Acute toxicity of cyanide (KCN) on two types of marine larvae: *Acropora* sp. planulae and D-veliger larvae of *Tridacna squamosa*

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Abstract. Cyanide fishing has been intensively used to catch aquarium fishes and fish for human consumption around coral reefs in the South East Asian countries, such as Indonesia. There is little known about the lethal effect of cyanide on the early life stages of marine organisms. We assessed mortality and morphological changes of *Acropora* sp. planulae and fluted giant clam (*Tridacna squamosa*) D-veliger larvae exposed to potassium cyanide (KCN) concentrations close to those used in cyanide fishing. The KCN concentration exposure treatments for coral planulae were 50, 100, 300, 600, and 1000 mg L\(^{-1}\); and those for giant clam D-veliger larvae were 18.875, 37.5, 75, 300, and 600 mg L\(^{-1}\). The 24 hour static-acute toxicity test was used with four replicates for each concentration. The 24h-LC50 was calculated based on Finney’s Probit Analysis Method, and the 24h-LC50 for coral planulae and giant clam veliger were 121.854 and 84.421 mg L\(^{-1}\), respectively. The D-veliger larvae of *Tridacna squamosa* were more sensitive to KCN exposure than *Acropora* sp. planulae. In addition to mortality, we observed that, in both the planulae and D-veliger larvae, morphological abnormalities increased in frequency and severity with increasing KCN concentration, even at the lowest KCN concentrations.

1. Introduction

Cyanide fishing has been intensively used to catch fish in and around coral reefs in many South East Asian countries, such as Indonesia. These fish are destined for the marine ornamental trade and human consumption, with one driver being the high market demand for live reef food fish trade in several Asian countries [1,2,3]. This illegal practice of poison fishing aims to stun the fish in order to capture it alive. When cyanide fishing, fishermen tend to use high concentrations (1.5-120 g L\(^{-1}\)) of sodium cyanide or potassium cyanide [4], produced by dissolving tablets in seawater. This solution is generally squirted into coral crevices to stun the target fish [5]. The high concentrations of cyanide used are not intended to be fatal to the target fish, but may cause lasting damage to internal organs of the target fish itself and have lethal or sub-lethal effects on other organisms. Despite the relatively low rate of coral degradation due to cyanide fishing alone compared to some other methods of destructive fishing [6] this poison can cause corals to bleach and kill polyps [7,8,9,10]. Corals can also be damaged when fishermen break them apart to extract the stunned fish [11]. The use of cyanide fishing can also have potentially devastating effects on smaller (non-target) fish and invertebrates in the surrounding coral reef habitat [12]. Giant clams are one invertebrate that...
can be impacted by the use of cyanide fishing [13,14,15]. It has also been reported [16] that habitat degradation due to destructive fishing (blast fishing and poison fishing) can have a negative effect on clam population dynamics, hampering the settlement and recruitment of giant clam larvae.

Corals (Ordo: Scleractinia) and giant clams (Family: Tridacnidae) reproduce sexually; the process begins with the spawning of gametes (egg and sperm cells). While some corals are brooders with internal fertilization, for most corals fertilization occurs in the water column, as for giant clams. In both modes of reproduction, the fertilized eggs develop into planktonic larvae. A single pair of broadcast-spawning adult corals can produce thousands of juveniles from one annual reproductive event [17]. Coral larvae at the planula stage and giant clam larvae of the D-veliger stage are both 6-7 days old when they begin to settle on the substrate; this settlement stage is a critical phase in the lifecycle of these benthic invertebrates [18,19]. When fishermen use cyanide to catch coral reef fish, the threat of coral death and of coral recruitment failure is unavoidable [11].

The toxicity of cyanide to corals has mostly been studied in the adult stage. Reported impacts include the inhibition of photosynthesis and calcium in Acropora cervicornis [20]; disruption of photosynthesis and loss of zooxanthellae in Pocillopora damicornis, Porites lichens, Stylophora pistillata, Acropora aspera, and Plesiastrea versipora [7,8,9]; mitochondrial membrane disruption in Pocillopora damicornis [21]; and mortality of Acropora millepora [22].

There are still very few studies of the toxicity of cyanide on the larval stages of coral and giant clams, despite the reported sensitivity of these early life stages to other pollutants [19,23,24,25]. In particular, there is limited information on the impact of high concentrations of cyanide on coral and giant clam larvae. Therefore, in this study, we aimed to assess the larval mortality and morphological abnormalities of Acropora sp. planulae and D-veliger of Tridacna squamosa larval exposed to concentrations of potassium cyanide (KCN) close to those generally used in cyanide fishing.

2. Materials and Methods

Experimental animals used were planula stage larvae (6-7 d) of Acropora sp. and D-veliger stage (6 d) larvae of Tridacna squamosa. The larvae were obtained through captive breeding (induced spawning) in the hatchery of the Universitas Hasanuddin Marine Science Station on Barranglompo Island, Makassar, South Sulawesi, Indonesia. Spawning of Acropora sp. was induced through flow-through physical induction in March 2016 [27]. The spawning of T. Squamosa was induced through a combination of physical and chemical treatment methods in August 2016 [18]. Prior to the experiment (up to day 6-7 for coral larvae and up to day 6 for clam larvae) the larvae of each species were kept in 1000 L tanks filled with filtered seawater (1μm mesh size) and aerated. Zooxanthellae were added to the clam larvae tank on day 3 but not to the coral larvae tank.

A stock solution of 1000 mg·L⁻¹ potassium cyanide (KCN, Merck) was prepared according to method 4500-CN in [28]. The stock solution was diluted with filtered natural seawater to provide nominal concentrations of 50, 100, 300, 600, and 1000 mg·L⁻¹ KCN for the coral planula toxicity test and nominal concentrations of 18.875, 37.5, 75, 150, and 300 mg·L⁻¹ KCN for the D-veliger giant clam toxicity test. A standard range finding test was not conducted due to the limited number of larvae produced from the induced spawning. Therefore the highest concentration used in the planula toxicity test was decided based on the lowest average concentration used by fishers and the lower concentrations were chosen based on [22]. The highest concentration used for D-veliger test, 300 mg L⁻¹ KCN, was based on the results of the planula toxicity test, which was conducted first, and was the level at which 100% planula mortality was observed.

The 24-h acute toxicity test for each type of larvae was prepared according to the protocols in Part 8010 of [28]. There were four replicates for each of the five concentrations plus control, and larvae were randomly distributed between the 24 experimental units. Each experimental unit consisted of 10 newly hatched-larvae placed in a 30 mL glass vial filled with a test solution at the appropriate KCN concentration. Water was not changed during the 24-h exposure tests. Mortality was observed at 6-hour intervals and water quality was monitored at the beginning and the end of the toxicity testing. A coral planula stage larva was considered dead when one or more of the following
diagnostic conditions was observed: no movement was observed; the larva had shrunk; the larva appeared fuzzy as a result of disintegrated or ruptured epidermis [29]. Similarly, a D-veliger stage clam larva was considered dead when one or more of the following diagnostic conditions was observed: no movement was observed; the soft tissue was transparent; the shell was empty because the mantle tissue and zooxanthellae had been expelled. Morphological abnormalities were observed using a microscope with 10x10 magnification and photographed at the end of the experiment. All glassware was initially washed with detergents, soaked in 10% HNO₃ overnight, and rinsed with distilled water.

Median lethal concentration (24 h-LC₅₀) and 95% fiducial confidence interval were calculated using Finney Probit Analysis [30]. Differences in mortality between the treatments and the control were assessed using the Dunnet Test. All results were presented as nominal concentrations.

3. Results and Discussion

3.1. Mortality

The water quality parameters recorded during the toxicity testing were: dissolved oxygen 5.9-6.3 mg L⁻¹; temperature 25.3-25.7°C, salinity 34-35 ppt and pH 8.1-8.5. All water quality parameters during the toxicity testing met the criteria of optimum growth and survival for tropical marine invertebrates [31]. Mortality occurred in all experimental units under all exposure concentrations. However, mortality in the control treatments was less than 10% for both planulae and D-veliger larvae. At the highest concentrations (600 and 1000 mg L⁻¹ KCN), planula mortality reached 100% within the first two (2) hours of exposure; at the lower maximum concentration of 300 mg L⁻¹ KCN, D-veliger mortality had reached 100% by six (6) hours of exposure. The concentration-response relationship showed the average mortality increased with increasing KCN concentrations in both tests (Figure 1).

![Graph](image-url)

**Figure 1.** Concentration-response relationship after 24 h exposure (*= significant differences, Dunnett Test, 2-sided, p<0.05).

The Dunnett test results showed that the mortality of planulae and D-veliger larvae was significantly higher than the control at 300 mg L⁻¹ KCN and above, and 75 mg L⁻¹ KCN and above,
respectively. The median lethal KCN concentrations (24 h-LC50) were 121.854 mg L\(^{-1}\) for planulae and 84.421 mg L\(^{-1}\) for D-veliger larvae (Table 1). Based on the value of 24 h-LC50, the D-veliger larvae *Tridacna Squamosa* were more sensitive to cyanide than the planulae of *Acropora sp.*

Table 1. Median 24h lethal concentration (24 h-LC50) of KCN to *Acropora sp.* planulae and *Tridacna squamosa* D-veliger stage larvae

| Test                              | 24 h - LC50 (mg L\(^{-1}\)) | 95% Fiducial Confidence Interval |
|-----------------------------------|------------------------------|---------------------------------|
| Planulae (*Acropora sp.*)         | 121.854                      | Lower 80.058, Upper 185.471     |
| D-veliger (*Tridacna squamosa*)   | 84.421                       | Lower 51.637, Upper 138.021     |

3.2. Morphological abnormality

Morphological abnormalities in larvae of *Acropora sp.* and *Tridacna squamosa* exposed to cyanide were photographed. A representative selection is shown in Figure 2. Normal elongated-shape planulae were observed in the control and 50 mg L\(^{-1}\) KCN treatments. After exposure to concentrations of 100 and 300 mg L\(^{-1}\) KCN, the planulae started to exhibit mortality during the first six hours of exposure, indicated by cell deformation and rupture. Oily substances began to be released from the planula cell at 300 mg L\(^{-1}\) KCN; the onset of this phenomenon coincided with a statistically significant increase mortality as defined by the Dunnett Test. At the higher concentrations of KCN (600 and 1000 mg L\(^{-1}\) KCN), all planulae died within two hours of exposure; the planulae cells began to disintegrate and form an organic stock (Figure 2 A.).

In contrast to the coral planulae, there were no morphological abnormalities (shell deformations) detected in D-veliger *Tridacna squamosa* exposed to KCN. However, some behavioural abnormalities and lower zooxanthellate abundance were observed (Figure 2 B). All D-veliger larvae were actively moving in the control; the larvae were slightly less active in 18.875 mg L\(^{-1}\)-KCN, but the algal symbiont (zooxanthellae) were fully present in both control and 18.875 mg L\(^{-1}\) KCN treatments. At higher concentrations, 37.5 and 75 mg L\(^{-1}\) KCN, there was less movement and fewer zooxanthellae were observed inside the translucent shells of the larvae. At the 150 and 300 mg L\(^{-1}\) KCN concentrations, all zooxanthellae were released from the tissues of the D-veliger larvae. Broken shells were also observed in the highest exposure (300 mg L\(^{-1}\) KCN) treatment.

3.3. Discussion

High concentrations of potassium cyanide (KCN) or sodium cyanide (NaCN) has been squirting out into the coral reefs area during the cyanide fishing. In this study, we were assessing high concentrations of KCN exposed to larval stage of *Acropora sp.* planulae and *Tridacna squamosa* D-veliger. We found median lethal concentration (24 h-LC50) of KCN exposed to D-veliger (84.421 mg L\(^{-1}\)) was lower than those exposed to planulae (121.854 mg L\(^{-1}\)), indicating high concentrations of KCN was more toxic to D-veliger larval stage of *T. squamosa* than to planulae of *Acropora sp.* While no information could be found on cyanide toxicity to giant clam, there have been several studies on corals. Table 2 presents key findings from a number of studies on the acute effects of cyanide exposure on corals and other aquatic organisms.

There was only one unpublished (grey literature) study found on the acute effect of sodium cyanide (NaCN) to the planula stage of coral *Stylophora pistillata* (https://repository.seafdec.org.ph/bit stream/handle/10862/2147/2147-Mingoa-LicuananSS2007-AEM37.pdf) and there were none done on giant clam larvae and adult stages. Lethal and sub-lethal effects of cyanide to coral ranged from loss of zooxanthellae and coral bleaching to mortality. An early study in the 1970’s [20] revealed calcification inhibition and photosynthesis impairment occurred when colonies of the corals *Acropora cervicornis* and *Acropora formosa* were incubated for 1-2 h in > 4.8 mg L\(^{-1}\) NaCN. Research using high concentrations of cyanide in the 1990’s [9]
observed loss of zooxanthellae, bleaching and mortality of *Pocillopora damicornis* and *Porites lichens* coral fragments at 5203 mg·L⁻¹ CN⁻³.

Figure 2. Morphological abnormalities in larvae exposed to KCN: (A) *Acropora sp.* planulae and (B) *Tridacna squamosa* D- veliger stage larvae
| Test species | Life stage | Test chemical/ duration | Effect measured | Concentration | Reference |
|--------------|------------|-------------------------|----------------|--------------|-----------|
| Coral, *Acropora* spp. | Planula | KCN, 24 h, static test | LC50, morphological abnormality | 121.854 mg L\(^{-1}\) | present study |
| Giant clam, *Tridacna squamosa* | D-veliger | KCN, 24 h, static test | LC50, morphological abnormality | 84.421 mg L\(^{-1}\) | present study |
| Mussel, *Mytilus galloprovincialis* | Veliger | CN, 48 h, static test | LC50 | 154 µg L\(^{-1}\) | [32] |
| Coral, *Stylophora pistillata* | Planula | NaCN, 24 h | dead and grossly deformed | 5 mM (245.036 mg L\(^{-1}\)) | http://www.haereticus-lab.org/wp-content/uploads/2016/10/ecotox-coral-planula-tox.pdf |
| Coral fragment, *Pocillopora damicornis* | Adult | CN\(^{-1}\), 1-30 min (incubation), observations 12 h and 24 h after incubation | Zooxanthellae loss, discolouration, mortality | 2x10\(^{-1}\) M (5203.48 mg L\(^{-1}\)) | [9] |
| Coral fragment, *Porites lichens* | Adult | CN\(^{-1}\), 1-30 min (incubation), observations during incubation time | all colonies died and discoloured | 2x10\(^{-1}\) M (5203.48 mg L\(^{-1}\)) | [9] |
| Coral colony, *Acropora cervicornis* and *A. formosa* | Adult | NaCN, 1-2 h incubation | Photosynthesis and calcification inhibited | >1x10\(^{-4}\) M (>4.9 mg L\(^{-1}\)) | [20] |
| Coral fragment, *Acropora millipora* | Adult | NaCN, exposed to cyanide for 60 or 120 s by directly dipping fragments into cyanide solution | mortality within 3 weeks at lower concentrations and 1 week at highest concentration; mild to severe bleaching, tissue detachment, swollen tissue | 50, 100, 300, or 600 mg L\(^{-1}\) | [22] |
| Coral tissue, *Pocillopora damicornis* | Adult | KCN, 4 h | EC50- 3 h mitochondrial membrane potential | 253.6 µg L\(^{-1}\) | [21] |
| Coral fragment, *Stylophora pistillata* and *Acropora aspera* | Adult | NaCN, 24 h | Photoinhibition and photosynthesis electron transport in zooxanthellae ceased | 2x10\(^{-1}\) M (9801.4 mg L\(^{-1}\)) | [8] |
| Coral fragment, *Plesiastrea versipora* | Adult | Incubation for 3 h | Photosynthesis disruption, coral discolouration and bleaching | >1x10\(^{-3}\) M (>0.49 mg L\(^{-1}\)) | [7] |
| Coral fish, *Chromis viridis* | Adult | KCN, 96 h, static-renewal | LC50 | 0.0413 mg L\(^{-1}\) | [4] |
Table 2: Summary of lethal cyanide concentrations for various species and life stages of adult corals.

| Test species | Life stage | Test chemical/ duration | Effect measured | Conceni, mg L⁻¹ | Reference |
|--------------|------------|-------------------------|-----------------|-----------------|-----------|
| Freshwater fish, Catla catla and Cirrhinus mrigala | Adult | NaCN, 96 h | LC50 | 0.280 - 0.458 | [33] |
| | | NaCN, 48 h | LC50 | 0.280 | [33] |
| | | NaCN, 24 h | LC50 | 0.280 | [33] |

Another study from the 1990’s [8] concluded that the concentrations of cyanide (9801 mg.L⁻¹ NaCN) squirited from bottles directly on to coral by fishermen will cause bleaching (leading to mortality) as a result of disruption of the photosynthetic electron flow in the symbiotic algae, causing the expulsion of zooxanthellae from the tissue of Stylophora pistillata and Acropora aspera. A more recent study using a high concentration of cyanide (50-600 mg.L⁻¹ CN⁻) showed that in situ and in vitro experiments resulted in the mortality of Acropora millipora fragments due to cellular damage [22]. It seemed that the cellular damage in coral tissue resulted in a loss of membrane integrity causing the release of zooxanthellae from the gastrodermal cells. This pathway was consonant with our results at higher concentrations (300, 600, and 1000 mg L⁻¹ KCN) leading to cell membrane rupture in Acropora sp. planulae. The experiments in [22] also found that Acropora sp. was the most sensitive to cyanide exposure of all coral genera studied (Goniopora sp., Favites abdita, Trachypyllia geoffrio, Plerogyra sp., Heliofungia actiniformis, Euphyllia divisa, and Sarcophytton sp.), as indicated by more rapid signs of stress and loss of zooxanthellae.

A recent study [21] using in vitro toxicity tests on tissue of the coral Pocillopora damicornis, found that a fairly high cyanide concentration (3h-LC50 = 5.1 mg.L⁻¹ KCN) was required to kill 50% of coral cells within 3 hours of exposure. Compared to [21], our 24-h LC50 of 121.9 mg L⁻¹ KCN for the exposure of Acropora sp. planulae was quite high, indicating that coral planulae might be somewhat more resistant to cyanide exposure than adult stages. However the methods used and the species were different; furthermore, it might be inappropriate to make a comparison between adult coral tissue and live coral planulae.

No previous publications on the acute toxicity of cyanide to giant clams could be found. However, there are some studies on other molluscs. For example, a study on the effect of cyanide to bivalve, mussel (Mytilus galloprovincialis) [32]. This study found that the cyanide concentration that killed 50% of the mussel veliger larvae within 48 hours (48 h-LC50) was 154 μg.L⁻¹. Compared to this figure, the 24h-LC50 of KCN to D-veliger T. squamosa (84.421 mg L⁻¹) in our study was higher. This might indicated that veliger larvae of T. squamosa are more resistant to cyanide than mussel veliger larvae; the difference could also be related to the longer exposure time (48 hours compared to 24 hours) in [32].

One result of our study was that KCN appeared to be more toxic to the D-veliger larval stage of T. squamosa than to the planula stage larvae of Acropora sp. This may be due to the timing of the acquisition of algal symbionts (zooxanthellae) and the level of dependence on symbiosis with zooxanthellae. Acropora larvae are known to be lecithotrophs, larvae that can go through metamorphosis without relying on any exogenous feeding [34]. On the other hand, giant clam veliger larvae are planktrotrophs, which means that nutrition from the algal symbiont is required to enable the metamorphosis to subsequent life stages [35,36]. Since the main action pathway of cyanide toxicity appeared to be through the impairment and disruption of the symbiotic algae, it would seem logical that the D-veliger of T. squamosa would be more sensitive than the planulae of Acropora sp.

4. Conclusion
This study indicated that, although both were affected at levels below common concentrations used in cyanide fishing, veliger phase of Tridacna squamosa larvae were more sensitive than Acropora planulae to cyanide toxicity, based on the respective LC50 values. Morphological abnormalities were only observed in coral planulae, while in giant clam D-veliger stage larvae the shells retained their...
shape at all concentrations, and remained intact until rupturing when mortality occurred. Mortality and cell deformation of Acropora planulæ were significant at KCN concentrations of 300 ppm and higher. Mortality and loss of zooxanthellæ in D-veliger stage T. squamosa larvae was significant at KCN concentrations of 75 ppm and above. These results indicate that cyanide fishing could significantly impact larval survival and quality and thus have a negative impact on the recruitment of reef-building corals and reef-associated benthic invertebrates such as giant clams.

References
[1] Burke L, Reytar K, Spalding M and Perry A 2011 Reefs At Risk Revisited (Washington DC: World Resources Institute)
[2] Ferrol-Schulte D, Gorris P, Baitoningsih W, Adhuri D S and Ferse S C 2015 Coastal livelihood vulnerability to marine resource degradation: A review of the Indonesian national coastal and marine policy framework Mar. Pol. 52 163-171
[3] Halim A 2002 Adoption of cyanide fishing practice in Indonesia Ocean. Coast. Manage. 45 313-323
[4] Arifin Z and Hindarti D 2006 Effects of cyanide on ornamental coral fish (Chromis viridis) Mar. Res. Indonesia. 30 15-20
[5] Vaz M C, Rocha-Santos T A, Rocha R J, Lopes I et al 2012 Excreted thiocyanate detects live reef fishes illegally collected using cyanidea non-invasive and non-destructive testing approach PloS one 7 e35355
[6] Mous P, Pet-Soede L, Erdmann M, Cesar H et al 2000 Cyanide fishing on Indonesian coral reefs for the live food fish market-what is the problem (Collected essays on the economics of coral reefs) (Kalmar, Sweden: CORDIO, Kalmar University) 69-76
[7] Jones R J and Hoegh-Guldberg O 1999 Effects of cyanide on coral photosynthesis: implications for identifying the cause of coral bleaching and for assessing the environmental effects of cyanide fishing Mar. Ecol. Progr. Ser. 177 83-91
[8] Jones R J, Kildea T and Hoegh-Guldberg O 1999 PAM chlorophyll fluorometry: a new in situ technique for stress assessment in scleractinian corals, used to examine the effects of cyanide from cyanide fishing Mar. Poll. Bull. 38 864-874
[9] Jones R J and Steven A L 1997 Effects of cyanide on corals in relation to cyanide fishing on reefs Mar. Fresh. Res. 48 517-522
[10] Rubec P J 1986 The effects of sodium cyanide on coral reefs and marine fish in the Philippines, The First Asian Fisheries Forum (Manila, Philippines: Asian Fisheries Society) pp. 297-302
[11] Pet J S and Pet-Soede L 1999 A note on cyanide fishing in Indonesia Nat. Can. 100 1-10
[12] Donnelly R, Davis D and Lam M 2000 Socio-economic and biological aspects of the live reef food fish trade and its development in Solomon Islands (Canberra: Report to ACIAR) 52 pp
[13] Guest J R, Todd P A, Goh E, Sivolanganathan B S and Reddy K P 2008 Can giant clam (Tridacna squamosa) populations be restored on Singapore's heavily impacted coral reefs? Aquat. Conserv. 18 570-579
[14] King M and Faasili U 1999 Community-based management of subsistence fisheries in Samoa Fish. Manage. Ecol. 6 133-144
[15] Nuryanto A and Kochzius M 2009 Highly restricted gene flow and deep evolutionary lineages in the giant clam Tridacna maxima Coral Reefs 28 607-619
[16] Mingoa-Liciuanan S S, Gomez E D 2002 Giant clam conservation in Southeast Asia Tropic. Coasts. 3 24-31
[17] Baird A H, Guest J R and Willis B L 2009 Systematic and biogeographical patterns in the reproductive biology of scleractinian corals Ann. Rev. Ecol. Evol. Syst. 40 551-571
[18] Braley R 1993 Pros and cons of methodologies used in the hatchery and land-based nursery phase of giant clam culture, (Australia: ACIAR Proceeding Australian Centre for International Agricultural Research) pp. 87-87.
[19] Negri A, Webster N, Hill R and Heyward A 2001 Metamorphosis of broadcast spawning corals in response to bacteria isolated from crustose algae Mar. Ecol. Prog. Ser. 223 121-131

[20] Chalker B and Taylor D 1975 Light-enhanced calcification, and the role of oxidative phosphorylation in calcification of the coral Acropora cervicornis Proc. R. Soc. Lond. B 190 323-331.

[21] Downs C A, Fauth J E, Downs V D and Ostrander G K 2010 In vitro cell-toxicity screening as an alternative animal model for coral toxicology: effects of heat stress, sulfide, rotenone, cyanide, and cuprous oxide on cell viability and mitochondrial function Ecotoxicology. 19 171-184

[22] Cervino J M, Hayes R L, Honovich M, Goreau T J et al 2003 Changes in zooxanthellae density, morphology, and mitotic index in hermatypic corals and anemones exposed to cyanide Mar. Poll. Bull. 46 573-586

[23] Byrne M 2012 Global change ecotoxicology: identification of early life history bottlenecks in marine invertebrates, variable species responses and variable experimental approaches Mar. Environ. Res. 76 3-15

[24] Negri A, Vollhardt C, Humphrey C, Heyward A et al 2005 Effects of the herbicide diuron on the early life history stages of coral Mar. Poll. Bull. 51 370-383

[25] Negri A P, Harford A J, Parry D L and van Dam R A 2011 Effects of alumina refinery wastewater and signature metal constituents at the upper thermal tolerance of: 2. The early life stages of the coral Acropora tenuis Mar. Poll. Bull. 62 474-482

[26] Pechenik J A 1999 On the advantages and disadvantages of larval stages in benthic marine invertebrate life cycles Mar. Ecol. Progr. Ser. 177 269-297

[27] Yusuf S, Zamani N P and Jompa J 2014 Perkembangan Larva Dalam Embriogenesis Karang Acropora Hasil Pemijahan Ex-Situ TORANI: Journal of Fisheries and Marine Science 24 48-54

[28] APHA 1999 Standard method for the examination of water and wastewater 20th edition, (Washington : APHA, AWWA, WE) pp 4-26

[29] Reichelt-Brushett A J and Harrison P L 2004 Development of a sublethal test to determine the effects of copper and lead on scleractinian coral larvae Arch. Environ. Con. Tox. 47 40-55

[30] Finney D J 1971 Probit analysis, 3rd ed (New York : Cambridge University Press)

[31] Ellis S 1997 Spawning and early larval rearing of giant clams (Bivalvia: Tridacnidae) (USA : Center for Tropical and Subtropical Aquaculture)

[32] Pavicic J and Pihlar B 1982 Toxic effects of cyanides (including complex metal cyanides) on marine organisms (Frances : Workshop on Pollution of the Mediterranean, Cannes) pp 2-4

[33] Alavandi S and Hosetti B B 2013 Influence of cyanide on some antioxidant enzymes of freshwater fish, Cirrhinus mrigala (Hamilton) J. Agricult. Sci. 58 177-84

[34] Morse A N, Iwao K, Baba M, Shimoike N, Iwao K, Baba M, Shimoike K et al 1996 An ancient chemosensory mechanism brings new life to coral reefs Biol. Bull. 191 149-154

[35] Fitt W K, Fisher C R and Trench R K 1986 Contribution of the symbiotic dinoflagellate Symbiodinium microadriaticum to the nutrition, growth and survival of larval and juvenile tridacnid clams Aquaculture 55 5-22

[36] Mies M, Braga F, Scozzafave M S, Lemos D E L D and Sumida P Y G 2012 Early development, survival and growth rates of the giant clam Tridacna crocea (Bivalvia: Tridacnidae) Braz. J. Oceanogr. 60 127-133