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Ambon Damsel (*Pomacentrus amboinensis*) as a Bioindicator Organism for the Great Barrier Reef: Responses to Chlorpyrifos

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The Great Barrier Reef World Heritage and Wet Tropics Rainforest areas are an extremely sensitive and important conservation region (Williams 2001). In terms of the potential for man's impact on this region, the sugar growing areas of Queensland are of concern. Sugar cane is grown mainly within 50 km of the coast and within 26 major drainage basins and river catchments covering around 230, 000 km^2 of land. Concern has been raised about agricultural compounds moving from areas of the mainland into coastal ecosystems (Klumpp and Westernhagen 1995; Haynes et al. 2000; Johnson and Ebert 2000). Although concentrations of pesticides in these coastal environments are generally low there is evidence of localised hot spots adjacent to ports and harbours, urban centres and areas adjacent to intensive agricultural activity in both sediments (Haynes et al. 2000) and fish tissues (Klumpp and Westernhagen 1995). Thus there is a need to develop sensitive biological techniques for monitoring effects of contaminants on the environmental health of the Great Barrier Reef ecosystem. Biological techniques provide a measure of an integrated response to the totality of contaminants that contribute to the toxic load over time, whether or not they are known and monitored. This is an extremely important point given that many of today's pesticides are not persistent and are rarely found during spot sampling, though they may be found when using continuous samplers (NRA 2000).

The early life stages (ELS) of fish (eg. egg, embryo, larva and fry) have been used extensively in toxicity testing because these stages of development are both well described and are generally considered to have equal or greater sensitivity compared with adult life stages (McKim 1977; Westernhagen 1988). The ELS toxicity test begins with embryos before the eyed stage of development and can utilise such endpoints as mortality, developmental defects, hatching success and length at hatching. Procedures for using ELS testing are now so well established that partial and complete life-cycle toxicity testing with fish have been used extensively in the establishment of water quality criteria for aquatic life (eg. EPA 1996). Despite this, very little testing has been carried out in Australian marine waters using local species under local conditions. This was highlighted in the recent Australian Guidelines for water quality monitoring and reporting (ANZECC and ARMCANZ 2000). The ambon damselfish, *Pomacentrus aboinensis* (Pomacentridae), was selected for it's potential as a bioindicator of the

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effects of toxic contaminants in Great Barrier Reef waters. It is widespread and abundant on the Great Barrier Reef, has frequently been used in field (eg. Jones 1990; Doherty and Williams 1988) and laboratory studies (eg. McCormick 1999; Job and Shand 2001) and is easily bred in captivity. McCormick (1999) considered it a "model" experimental animal as it can be induced to lay eggs on artificial substrate both in the field and the laboratory making collection of eggs a simple process. The eggs have a clear, uniform chorion making observations of development possible. All these basic factors suggest that *P. amboinensis* may be ideal for use in toxicity tests.

The chemical selected for testing in *P. amboinensis* was the organophosphorus insecticide chlorpyrifos [*O,* O-diethyl O-3, 5, 6-trichloro-2-pyridyl phosphorothioate]. This is a widely used, broad-spectrum insecticide, the use of which has increased mainly due to concerns about the adverse effects of organochlorine insecticides (Barron and Woodburn 1995). It is the most widely used insecticide in the sugar-cane growing regions of Queensland. It is estimated that 74,500 kg of active ingredient of chlorpyrifos is used annually by the Queensland sugar industry, which comprises approximately 90% of all insecticides used (Hamilton and Haydon 1996). Chlorpyrifos is considered a risk to aquatic systems due to its high toxicity to a range of aquatic species and relatively persistent nature (Racke 1993). Barron and Woodburn (1995) reviewed the ecotoxicology of chlorpyrifos and found that it is acutely toxic to marine fish at concentrations of 0.5 to 520 μg/L. Sublethal effects, such as reduced growth, have been shown in larval fathead minnows (*Pimephales promelas*) at chlorpyrifos concentrations as low as 0.5 μg/L (Brazner and Kline 1990), while Jarveinen et al. (1983) found reduced maturation and reproduction in *P. promelas* at 0.63 μg/L.

Pomacentrus amboinensis embryos were exposed under controlled conditions to a range of concentrations of chlorpyrifos and monitored for survival and sublethal effects such as the incidence of developmental abnormalities during early life history. The potential use of this species as a sensitive bioindicator of the effects of toxic contaminants in Great Barrier Reef waters is assessed.

MATERIALS AND METHODS

Pomacentrus amboinensis eggs used in this experiment were collected from the captive breeding population maintained at the School of Biological Sciences, James Cook University (JCU) in Townsville. As *P. amboinensis* is a protogynous hermaphrodite the males guard demersal nests during a summer breeding season and associated with each nesting site are between one and six females. Natural nests are readily replaced with artificial nesting surfaces consisting of half a 150 mm diameter terracotta water pipe (300 mm long), split lengthwise. These breeding associations can readily be transplanted to the laboratory, where breeding pairs can be established and the fish induced to spawn throughout the year. Females can produce up to 3000 eggs, 2-3 times a weeks which are laid in a single layer and form discrete patches that can be readily collected by removing the artificial nesting surfaces and replacing it with new nesting material.

Eggs from a single clutch, attached to the nesting material were collected from JCU early each morning and transported in 50 L of aerated seawater to the aquarium facility (an open flow-through system) at the Australian Institute of Marine Science (AIMS). At AIMS the eggs were carefully removed from the nesting substrate and separated from each other using fine dissecting needles. All eggs were checked to ensure that only healthy, fertilised eggs were used for the toxicity testing.

Test chambers were 900 mL glass crystallising dishes placed in a circulating constant-temperature water bath. Chambers were covered to reduce evaporation and were not aerated. A range of chlorpyrifos concentrations was made up by adding appropriate amounts of technical grade chlorpyrifos (99.5% w/w purity; DowElanco Australia Ltd.) in 50μL of acetone to the exposure chambers containing 700 mL of 1 μm filtered seawater. There were eight nominal chlorpyrifos concentrations – 50, 125, 250, 500, 750, 1000, 1250, 2000 μg/L plus a control, which received 50μL of acetone without chlorpyrifos and there were triplicate samples for each treatment and control. Water quality parameters were monitored regularly during the experiments and were as follows: dissolved oxygen > 85% saturation, pH 8.1±0.1, temperature 27±1°C and salinity 33.3±0.2 ‰.

Static toxicity tests of 144 hr-duration were carried out to determine the effect of chlorpyrifos toxicity on embryos and larvae of *P. amboinensis*. By the end of 144 hr exposure period all embryos had hatched and mortality was so high at the highest concentrations that it was decided to end the test. Fertilised eggs less than 10 hr old were randomly assigned to each of the test chambers containing chlorpyrifos and the control until there were 20 eggs in each. A pilot study was conducted under these standard test conditions to determine breakdown of chlorpyrifos and it was found that between 72-86 % of the added chlorpyrifos remained after 6 days (Humphrey, unpublished data). Hence nominal concentrations were assumed to be a reliable measure of actual chlorpyrifos exposure. The numbers of dead, immobilised or abnormal animals in each test chamber were counted after 72 and 120 hr. Eggs were examined under a stereomicroscope (60 ×) and the methodology for detecting the incidence of abnormal embryos follows that of Westernhagen et al. (1988). All embryos were observed within a 2 hr period. Dead embryos (no heartbeat) when found were removed promptly. After 96 hr, heart rates were measured over one minute in five embryos from each of the treatments using a Hi-8 video system connected to a stereo dissecting microscope.

Upon completion of hatch (~120-144 hrs after spawning), the total numbers of larvae in each replicate were counted. Dead or deformed (spinal curvature, melanism, craniofacial and skeletal malformations) larvae were counted and subtracted from the total for a determination of viable hatch using the formula: -

$$Percent\ viable\ hatch = \frac{no.of\ larvae - no.of\ abnormal\ larvae}{no.of\ fertile\ eggs} \times 100$$

At this point the remaining larvae from each chamber were killed by immersion in ice water and their standard lengths (SL – from the anterior margin of the mouth to the tip of the hypural joint) measured.

Effects of exposure to a range of chlorpyrifos concentrations on embryonic development after 72 and 120 hr, length at hatch, hatch rate, viable hatch and heart rate were analysed by one-way ANOVA. Data in proportions were square-root arcsine transformed prior to analysis to meet the assumption of normality implicit in analysis of variance (Zar 1996). Results were considered to be significantly different if $p \leq 0.05$. If significantly different, the data were compared using the Tukey post hoc comparison test (Zar 1996), which were considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Pomacentrus amboinensis embryos exposed to chlorpyrifos showed higher incidences of developmental abnormalities (spinal curvature, melanism etc) compared with controls, and such abnormalities often resulted in death. After 72 hr exposure to chlorpyrifos there was a significant effect on embryonic development, as measured by an increased number of abnormal embryos, at all chlorpyrifos concentrations above 750 μg/L (Fig. 1). Furthermore after 120 hr many of the abnormal embryos died during this time period, and embryos that had appeared normal after 72 hr exposure began showing distinct abnormalities (Fig. 1). Reduced developmental rates and erratic movement within the chorion relative to the controls also accompanied these increases in abnormality rates and mortality.

Abnormalities in developing embryos are considered a sensitive and ecologically relevant measure of toxicant effect and have thus been used extensively in monitoring pollution effects in parts of the north Atlantic (Longwell and Hughes 1980; Cameron et al. 1992) and along the Queensland coast (Klumpp and von Westernhagen 1995). Many of these studies have demonstrated that abnormality rates can be directly correlated with the degree of contamination of water and parental fish stocks (Cameron and Berg 1992).

Chlorpyrifos exposure decreased the survival of *P. amboinensis* embryos and this was reflected in the hatching success, which decreased with increasing concentration of chlorpyrifos (Table 1). At the highest chlorpyrifos concentrations used (1250 & 2000 μg/L) no embryos survived to hatch. Chlorpyrifos also had an effect on the condition of the hatched fish, reflected in the rates of viable hatch (Table 1). The lowest nominal chlorpyrifos concentration that caused significant reduction in viable hatch, 125 μg/L, was considerably higher than that found in other studies (eg. Goodman et al. 1985). There are a number of factors that may explain this. Firstly the eggs were comparatively well developed (approximately 10 hr) by the time they were placed into the exposure chambers. It has been found that the early cleavage stages are considered the most sensitive of the newly formed zygote with further developmental stages generally showing greater resistance (Perry et al. 1991).

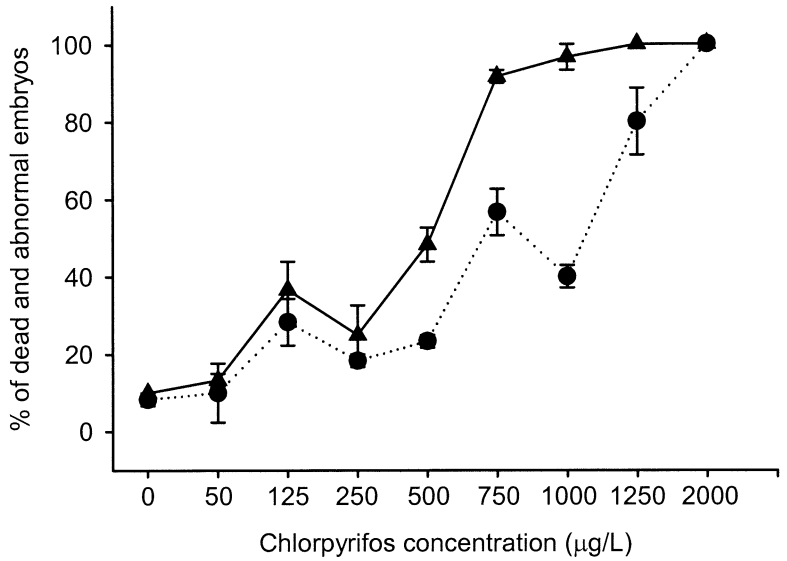

Figure 1. Percentage of dead or abnormal embryos after exposure to chlorpyrifos after 72 hr (●) and 120 hr (▲). After 72 hr there was a significant effect (α=0.05) due to chlorpyrifos at 750, 1000, 1250 and 2000 μg/L and after 120 hr there was a significant effect (α=0.05) due to chlorpyrifos at 125, 500, 750, 1000, 1250 and 2000 μg/L.

In addition, relative toxicity of chlorpyrifos in the present study could have been underestimated because nominal concentrations have been used and actual exposure concentrations are expected to be as much as 20-30% lower. Moreover, it has been found that reported toxicity values for chlorpyrifos are likely to be 2-5 times lower in flow-through systems with measured values than in static tests with nominal values (Borthwick et al. 1985; Mayer 1987). We conclude that the nominal values presented in this paper are probably quite conservative and that the actual toxicity of chlorpyrifos to *P. amboinensis* is likely to be greater than that presented here. Future development of this test procedure will involve the use of a flow-through system with measurement of contaminant concentration.

Length at hatching was as sensitive as percentage hatch and percentage viable hatch as a reliable measure of effect of chlorpyrifos on *P. amboinensis*, with significant effects being found at concentrations of 125 μg/L and above (Table 1). No lengths could be measured for fish in the higher concentrations as not enough fish survived. Length at hatching may be a useful indicator because previous studies have shown that chlorpyrifos effects growth in early life stage tests (Jarvinen and Tanner 1982). A number of other studies have also found growth to be one of the most sensitive endpoints in early life stage toxicity tests (Norberg and Mount 1985; Brazner and Kline 1990).

Table 1. Effects of chlorpyrifos exposure on hatch rate, viable hatch, length at hatch and heart rate of *Pomacentrus amboinensis* embryos and larvae.

Chlorpyrifos concentration (μg/L)	% Hatch	% Viable hatch	Length at hatch (mm SL)	Heart rate after 72 hr (beats/min)
Control	90.0	88.3	3.18	204
	(0)	(1.67)	(0.04)	(4.09)
50	86.7	86.7	3.12	222
	(1.67)	(4.41)	(0.03)	(4.97)
125	63.3*	56.7*	2.77*	217.2
	(7.26)	(6.01)	(0.04)	(1.81)
250	75.0	70.0	2.61*	193.6
	(7.64)	(10.4)	(0.04)	(1.46)
500	51.7*	41.7*	2.23*	178*
	(4.41)	(3.33)	(0.06)	(2.17)
750	8.33*	3.33*	-	153.6*
	(1.67)	(1.67)		(1.39)
1000	3.33*	0*	-	134.8*
	(3.33)*	(0)		(0.08)
1250	0*	0*	-	131.6*
	(0)	(0)		(1.16)
2000	0*	0*	-	-
	(0)	(0)		

Each entry represent the average value for each treatment and numbers in parentheses denote standard errors. * denotes values that are significantly different from controls. Data for hatch and viable hatch are shown as untransformed means, but analyses were based on arcsine square root transformed data.

Heart rate in embryonic *P. amboinensis* was a less sensitive indicator of chlorpyrifos toxicity. Heart rate was significantly affected at chlorpyrifos concentrations at or above 500 μg/L with heart rate in the controls of 204 beats per minute being reduced to 178 beats per minute at 750 μg/L and 131.6 beats per minute at 1250 μg/L (Table 1). However in other studies heart rate has regularly been shown to be a sensitive endpoint (eg. Handy and Depledge 1999) and has been used to monitor toxic effects of effluents (Weis et al. 1989).

This study has shown that development in early life stages of *P. amboinensis* has definite potential to be used as a sensitive bioindicator of the effects of toxic contaminants in the coastal waters of the Great Barrier Reef. It is a common and widely distributed fish on the Great Barrier Reef that can easily be bred throughout the year in captivity. The eggs produced can easily be harvested and observed for effects such as physiological response and morphological abnormalities. At present we are using this species in laboratory bioassays to test the toxic potential of common contaminants along the north Queensland Coast. This study has demonstrated that *P. amboinensis* embryos are relatively sensitive to chlorpyrifos, the commonly used insecticide in this region.

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