. It was also indicated that TPTH effects are accumulated and it is possible that a full life-cycle chronic study may imply a statistically significant adverse effect at lower concentrations than the tests. The study of bioconcentration and elimination of TBT and TPT in several fish species were carried out by Yamada and Takayanagi (1992). It was found that the distribution pattern of TPT was different from that of TBT suggested that the metabolic pathways and activities of these two organotin compounds are dissimilarity.

de Vries *et al.* (1991) exposed yolk sac fry of rainbow trout (*Oncorhynchus mykiss*) to various organotin compounds : tributyltin chloride (0.12-15 ng/L), triphenyltin chloride (0.12-15 ng/L), dibutyltin dichloride (160-4000 ng/L), trimethyltin

chloride (3-75 ng/L), tricyclohexyltin chloride (0.12-15 ng/L), and diphenyltin dichloride (160-4000 ng/L) for 110 days. Significantly decreased in body weight was observed in all groups exposed to organotin compounds in comparison to that of the control. Moreover, increased mortality rates were also observed in treated rainbow trout. In the groups that little or minimal mortality occured, body weights of the exposed animals were approximately as the same as those of the controls. The highest exposure levels of various organotin compounds that did not affect in changing body weight were 160 ng/L DBTC, 800 ng/L DPhTC and 0.6 ng/L TBTC. The body weights of the fish exposed to TCHTC were decreased even at the lowest doses of 0.6 ng/L.

Effect of TBT on growth of other aquatic animals were also reported. For instance, Weis *et al.* (1986) exposed the fiddler crab (*Uca pugilator*) to TBTO at the concentration of 0.5, 5.0, and 50 μg/L under static renewal (twice weekly) conditions. Regenerations of a chelae and 5 walking legs was induced. Although some growth retardation was observed, the most striking effect was the development in regeneration of deformed limbs, e.g., backward curling or complete absence of the dactyl of the claw, chelae or walking legs, stunted, unjointed, or bent in the wrong direction. No deformities in the control groups were observed during the experiments. Hall, *et al.* (1988) exposed amphipods (*Gammarus* sp.) to TBT at the concentrations of 0, 29, 49, 120, 283, and 579 ng/L for 24 days in continuous -flow conditions. The average weight of *Gammarus* in a control condition was 2.8 times greater than that of tested organisms (exposed to TBT at 579 ng/L). However, a significant difference was only reported at 49 ng/L. It was suggested that TBT may affect weight gain in *Gammurus*, despite the fact that growth responses were not dependent solely on the concentration of TBT.

In conclusion, considering that the feed quality, feeding rate and water quality in each aquaria in the experiments were, however, the same in all treatment, it is therefore likely that the differences in growth among treatments should be a result of the potential of TBTO. The size of *L. calcalifer* used in this experiment was relatively

small. Thus the fish might still be in the lag phase, during the experiment, and possibly when it was terminated, the testing period was possibly too short, so the exponential growth was not obtained.

However, it can be concluded that the TBTO concentrations used in sublethal toxicity tests can cause growth retardation in *L. calcalifer* in the 8 weeks period. Prolonged exposure to such concentrations may be harmful to *L. calcalifer*. Therefore, futher investigated experiments need to be carried out to address this controversy.

Table 4.7 Mean length measurements from growth test

Concentration	Length (cm) ± S.D.				
	Week 0	Week 2	Week 4	Week 6	Week 8
Control	2.7739 ± 0.3255	3.2739 ± 0.3179	3.7957 ± 0.3176	4.0283 ± 0.3016	4.4444^{a} ± 0.3116
0.03 µg/L	2.7089 ± 0.3463	3.2911 ± 0.3878	3.6818 ± 0.4239	3.9545 ± 0.4501	4.2205^{b} ± 0.4322
0.05 µg/L	2.8068 ± 0.4250	3.3233 ± 0.4685	3.6860 ± 0.4877	4.0070 ± 0.5307	4.2837^{ab} ± 0.5145
0.10 µg/L	2.6622 ± 0.2923	3.1933 ± 0.2950	3.6267 ± 0.3714	3.8733 ± 0.3726	4.1341^{b} ± 0.3563

The same superscript indicates no significant difference in the column at $p \leq 0.05$

Table 4.8 Mean weight measurements from growth test

Concentration	Weight (g) ± S.D				
	Week 0	Week 2	Week 4	Week 6	Week 8
Control	0.4633 ± 0.1389	0.8426 ± 0.2761	1.2472 ± 0.3065	1.4509 ± 0.3366	2.0250^{a} ± 0.3923
0.03 µg/L	0.4808 ± 0.2085	0.8447 ± 0.2747	1.1842 ± 0.4267	1.4827 ± 0.5139	1.8103^{ab} ± 0.5849
0.05 µg/L	0.5427 ± 0.2516	0.8669 ± 0.3553	1.2305 ± 0.4422	1.4485 ± 0.5155	1.8286^{ab} ± 0.6673
0.10 µg/L	0.4364 ± 0.1291	0.7491 ± 0.2031	1.1275 ± 0.2832	1.3971 ± 0.3853	1.6531^{b} ± 0.3681

The same superscript indicates no significant difference in the column at $p \leq 0.05$

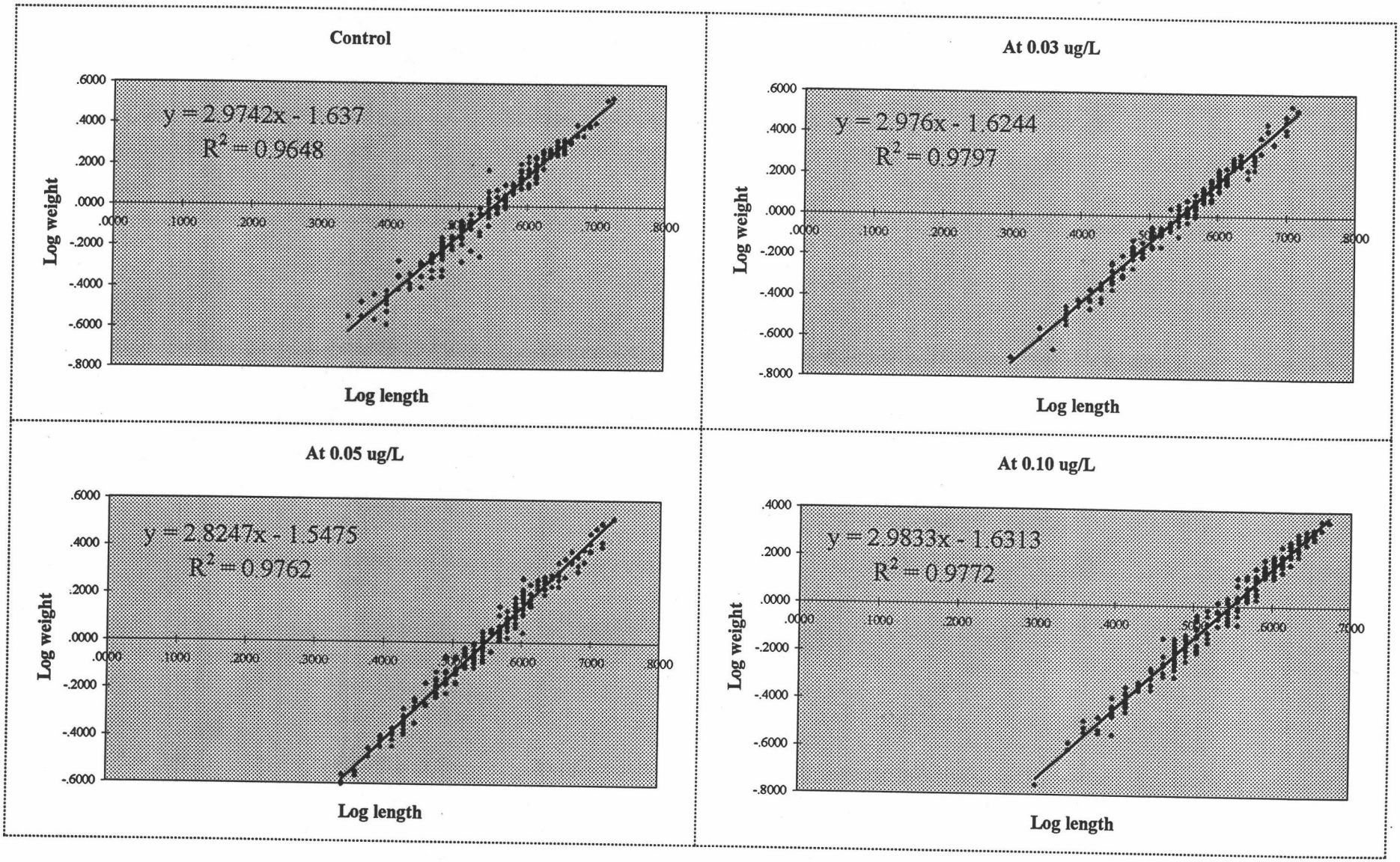

Figure 4.4 Length-weight relationships for each TBTO treatment from an 8-week testing period

Table 4.9 Growth rate by length estimated for each treatment at different sampling period (% increased in two weeks)

Time (Week)	Treatment			
	Control	0.03 µg/L	0.05 µg/L	0.10 µg/L
0-2	18.02	21.49	18.40	19.95
2-4	15.94	11.87	10.91	13.57
4-6	6.13	7.41	8.71	6.80
6-8	10.33	6.73	6.90	6.73
Average	12.60	11.87	11.23	11.76
All week (0-8)	60.22	55.80	52.62	55.29

Table 4.10 Growth rate by weight estimated for each treatment at different sampling period (% increased in two weeks)

Time (Week)	Treatment			
	Control	0.03 µg/L	0.05 µg/L	0.10 µg/L
0-2	81.87	75.69	59.74	71.65
2-4	48.02	40.19	41.94	50.51
4-6	16.33	25.21	17.72	23.91
6-8	39.57	22.09	26.24	18.32
Average	46.45	40.80	36.41	41.10
All week (0-8)	337.08	276.52	236.94	278.80

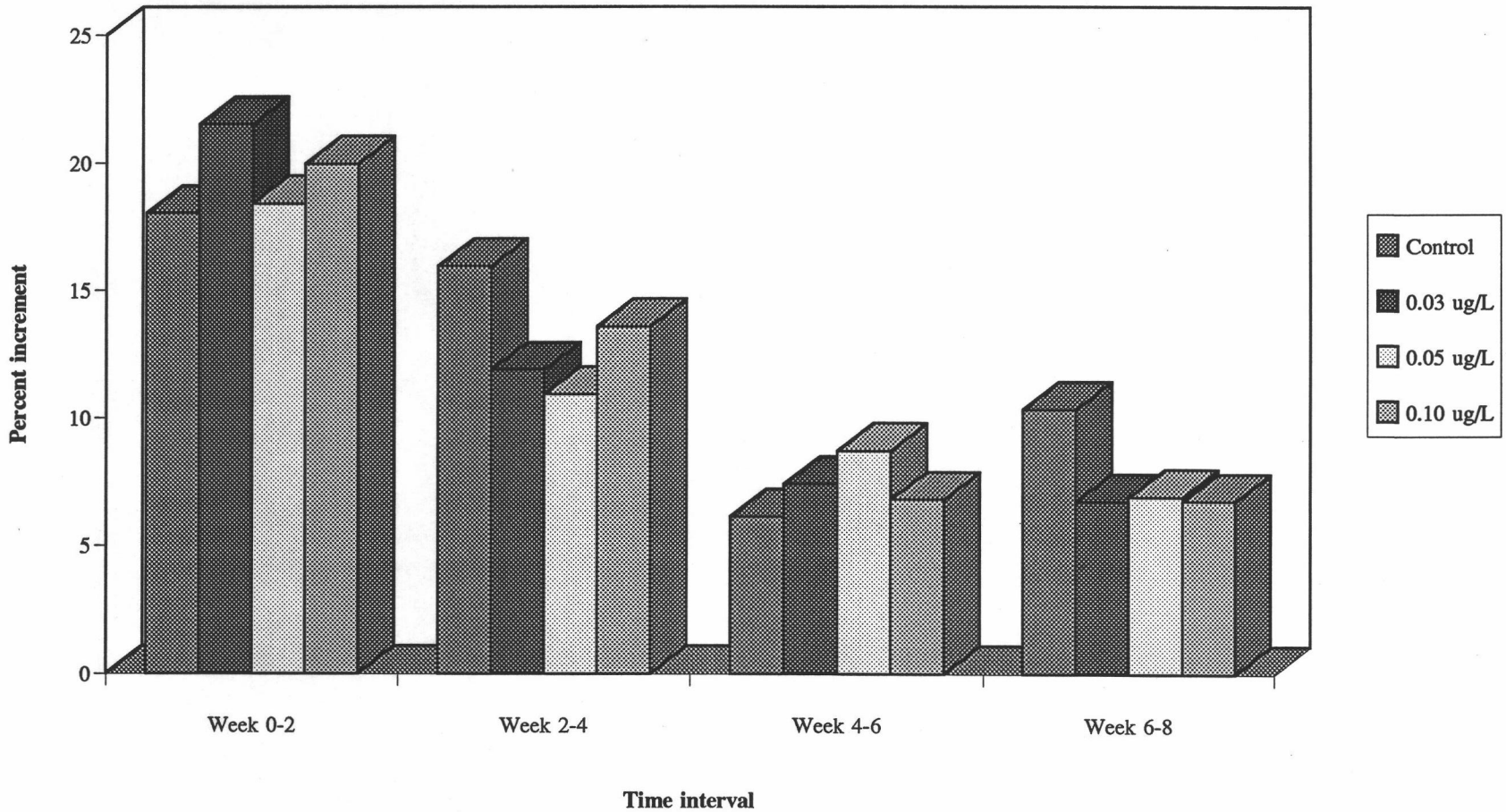

Figure 4.5 Growth rate by length of *L. calcalifer* during 8-week test period

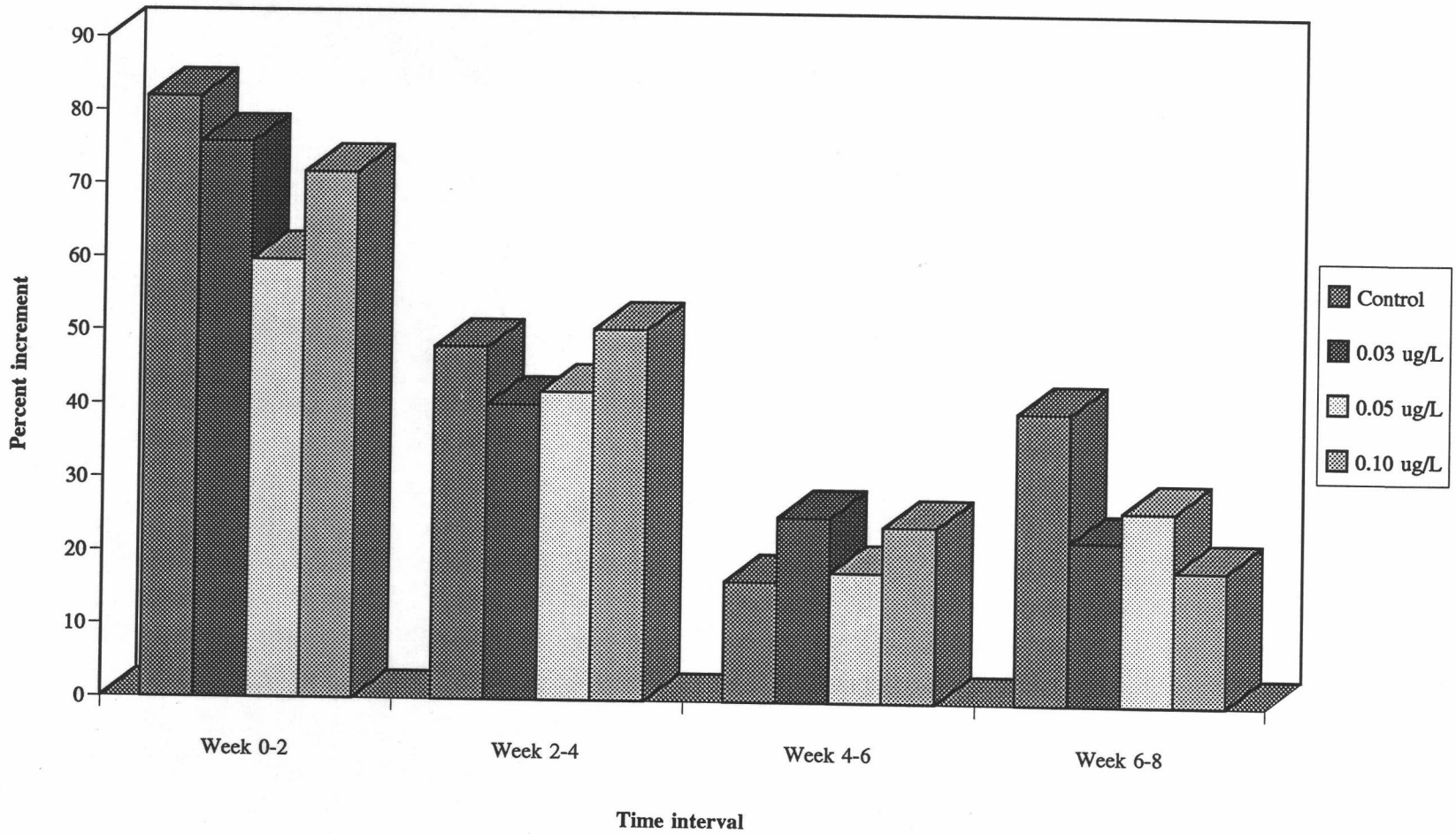

Figure 4.6 Growth rate by weight of *L. calcalifer* during 8-week test period

Survival rate of *L. calcalifer* during sublethal toxicity test

Survival rate of *L. calcalifer* at each experimental treatment was not statistically significant ($p \leq 0.05$). It may be concluded that the sublethal levels of TBTO had no effect on survival rate during 8 weeks. The survival rates are presented in Figure 4.7.

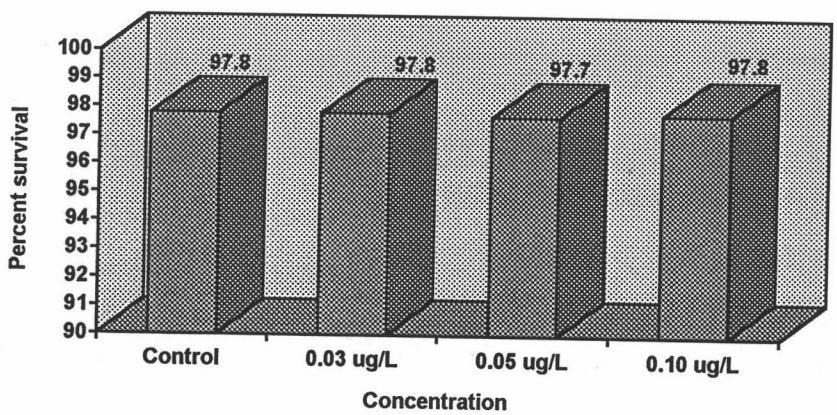

Figure 4.7 Percent survival of *L. calcalifer* after exposed to TBTO concentrations (μg/L) after 8-week testing period