Cyanide fishing has been demonstrated to kill corals and to contribute to high delayed mortality of marine aquarium fish. Moreover, Cervino *et al.* (2003) demonstrated that sodium cyanide at concentration of 50 mg $1^{-1}$ of cyanide ion for one to two minute exposures caused mortality to corals *(Acropora millepora, Goniopora* sp., *Favites abdita)* and anemones *(Aiptasia pallida)*. Cyanides are extremely toxic to fish, but about 50% of the fish survive the initial exposure if rapidly moved to clean water (Rubec, 1986). Concentrations greater than 5 mg $1^{-1}$ are lethal under exposure time exceeding several minutes.

AMEQC-WG (1998) recommended that interim water quality criterion for the protection of marine aquatic life for free cyanide in seawater is 7.0 µg $1^{-1}$. This concentration in marine waters is intended to protect all forms of marine life and all aspect of the marine life-cycle in the ASEAN region. However, the AMEQC-WG (1998) also states that there is still data gap on the acute and chronic effect of cyanide to tropical fish and invertebrates. Hence, the present study tries to fill the data gap on toxicity of cyanide (KCN) on ornamental coral fish, *Chromis viridis.*

## MATERIALS AND METHODS

Ornamental coral fish, *(Chromis viridis* Cuvier), were obtained from commercial supplier. The fish were acclimatized to laboratory conditions and fed natural food (mysid shrimp) for at least two weeks before they were used in any experiment. They were not fed 24 hours prior to and during the tests. During acclimation period, fish mortality was maintained not to exceed 10%, otherwise the fish batch was discarded.

A 1000 mg $1^{-1}$ potassium cyanide stock solution (KCN) was prepared according to method 4500-CN-E-3b, (APHA, 1992). Test solutions were prepared from intermediate stock solution of 100 mg $1^{-1}$ $CN^-$. A 10,000 mg $1^{-1}$ stock solution of cadmium was prepared by dissolving 17.909 g of cadmium chloride ($CdCl_2.H_2O$ EMERCK, Art. 2011) in 1000 ml of distilled water. A series of test Concentrations were prepared by diluting an appropriate amount of stock solution.

### Dosing concentrations of exposure

Static-renewal toxicity tests were conducted according to the ASTM (1992) procedures with some modifications. The tests were conducted in three replicates. Renewal of seawater was conducted every 24 hours, based on previous study that free cyanide and total cyanide were lost from test solution during a 96-h exposure period (Otico *et al.*, 1999). The tests were conducted in 401 glass aquaria filled with 201 of seawater and immersed in a running water system. The glass aquaria were fitted with flexi-glass lids. A range-finder test was performed to test the sensitivity of organisms at five concentrations, each differing by a factor of 10: *i.e.* 0, 0.1, 1.0, 10.0, 100.0 and were run for 96 hours. The purpose of range-finder test was to determine the upper and lower limits of cyanide concentrations needed in performing the definitive toxicity test.

Definitive tests were performed based on the result of the range-finder test. The nominal test concentrations were consecutively 10,18,32,56, and 100 µg $1^{-1}$ $CN^-$. As a reference test, cadmium solutions were used at concentrations of 3.2, 5.6, 10.0, 18.0, and 32 mg $1^{-1}$. Ten fish were randomly picked and subjected to each of the treatment concentration and control. Observation offish mortality, temperature, dissolved oxygen and pH was made daily. Dead fish was removed immediately from the test solution. All tests were completed in 96 hours to determine the $LC_{50}$.

### Water conditions for exposure tanks

Temperature and salinity of the test were maintained under marine tropical condition. Water quality was monitored daily and kept within the following ranges: water temperature 26 - 29°C, pH 7.5 - 8.5, dissolved oxygen 1.52 - 5.83 mg $1^{-1}$, and salinity 33 - 37 psu. Lighting was based on natural condition that was approximately between 12 h light and 12 h dark.

### Statistical test

The 96-h median lethal concentration (96-h $LC_{50}$) of cyanide and cadmium with their 95% confidence limits were calculated based on

Low and no-effects observed concentrations (LOEC and NOEC) of cyanide and cadmium were calculated based on nominal chemicals concentration. LOEC and NOEC values of cyanide for *Chromis viridis* were 56 µg $1^{-1}$ and 32 µg $1^{-1}$, respectively. While LOEC and NOEC values of cadmium for the fish were 3.2 mg $1^{-1}$and <3.2 mg $1^{-1}$, respectively.

## DISCUSSION

Cyanide is known to impair enzyme systems that facilitate oxygen metabolism *{e.g.* cytochrome oxydase) and other physiological functions in fish and invertebrates, and to damage the liver, spleen, heart and brain of the fish (Dampster and Donalson, 1974). Early research found that cyanide to be acutely toxic at concentration greater than 100 - 300 µg $1^{-1}$ causing death within 96 h (Doudoroff, 1980 *in* Rubec, 1986). Chronic toxicity also occurs when fish exposed to cyanide do not die within 96 h, but suffers stress which leads to their subsequent death. Prolonged exposure to low concentrations of hydrocyanic acid, HCN, (5 -10 µg $1^{-1}$) causes adverse effects on fish egg, fry and adult fish, including reduction of hatching success and survival, reduction of growth, impairment of swimming performance, and inhibition of reproduction (Rubec, 1986). Rubec (1986) also stated that many of the above effects can be traced to the fact that cyanide interferes with oxygen metabolism by blocking key enzyme systems such as cytochrom oxydase, reduces the capacity of hemoglobin to carry oxygen in the blood and blocks enzymatic pathways in the liver.

Present study shows that when KCN is dissolved in water, it dissociates to HCN that is rapidly taken up by the fish. Within a few hours after exposure, an enzyme called thodanase (thiosulfate sulfur transferase) converts the HCN to thyocyanate ($SCN^-$). Fish exposed to $SCN^-$ shows sign of convulsion, gasping, loss of equilibrium and buoyancy, flaring of the opercula, and within minutes, cessation of ventilation and extreme rigor.

Few Studies have been done on the effect of cyanide on coral fish both in Indonesian and in Southeast Asian regions. First study on marine fish confirmed that cyanide was very harmful to milkfish, *Chanos chanos* (Otico *et al.*, 1999). The present study was the first attempt to fill a gap on toxicity of cyanide to ornamental coral fish in Indonesia. Under static renewal toxicity test, cyanide was very toxic to *Chromis viridis* with $LC_{50}$ (at 96 h) of 41.3 µg $1^{-1}$. This cyanide concentration was 300 times more toxic to *Chromis viridis* than that reference test, Cd (96-h $LC_{50}$ of Cd, 13.3 mg $1^{-1}$). Compared to previous study (Otico *et al.*, 1999), juvenile of *Chromis viridis* was more sensitive to cyanide than the milkfish fry *(Chanos chanos).* The 96-h $LC_{50}$ of cyanide for milkfish fry (1.0 - 1.5 cm, 0.01 -0.02 g wet weight) was 0.53 mg $1^{-1}$.

From the above studies it was apparent that estuarine and coral fish had different sensitivity to cyanide contamination. The study also supports general hypothesis that toxicity of cyanide differs for different fish sizes, ages and species. This evidence also indicated that using non-local or non-endemic species to develop water quality criteria for cyanide and other toxic substances should be done cautiously.

Our sample of fish *(Chromis viridis)* was obtained from a commercial aquarium distributor. It was assumed that the fish was caught using environmentally friendly method. If this were true then the fish would be really sensitive to cyanide. In contrast, if the fish have had experience exposure to cyanide, then the fish might be more sensitive to cyanide. As Sprague (1985) stated that acclimation at one-third of the lethal level of cyanide, the fish became more sensitive by about 30% in the first week. With continued acclimation, their tolerance climbed back to the original level by the end of 3 weeks.

It was estimated that LOEC and NOEC of cyanides to C. *viridis* were at concentration of 56.0 µg $1^{-1}$and 32.0 µg $1^{-1}$, respectively. The lowest observable effect concentration (LOEC) value of cyanide for *Chromis viridis* (32.0 µg $1^{-1}$) indicates that the fish was very sensitive to cyanide contamination. For comparison, toxicity of cyanide to tropical marine fishes and invertebrates is presented in Table 3. Among the fishes that have been studied, C. *viridis* is very sensitive to

**Table 3.** Toxicity of cyanide to tropical and marine fish and invertebrates

| Test species | Life stage | Test duration | Effect measured | Test system | T (°C) | S (psu) | DO (ppm) | Con. ($\mu g\ l^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Milkfish (*Chanos chanos*) | Fry | 96 h | $LC_{50}$ | S-R, U | - | - | - | 530 | Otico *et al.* 1999 |
| Atlantic silverside (*Menidia menidia*) | - | - | $LC_{50}$ | FT, M | 25 | - | - | 59 | U.S. EPA, (1985) *in* AMEQC-WG (1998) |
| Coral fish (*Chromis viridis*) | Juvenile | 96 h | $LC_{50}$ | S-R,U | 26.0 – 28.5 | 33-37 | 3.74 ± 0.82 | 41.3 | Present study |
| Mussel (*Mytilus galloprovincialis*) | Embryo | 48 h | $EC_{50}$ | S, M | 20 | NS | - | 10.6 | Pavicic and Pihlar, (1983) *in* AMEQC-WG (1998) |
| Mussel (*Mytilus galloprovincialis*) | Veliger | 48 h | $LC_{50}$ | S, U | 20 | NS | - | 154 | Pavicic and Pihlar, (1983) *in* AMEQC-WG (1998) |
| Mysid (*Mysidopsis bahi*) | 24 h old | 96 h | $LC_{50}$ | FT, M | 24 | 30 | - | 113 | Lussier *et al.* (1985) *in* AMEQC-WG (1998) |
| Amphipod (*Ampelisca abdita*) | - | - | - | S,U | 20? | - | - | 704<br>1150<br>1220 | U.S. EPA, (1985) *in* AMEQC-WG (1998) |

Note: S = static, S-R = static-renewal, FT = flow-through, U = unmeasured (nominal), M = measured (actual), NS = natural seawater, Conc = lethal concentration

cyanide. The most sensitive among studied organisms is the larvae of blue mussel *(Mytilus galloprovincialis).* Further studies are needed to understand the response of fish during toxicity experiment. Aspects to be studied include feeding behavior (cannibalism), biochemical response to cyanide, and technical aspects involved in toxicity test using flow-through system in order to mimic natural condition.

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