

Acute Toxic Effects of Malathion on the 21st Stage Larvae of the Marsh Frog

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Abstract: Currently, amphibian decline is accepted as a global problem. One of the most important proposed causes for the decline is the use of agrochemicals, especially pesticides. Nevertheless, information about the impact of pesticides on amphibian populations remains scarce. The present study sought to identify the effects of malathion, an organophosphorus insecticide, on 21st stage larvae of the marsh frog, *Rana ridibunda*, with an acute toxicity test using a static system. Each experimental group contained 10 healthy larvae and was exposed to different concentrations of technical and formulation grade malathion for 96 h. Concentrations of malathion that caused 50% mortality (LC_{50}) at 96 h were estimated using a probit analysis program. The results showed that formulation grade malathion ($LC_{50} = 29$ ppm) was more toxic than technical grade malathion ($LC_{50} = 38$ ppm). Malformations, certain signs of toxicity (hyperactive symptoms, loss of balance, motionlessness, and death), and growth retardation were observed in malathion-treated larvae.

Key Words: Acute toxicity test, malathion, organophosphorus insecticide, Amphibia, anuran larvae, marsh frog, *Rana ridibunda*

Ova Kurbağası'nın 21. Evre Larvaları Üzerine Malathion'un Akut Toksik Etkileri

Özet: Günümüzde, amfibilerde gözlenen azalmalar evrensel bir problem olarak kabul edilmektedir. Bu azalmalara neden olabilecek sebeplerin en önemlilerinden biri, zirai kimyasallar ve özellikle de pestisitlerdir. Bununla birlikte, pestisitlerin amfibi popülasyonları üzerindeki etkileriyle ilgili bilgiler hala yeterli değildir. Bu çalışma; statik sistem esas alınarak yapılan akut toksisite testleriyle organikfosforlu bir insektisit olan malathion'un Ova Kurbağası, *Rana ridibunda*'nın 21. evre larvaları üzerindeki etkilerini ortaya koymaktadır. Sağlıklı 10 larvadan oluşan her bir deneme grubu, 96 saatlik deney süresince, teknik ve ticari malathion'un farklı konsantrasyonlarına maruz bırakılmıştır. Probit analiz programı kullanılarak, 96 saatte %50 ölüme sebep olan malathion konsantrasyonları (LC_{50}) hesaplanmıştır. Bu çalışma sonuçları, 21. evre larvalar üzerinde ticari malathion'un ($LC_{50} = 29$ ppm), teknik malathion'dan ($LC_{50} = 38$ ppm) daha zehirli olduğunu göstermiştir. Malathion'a maruz bırakılan larvalarda şekil bozuklukları; hiperaktivite semptomları, denge kaybı, hareketsizlik, ölüm gibi bariz toksisite belirtileri ve bu larvaların gelişimlerinde gecikme gözlenmiştir.

Anahtar Sözcükler: Akut toksisite testi, malathion, organofosforlu insektisit, amphibia, anur larvası, Ova Kurbağası, *Rana ridibunda*

Introduction

Since the late 1980s there has been a growing realization that populations of many amphibian species from a variety of taxa are declining at an alarming rate. Recent reports suggest that the frequency of mass incidences of frog deformities has increased, and population declines have been observed within the last 2 decades (Blaustein and Wake, 1990, 1995; Bishop and Pettit, 1992; Materna et al., 1995; Houlahan et al., 2000; Gardner, 2001; Blaustein et al., 2003). To

prevent further decline it is very important to establish what factors are responsible. While evidence of widespread reductions in the numbers of amphibians is rapidly accumulating, the cause remains unclear. Postulated causes of the deformities and population declines include ultraviolet radiation (Blaustein et al., 1997; Blaustein et al., 2003), parasites (Rollins-Smith et al., 2002a; Rollins-Smith et al., 2002b), and agrochemicals, especially pesticides (Ouellet et al., 1997; Sparling et al., 2001).

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One of the non-target biological groups most affected by pesticides is amphibians (Fulton and Chambers, 1985; Berrill et al., 1994; Sparling et al., 2001). Much of the amphibian life cycle occurs in ponds, streams, and temporary pools that are often associated with agricultural areas exposed to pesticide applications. Furthermore, breeding and larval development of most amphibians occurs during spring and summer months, which coincides with heavy pesticide application for agricultural purposes. However, information about the impact of pesticides on amphibian populations remains scarce. Amphibians constitute an important part of the vertebrate biomass in many ecosystems and are important as predators and prey; therefore, the impact of pollution upon these animals are relevant to an understanding of the health of the entire ecosystem. This study was designed to identify the acute toxic effects of malathion, an organophosphorus insecticide widely used in Turkey, and to contribute to the knowledge of the effects of pesticides on amphibians.

Malathion and similar related compounds are, in part, replacing chlorinated hydrocarbons. Organophosphorus chemicals degrade relatively quickly and are not associated with the problem of persistence, as are chlorinated hydrocarbons. However, organophosphorus chemicals have an acute toxicity that may be fatal, and they are acetylcholinesterase inhibitors, which adversely affect the nervous system (Chambers and Levi, 1992).

The purpose of this study was to determine the LC₅₀ of malathion on 21st stage larvae of the marsh frog, *R. ridibunda*, and to describe the acute toxic effects of this organophosphorus insecticide.

Materials and Methods

Egg masses of *R. ridibunda* were collected from a clean stream located within the Ege University Campus, İzmir, Turkey, and reared under natural lighting in glass aquaria at room temperature using gently aerated and dechlorinated tap water. The water temperature largely corresponded to the room temperature, which was recorded continuously with a thermograph during the experimental period. Experiments were performed on 21st-22nd fully-aquatic stages of pre-feeding larvae (5-7 mm). The stages of the larvae were determined according to Gosner (1960).

Technical (95%) and formulation grade (25% WP, Koruma) malathion were used. The stock technical grade malathion solution was prepared by dissolving in dechlorinated tap water and dilutions were made from the stock solution in order to reach the following test concentrations (in ppm) of 28, 30, 32, 34, 36, and 38. Formulation grade malathion was also dissolved in dechlorinated tap water for preparing the test concentrations (in ppm) of 22, 24, 26, 28, 30, and 32. The concentration of formulation grade malathion was calculated from the percentage of the active ingredients. Solutions were freshly prepared before use.

A static system was used in each acute toxicity test as a method of exposure. In all, 3 sets of experiments were conducted simultaneously. One served as a control group; larvae in dechlorinated tap water without insecticide (n = 10). The second and the third sets corresponded to experimental larvae placed in different technical and formulation grade malathion solutions, respectively. We randomly selected 10 healthy larvae and transferred them to a glass petri dish containing 100 ml of malathion solution of the desired concentration, where they remained for 96 h. The petri dishes were covered with glass plates to obtain a negligible air-water interface and to minimize evaporation. For each concentration, including the control, 3 replicates were used and the mean values of these were taken into account in estimations. Observations were made at 24-h intervals during 96 h of exposure. Death was considered the endpoint. Every 24 h the number of dead animals was recorded, which were removed from the petri dishes, and their external aspects recorded. The morphological and behavioral characteristics of the living animals were also recorded.

At the end of the experiment, lengths of the living larvae were measured under a stereo microscope with an ocular micrometer and compared to the controls. Mortality data from the replicate samples from each malathion concentration were pooled prior to calculating the LC₅₀ and the 95% confidence limits. The 96-h LC₅₀ value and the 95% confidence limits were determined through probit analysis, using SPSS v.10.0 for Windows. All regressions were significantly linear (χ^2 , $P < 0.05$).

Results

No mortality occurred in the control group, 28 ppm technical and 22 ppm formulation grade malathion groups throughout the 96-h experimental period. There was a significant positive correlation between increasing malathion concentration and mortality ($P < 0.05$). The 96-h LC_{50} values observed for technical and formulation grade malathion in 21st stage larvae were 38 and 29 ppm, respectively (Table 1).

Retardation of growth was observed in 32, 34, 36, and 38 ppm technical grade, and 26, 28, 30, and 32 ppm formulation grade malathion-treated larvae. Higher malathion concentrations caused more larvae to remain at the 24th stage, while the controls progressed to the 25th stage (compare Figures 1 and 2). Additionally, malathion-treated larvae were smaller than the controls. Malathion decreased larval length, although the differences in growth were never more than several mm (Table 2).

Malformations occurred in larvae exposed to technical grade malathion at the concentration of 32 ppm and above, and formulation grade malathion at concentrations of 26 ppm and above. The malformations recorded in this study were tail deformations, abnormal gut coiling and generalized edema, which was observed most frequently in the head and trunk. While control larvae are shown in Figure 1, some morphological abnormalities found in malathion-treated larvae are shown in Figures 2-4. Figure 2 shows malathion-treated larvae suffering from growth retardation. Head, trunk, and tail deformations, as well as edema are evident in Figures 3-4. Abnormal gut coiling is seen in Figure 4 b and c.

The larvae exposed to malathion showed certain signs of toxicity during the experimental period. At the beginning of the experiments these signs were characterized by hyperactivity, such as fast swimming in

Table 1. The 96-h lethal concentrations (LC_{10} , LC_{50} , and LC_{90}) in ppm for *R. ridibunda* larvae exposed to technical and formulation grade malathion.

	LC_{10} (95% CI)	LC_{50} (95% CI)	LC_{90} (95% CI)
Technical Grade Malathion	29 (16.39-32.05)	38 (35.11-48.25)	46 (40.46-77.81)
Formulation Grade Malathion	25 (20.93-26.13)	29 (27.62-30.82)	33 (31.34-38.48)

Table 2. Effects of technical and formulation grade malathion on the growth of *R. ridibunda* larvae. All lengths in different concentrations are significantly less than the controls' ($P < 0.05$).

Technical Grade Malathion Concentration (ppm)	Total Length Mean \pm SD (mm)	Formulation Grade Malathion Concentration (ppm)	Total Length Mean \pm SD (mm)
38	7.37 \pm 1.03	32	7.45 \pm 0.71
36	7.71 \pm 0.30	30	7.72 \pm 0.33
34	7.80 \pm 0.33	28	7.83 \pm 0.45
32	7.88 \pm 0.49	26	7.93 \pm 0.50
30	8.04 \pm 0.64	24	8.15 \pm 0.35
28	8.32 \pm 0.52	22	8.35 \pm 0.56
control	9.00 \pm 0.47	control	9.08 \pm 0.44

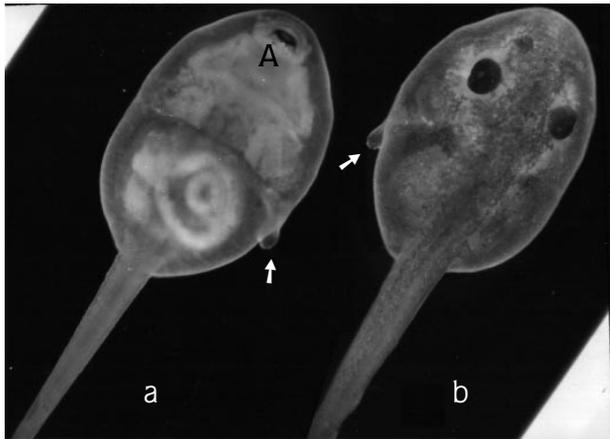


Figure 1. Ventral (a) and dorsal (b) views of an untreated larva (the control larvae reached the 25th stage). → : spiraculum; A: mouth.

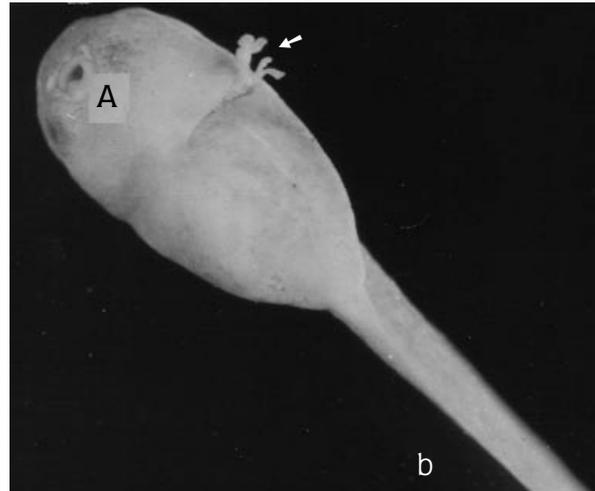
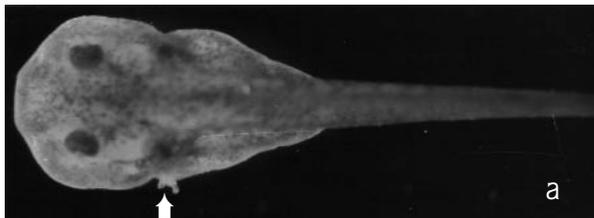


Figure 2. Photographs of 26 ppm formulation grade (dorsal, a) and 34 ppm technical grade (ventral, b) malathion-treated larvae, respectively. They only reached the 24th stage (growth retardation is evident). → : external gills; A: mouth.

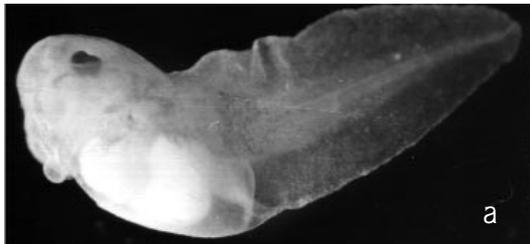


Figure 3. Malformed 25th stage larvae exposed to 26 ppm formulation grade (ventrolateral, a), 38 ppm technical grade (dorsal, b), and 28 ppm formulation grade (dorsal, c) malathion.

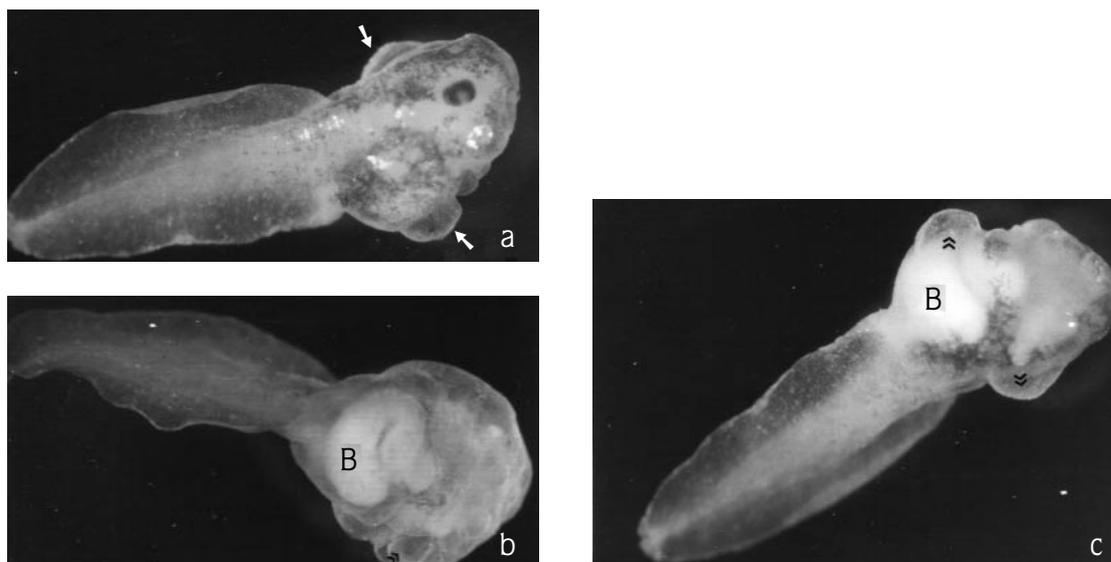


Figure 4. Significantly malformed 25th stage larvae exposed to 26 ppm formulation grade (dorsolateral, a) and 38 ppm technical grade malathion (b: ventral; c: ventrolateral). Note the generalized edema and abnormal gut coiling. →, »: edema; B: gut.

a spiral orbit and spontaneous twitching. Then, loss of balance was observed with poor swimming ability. Most of the larvae were observed laying/swimming on their sides or backs. Later, larvae became motionless. Finally, death was defined as a lack of response to mechanical stimuli.

Discussion and Conclusion

This experimental study revealed the toxicity of malathion to 21st stage marsh frog larvae in a 96-h acute toxicity test. The LC₅₀ of malathion determined in the present study is different from what has been previously published. Relyea (2004) examined survival and predatory stress of 6 tadpole species (*Rana sylvatica*, *Rana pipiens*, *Rana clamitans*, *Rana catesbeiana*, *Bufo americanus*, and *Hyla versicolor*) exposed to 6 different concentrations of malathion. He found that malathion was moderately toxic to all tadpoles and that the LC₅₀ value ranged between 1.25 and 5.9 mg/l. The reported 96-h LC₅₀ of malathion for larval *Rana boylii* was 2.137 mg/l (Sparling and Fellers, 2007). Therefore, in comparison, *R. ridibunda* has higher LC₅₀ values for malathion. The high LC₅₀ of *R. ridibunda* could indicate that this species is fairly resistant to organophosphorus insecticides. Therefore, amphibian responses to pesticide

exposure are likely to be complex, and dependent on species and developmental stage, as well as the timing, duration, and level of pesticide exposure (Berrill et al., 1993).

The active compounds used in commercial formulations of pesticides for agriculture are often contaminated by by-products or synthesis intermediates. This does not allow evaluation of their toxicology as some contaminants could be even more toxic than the active compound or could act synergistically. Therefore, in the present study the toxicity of technical and formulation grade malathion were evaluated and the results of this bioassay showed that formulation grade malathion (LC₅₀ = 29 ppm) was more toxic to 21st stage *R. ridibunda* larvae than technical grade malathion (LC₅₀ = 38 ppm). Similarly, it was also reported that formulation grade malathion with an LC₅₀ of 18.60 ppm was more toxic than technical grade malathion with an LC₅₀ of 30.18 to 25th stage *R. ridibunda* larvae (Sayim and Akyurtlaklı, 1999).

At the end of the present study's experimental period, growth retardation and decreased larval length were observed in 21st stage larvae treated with technical and formulation grade malathion. Similarly, reduced growth rates and tadpole mortality have been documented after

exposing eggs and larvae to organophosphorus pesticides (Mohanty-Hejmandi and Dutta, 1981; Mizgirev et al., 1984; Sayım and Kaya, 2006). Decrease in survivorship and growth rate in *R. catesbeiana* and *Limnonectes limnocharis* tadpoles exposed to malathion were reported in other studies (Fordham et al., 2001; Gurushankara et al., 2007). Such a delay in larval growth can have a significant detrimental impact later in the life of a frog by decreasing its survival rate, its adult size (Berven, 1990), its sexual maturation rate (Smith, 1987), mate selection ability (Forester and Czarnowsky, 1985), and locomotion ability for predator evasion (Goater et al., 1993). In the case of malathion exposure, similar to the findings of the current study, decreasing larval length was also determined in 25th stage *R. ridibunda* and *L. limnocharis* larvae in other studies (Sayım and Akyurtlaklı, 1999; Gurushankara et al., 2007).

Morphological abnormalities (such as head, trunk, and tail deformities), generalized edema, and abnormal gut coiling were observed in 21st stage *R. ridibunda* larvae exposed to malathion. Similarly, Pawar et al. (1983) reported that malathion caused gross morphological changes, such as abnormalities in the head, trunk, and tail, in *Microhyla ornata*. In accordance with these studies, abnormal tail flexure, abnormal gut coiling, and bent notochords were observed in *Xenopus laevis* larvae exposed to malathion in other studies (Snawder and Chambers, 1993; Bonfanti et al., 2004). Bonfanti et al. (2004) stated that malathion must be considered a powerful teratogenic compound. Sayım and Kaya (2006) also reported similar morphological alterations characterized by edema, especially on the head and trunk in 21st stage *Hyla arborea* exposed larvae in response to dimethoate, an organophosphorus insecticide. Bishop (1992) also showed that tail deformities and edema were observed when stage 19-25 *Rana temporaria* and *X. laevis* tadpoles were exposed to carbaryl. However, it was reported in another study that no malformations were observed in the 25th stage *R. ridibunda* larvae exposed to malathion (Sayım and Akyurtlaklı, 1999). Tail deformities in *R. pipiens* embryos treated with chlorpyrifos, an organophosphorus insecticide, were reported (Gaizick et al., 2001).

During the present study, certain signs of toxicity, such as hyperactive symptoms at the beginning, then loss of balance, followed by motionlessness and finally death, were observed in the behavior of 21st stage *R. ridibunda*

larvae exposed to malathion. Similar behavioral effects in response to laboratory exposure to carbaryl, carbofuran, malathion, and dimethoate in amphibian larvae were reported in other studies (Bishop, 1992; Sayım and Akyurtlaklı, 1999; Sayım and Kaya, 2006). These behavioral effects are not surprising as most of these pesticides are neurotoxins. It is also known that behavioral changes of these types increase the chances of frog larvae predation (Bishop and Pettit, 1992).

Amphibian populations are under increasing threat worldwide due to habitat loss and habitat degeneration. It has been argued that pesticides probably change the quality and quantity of amphibian foods and habitats (Berrill et al., 1993). Frogs have been shown to be very susceptible to developing mutations from exposure to malathion, which causes malformed tails and heads, and unusual swimming patterns (i.e., in tadpoles of *M. ornata*, Pawar et al., 1983). These results for *M. ornata* are in accordance with those for *R. ridibunda*. Moreover, this study also suggested that malathion caused growth retardation, malformations, and behavioral changes in *R. ridibunda* larvae. So it was concluded that the chemical contamination, at lethal or sub-lethal levels, can alter natural regulatory processes, such as juvenile recruitment, in amphibian populations. Therefore, chemical exposure should be considered as contributing to the declines in amphibian populations. Additionally, amphibians control many insect populations and serve as a connection between aquatic and terrestrial food webs. In order to ensure the continuity between the webs, it is important to protect amphibians from decline. Monitoring amphibian populations to assess the extent and cause of declines is confounded by a number of ecological and methodological limitations (Gardner, 2001). Thus, laboratory studies have a useful role to play in establishing the baseline sensitivity of various amphibian life stages and species to pesticides because other environmental stressors can be eliminated from the assessment. However, it is suggested that subsequent in situ studies may then establish their relevance to environmental conditions.

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References

- Berrill, M., Bertham, S., Wilson, A., Louis, S., Brigham, D. and Stromberg, C. 1993. Lethal and sublethal impacts of Insecticides on amphibian embryos and tadpoles. *Environmental Toxicology and Chemistry*. 12: 525-539.
- Berrill, M.S., Bertram, S., McGillivray, L., Kolohon, M. and Paull, B. 1994. Effects of low concentrations of forest-use pesticides on frog embryos and tadpoles. *Environmental Toxicology and Chemistry*. 13: 657-664.
- Berven, K.A. 1990. Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology*. 71: 1599-1608.
- Bishop, C.A. 1992. The effects of pesticides on amphibians and the implications for determining causes of declines in amphibian populations. In: *Declines in Canadian amphibian populations: designing a national monitoring strategy* (eds. C.A. Bishop, K.E. Pettit) Minister of Supply and Services Canada. Ontario, pp. 67-70.
- Bishop, C.A. and Pettit, K.E. 1992. Declines in Canadian amphibian populations: designing a national monitoring strategy, Minister of Supply and Services Canada, Ontario.
- Blaustein, A.R. and Wake, D.B. 1990. Declining amphibian populations: A global Phenomenon? *Trends in Ecology and Evolution* 5: 203-204.
- Blaustein, A.R. and Wake, D.B. 1995. The puzzle of declining amphibian populations. *Scientific American* 272: 52-63.
- Blaustein, A.R., Kiesecker, J.M., Chiver, D.P. and Anthony, R.G. 1997. Ambient UV-B radiation causes deformities in amphibian embryos. *Proceedings of the National Academy of Sciences* 94: 13735-13737.
- Blaustein, A.R., Romansic, J.M., Kiesecker, J.M. and Hatch, A.C. 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. *Diversity and Distributions*, 9: 123-140.
- Bonfanti, P., Colombo, A., Orsi, F., Nizzetto, I., Andrioletti, M., Bacchetta, R., Mantecca, P., Fascio, U., Vailati, G. and Vismara, C. 2004. Comparative teratogenicity of chlorpyrifos and malathion on *Xenopus laevis* development. *Aquatic Toxicology* 70: 189-200.
- Chambers, J.E. and Levi, P.E. 1992. *Organophosphates: Chemistry, Fate and Effects*, Academic Press, New York, USA.
- Fordham, C.L., Tessari, J.D., Ramsdell, H.S. and Keefe, T.J. 2001. Effects of malathion on survival, growth, development, and equilibrium posture of bullfrog tadpoles (*Rana catesbeiana*). *Environmental Toxicology and Chemistry* 20: 179-184.
- Forester, D.C. and Czarnowsky, R. 1985. Sexual selection in the spring peeper, *Hyla crucifer* (Amphibia: Anura): Role of the advertisement call. *Behaviour* 92: 112-128.
- Fulton, M.H. and Chambers, J.E. 1985. The toxic and teratogenic effects of selected organophosphorus compounds on the embryos of three species of amphibians. *Toxicology Letters* 26: 175-180.
- Gaizick, L., Gupta, G. and Bass, E. 2001. Toxicity of chlorpyrifos to *Rana pipiens* embryos. *Bulletin of Environmental Contamination and Toxicology* 66: 386-391.
- Gardner, T. 2001. Declining amphibian populations: a global phenomenon in conservation biology. *Animal Biodiversity and Conservation* 24: 25-44.
- Goater, C.P., Semlitsch, R.D. and Bernasconi, M.V. 1993. Effects of body size and parasite infection on the locomotory performance of juvenile toads. *Oikos* 66: 129-136.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16: 183-190.
- Gurushankara, H.P., Krishnamurthy, S.V. and Vasudev, V. 2007. Effect of malathion on survival, growth, and food consumption of Indian cricket frog (*Limnonectes limnocharis*) tadpoles. *Archives of Environmental Contamination and Toxicology* 52: 251-256.
- Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Meyers, A.H. and Kuzmin, S.L. 2000. Quantitative evidence for global amphibian population declines. *Nature* 404: 752-755.
- Materna, E.J., Rabeni, C.F. and LaPoint, T.W. 1995. Effects of the synthetic pyrethroid insecticide on larval Leopard frogs. *Environmental Toxicology and Chemistry* 14: 613-622.
- Mizgireuv, I.V., Flax, N.L., Borkin, L.J. and Khudoley, V.V. 1984. Dysplastic lesions and abnormalities in amphibians associated with environmental conditions. *Neoplasma* 31: 75-181.
- Mohanty-Hejmandi, P. and Dutta, S.K. 1981. Effects of some pesticides on the development of the Indian bull frog *Rana tigrina*. *Environmental Pollution* 24: 145-161.
- Ouellet, M., Bonin, J., Rodrigue, J., DesGranges, J.L. and Lair, S. 1997. Hindlimb deformities in free-living anurans from agricultural habitats. *Journal of wildlife diseases* 33: 95-104.
- Pawar, K.R., Ghate, H.V. and Katdare, M. 1983. Effect of malathion on embryonic development of the frog *Microhyla ornate*. *Bulletin of Environmental Contamination and Toxicology* 31: 170-176.
- Relyea, R.A. 2004. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. *Environmental Toxicology and Chemistry* 23: 1080-1084.
- Rollins-Smith, L.A., Carey, C., Longcore, J., Doersam, J.K., Boutte, A., Bruzgal, J.E. and Conlon, J.M. 2002b. Activity of antimicrobial skin peptides from ranid frogs against *Batrachochytrium dendrobatidis*, the chytrid fungus associated with global amphibian declines. *Developmental and Comparative Immunology* 26: 471-479.
- Rollins-Smith, L.A., Doersam, J.K., Longcore, J.E., Taylor, S.K., Shamblin, J.C., Carey, C. and Zasloff, M.A. 2002a. Antimicrobial peptide defenses against pathogens associated with global amphibian declines. *Developmental and Comparative Immunology* 26: 63-72.

- Sayım, F. and Akyurtlaklı, N. 1999. Acute toxicity of malathion on the 25th stage larvae of *Rana ridibunda*. Journal of Fisheries and Aquatic Sciences 16: 19-29.
- Sayım, F. and Kaya, U. 2006. Effects of Dimethoate on Tree Frog, *Hyla arborea* Larvae. Turkish Journal of Zoology 30: 261-266.
- Smith, D.C. 1987. Adult recruitment in chorus frogs: effects of size and date at metamorphosis. Ecology 68: 344-350.
- Snawder, J.E. and Chambers, J.E. 1993. Osteolathyrogenic effects of malathion in *Xenopus* embryos. Toxicology and Applied Pharmacology 121: 210-216.
- Sparling, D.W. and Fellers, G. 2007. Comparative toxicity of chlorpyrifos, diazinon, malathion and their oxon derivatives to larval *Rana boylei*. Environmental Pollution 147: 535-539.
- Sparling, D.W., Fellers, G.M. and McConnell, L.L. 2001. Pesticides and amphibian population declines in California, USA. Environmental Toxicology and Chemistry 20: 1591-1595.