Asian Journal of Biological and Life Sciences | Original Research

Effects of in situ firecrackers explosion on copepods (Crustacea, Copepoda) and *Siganus guttatus* (Pisces, Siganidae) larvae

Ephrime B. Metillo*, Sheldon Rey Boco, Mac Euan D. Malugao

Department of Biological Sciences, Mindanao State University-Iligan Institute of Technology, Iligan City 9200, Philippines.

*E-mail : ephrime.metillo@g.msuiit.edu.ph Contact No. : +63632214056

Submitted : 01.05.2016 Accepted : 13.07.2016 Published : 30.08.2016

Abstract

Underwater explosives are used in illegal fishing, military operations, harbor construction and seismic exploration for oil and gas. However, very little is known about the impact of underwater explosions on fish larvae and zooplankton. An *in situ* experiment was conducted to assess the effects of low intensity sound from firecrackers on copepod zooplankton and 20-day old rabbitfish (*Siganus guttatus*) larvae. Either rabbitfish larvae or copepods were ensonified with separate low, medium, and high explosions of firecrackers detonated inside a blast container placed in the middle of a 0.125 m^3 experimental cage with ambient seawater. Sound was recorded using a shockproof underwater video camera, and the recorded sound was converted into sound pressure levels in decibels (dB) and Pascals (Pa) using the Goldwave software. Mortality of copepods was determined using neutral red vital stain. No direct mortality effect was observed among fish larvae immediately after a blast, but across the three blast treatments 75-100% of larvae showed abnormal swimming behavior, abdominal distension, and bladder and intestinal injuries. Mortality of copepods increased with increasing level of explosion, and values reached up to 90%. The level of sound intensity in this study is several orders of magnitude lower than that at the core area of an average blast fishing explosion, but our results may reflect impact at the periphery of dynamited areas where reduced sound intensities may still cause high mortalities on copepods and fish larvae and very likely other zooplankton taxa.

Key words : explosives, fish larvae, Copepoda, zooplankton, *Siganus guttatus*, sound

INTRODUCTION

Apart from vision and olfaction, optimal audition is also a sensory and communication modality among organisms in the marine ecosystem [1,2]. However, anthropogenic activities often significantly increase the level of underwater sound, for instance, noise generated from recreational boating, dynamite fishing, channel and harbor construction, and seismic exploration for oil and gas [3,4,34]. Impacts of marine underwater explosions on animals have focused primarily upon the mortality of adult fish, turtles, and marine mammals[5,6]. This has increased awareness of the public on the destructive effects of underwater explosions [7], but the auditory impacts of coastal activities on marine organisms have been generally ignored or overlooked[4].

Dynamite or 'blast' fishing has been outlawed worldwide, but it remains a major threat to sustainable fisheries in parts of Asia [8,35] including the Philippines [9]. Impacts of blast fishing are usually documented on sessile macroscopic coral reef communities [10]. Despite their pivotal role in the trophic dynamics and structure of the marine ecosystem, zooplankton, particularly copepods and ichthyoplanktonic larval fish are rarely tested for their response to underwater explosion and its propagated blast wave and, if any, studies are mostly found in the grey literature [11,12]. A study has shown that because of their relatively small sizes, zooplankters are susceptible to underwater sound than the relatively larger animals as there is an inverse relationship between sound wave effects and body size [13]. In contrast, low intensity underwater explosions were reported not to reduce zooplankton abundance and fish embryo survival in Lake Pend Oreille, Idaho[11].

Vertebrates with gas-filled internal organs are vulnerable to and can be killed by underwater explosives [14]. However, underwater explosions do not seem to affect fish larvae [6], but a number of studies suggested otherwise [13,15,16, 17,18,19,20]. Mortality models to assess lethal impacts of lethal explosion on large juveniles and adult fishes can be applied to fish larvae as well[13,17,18,21,22]. However, to be able to assess lethal impacts and larval mortality with accuracy, empirical knowledge on underwater explosion effects on fish larvae is a requisite.

The effect of sound produced by underwater firecrackers explosion on marine zooplankton, particularly copepods and fish larvae, is still not clearly understood. Hence, this study is an attempt to determine the effect of firecrackers explosion within 0.25m radius on zooplanktonic copepods and rabbitfish (*S. guttatus*) larvae.

MATERIALS AND METHODS

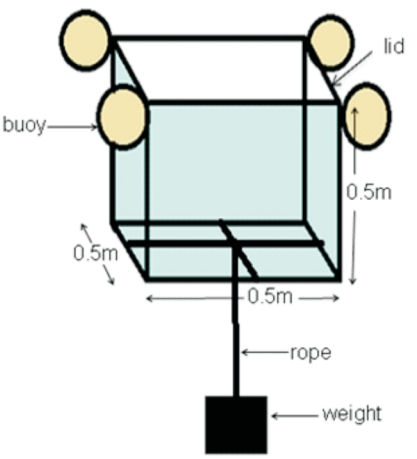


Figure 1: Floating cage used in the exposure of copepods and rabbitfish (*S. guttatus*) larvae to low, medium, and high intensity of firecrackers explosion.

Experimental box

All experiments were made using a transparent box with length, width, and height dimensions of 0.5m x 0.5m x 0.5m, respectively (Figure 1).The frame was made of wood and the faces including the lid of the box were made of 0.5mm thick plastic acetate. The box was filled with ambient seawater 5 cm below the brim so that the total volume of seawater in the cage amounted to 0.1 m^3. The box was made stationary at a depth of 1.5 m (chest depth) in a sublittoral area using enough weight attached at the center of the bottom face. Attached to each upper corners of the box are styrofor buoys to keep the box afloat.

Collection of Zooplankton

Zooplankters were collected using a conical plankton net (300µm mesh size; 0.5m mouth diameter)towed for 2-3 minutes near at the experimental site in Dalipuga, Iligan City, Mindanao, Philippines (8° 14' 48.63"- 8° 14' 59.07"N; 124° 14' 33.79"- 124° 14' 34.67" E) on 13-16 December 2013. The net was towed at a speed of ≤1 m s^{-1} to avoid damaging the animals. Before every tow, the net was rinsed out thoroughly to minimize accidental carryover of dead animals from earlier tows. Tow duration was kept as short as possible while still collecting an adequate sample size [23]. After each tow, the cod-end of the net was removed and its contents were transferred carefully with minimum turbulence into the experimental box. Before transferring the zooplankton the inside of the net was not hosed down as it can kill the animals or it can introduce dead animals into the cod-end.

Collection and preparation of S. guttatus larvae

Twenty two (22) days-old *S. guttatus* larvae were purchased from the Aquaculture Department of the Mindanao State University Naawan and transported within 30 minutes to the experimental site. Larvae were placed in a polyethylene terephthalate (PET) bag that was tied with trapped oxygen. Upon arrival, the bag with fish larvae was emptied carefully into a container and fish larvae were allowed to acclimatize for 20 minutes. Acclimation was confirmed when fish began swimming as a school. Fish larvae that were weak and dying were not included in the experiment. A plastic strainer was used to collect larvae in the container. Captured larvae were then transferred into a dipper with in situ filtered seawater. Finally, 15 larvae per replicate were poured into the experimental box.

Firecracker charge and detonation

Experiments with copepods and fish larvae were conducted during high tides and calm waters on 13-20 December 2013. Prior to experiments physico-chemical parameters (salinity, temperature, dissolved oxygen in % saturation and ppm, and pH) of the seawater were determined outside and inside the experimental box using portable meters (Atago [Japan] salinometer, Lutron PDO-519 [China] dissolved oxygen and temperature meter, Eutech [Germany] pH meter). Triangular firecrackers (EB and A Fireworks, Philippines) with known average gunpowder and cartridge (paper cover) weights (0.11 and 1.13 g, respectively) were used to produce underwater explosion. A dry detonation chamber made of 4 L polyethyleneterephthalate (PET) bottles with 1mm ply thickness was used to contain the explosion in the middle of the experimental box. The PET bottle prevented chemical contamination in the seawater medium. Detonation was started after placing test animals inside the experimental box. The experimental design comprised control (no explosion), and treatments comprising different intensity of explosion according to the amount of KNO_3 so that low, moderate and high sound intensity were set at 0.25g, 0.45 g, and 0.90 g KNO_3, respectively. Each control and treatment was replicated five (5) times. Sound was recorded underwater using a shockproof Panasonic® (Lumix DMC-FT30) underwater video camera positioned 0.1 m away from the detonation chamber inside the experimental box to get maximum recording of sound [24].All sound recordings were analyzed in the laboratory using the free software GoldWave version 6.21 [25]. Detonation started with a firecracker lighted at the mouth of the PET bottle and instantaneously dropped once lit. After each successful blast, the bottle was covered and removed to avoid contamination inside the experimental box and the working area.

Assessment of the effects of sound on test animals

Immediately after detonation, all test copepods were carefully concentrated into a plastic jar, and immediately stained with neutral red vital stain [23]. Stock solution was prepared by adding 0.1 g neutral red powder to 10 mL deionized water and slowly stirring the solution under dim light overnight to completely dissolve the powder. After preparation, the neutral red stock solution was stored in the dark at room temperature in a sealed amber borosilicate glass vial. After ensuring a final concentration of 0.15%, neutral red stain was added to test copepod samples that were then kept in a dark chamber. For samples with an exceptionally high number of animals (or in samples with high concentrations of phytoplankton or detritus), additional neutral red stock solution was added to increase stain uptake without causing harm to the animals. The water had to appear bright red and not pink (too little stain) or brown (too much stain). Test animals were allowed to take up the stain for 15 minutes at in situ temperature. Afterwards, samples were concentrated onto fine nylon mesh and rinsed briefly with 1um-filtered seawater to remove excess stain. Stained samples in nylon mesh were then placed flat and sample side up in Petri dishes and stored on ice in the dark.

In the laboratory, samples for analyses were acidified to pH <7 to develop the stain's color inside the animals. Acidification was done using any acidic solution (highly recommended: 1:10 1M HCl solution to sample volume ratio). Samples were then viewed under a dissecting microscope. Microscopy lighting was an important factor since excessive lighting caused stained animals to appear pale and unstained animals to appear pink. Dark field lighting was used in combination with a red overhead light to improve stain visibility of copepod nauplii, copepodites and adults. Animals alive at the time of staining are stained bright red in part or all of their tissues (mainly prosome tissue for copepods); animals dead before the staining will appear unstained or cloudy white. Percentage mortality of the copepods was quantified by dividing the total number of unstained (dead) copepods by the total number of unstained and stained (live) copepods, and the quotient multiplied with 100.

For fish larvae, a plastic strainer was used to capture the animals after a blast. The process was carefully done to minimize the stress, and avoid further damage. Captured larvae were immediately transferred into a small glass tank with ambient seawater for swimming and behavioural observation. Larvae were then poured into a plastic bottle and preserved with 10% formalin in filtered seawater. Samples were transported to the laboratory within 20 minutes for microscopic examination.

Data Analysis

One-way analysis of variance (ANOVA) was used to

determine variation among treatments followed by Tukey's Honestly Significant Difference *post hoc* test to determine statistical difference of mean values between treatments. Homoscedastic Student's t-test was used in pairwise comparison of means. Except for percentage data that were arc sin-transformed, other data were log-transformed prior to ANOVA to establish normality and homogeneity of data. All statistical analyses used the software SPSS version 11 [26].

RESULTS

Physico-chemical parameters and explosions intensity

The *in situ* physico-chemical parameters (Table 1) such as pH, temperature, salinity, and dissolved oxygen obtained during the experiment are within the acceptable standard values [27,28]. The values of pH ($F = 0.08$, df = 3, $p > 0.97$), temperature ($F = 0.68$, df = 3, $p > 0.58$), salinity ($F = 0.41$, df = 3, $p > 0.75$), and dissolved oxygen in percent saturation ($F = 1.59$, df = 3, $p > 0.24$) and in ppm ($F = 2.86$, df = 3, $p > 0.08$) were not significantly different across control and explosion treatments. Thus, these parameters were not confounding factors nor contributed to the mortality of copepods and fish larvae during the in situ explosion tests.

Goldwave software output images of the sound recordings during in situ firecrackers explosions are shown in Figure 2. The mean values of sound intensity and pressure levels for the low, medium, and high *in situ* firecracker explosions were 113 dB re 0.001 kPa and, 120 dB re 0.006 kPa, and 127 dB re 0.01 kPa, respectively.

Impact of in situ firecrackers explosion on copepod zooplankton

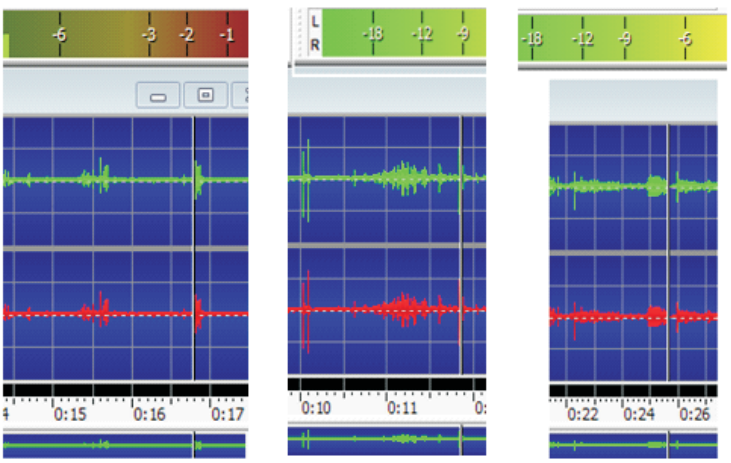
The neutral red stain was most suitable to accurately distinguish live copepods that stained red (Figure 3A) from dead individuals that did not take up the stain (Figure 3B). Mean copepod percent mortality were 9.8, 23.2, 36.0, 54.1 for control and low, medium and high intensity explosion treatments, respectively (Figure 4A). These values were significantly different ($F = 4.33$, df = 3, $p < 0.03$) indicating that the different levels of explosives have caused the mortality of copepods. Thus, percentage copepod mortality is directly dependent on the strength of the explosives.

Impact of in situ firecrackers explosion on fish larvae

Compared to intact control larvae (Figure 3C), individuals showed bloated swim bladder and distended abdomen at low blast charges, and abdominal distension and extrusion of intestine at medium and high explosion treatments (Figure 3D).The number of non-distended and distended fish larvae was significantly different (t-test, $p < 0.001$). Similarly, the number of larvae with distended stomach varied significantly ($F = 6.15$, df = 3, $p < 0.02$) across treatments due mainly to the control having unaffected individuals (Figure 4B). The number of larvae with distended abdomens was not statistically different among blast exposures ($p > 0.12$ for all). Similarly, the number of larvae with undistended

Table 1: Physical and chemical factors recorded during experiments testing the effects of low, medium and high levels of blast intensity on zooplankton copepods and rabbitfish (*S. guttatus*) larvae.

	Control	Low	Medium	High
Temperature (°C)	29.25 ± 0.47	29.78 ± 0.53	30.03 ± 0.42	30.08 ± 0.41
Salinity (PSU)	27.75 ± 1.80	26.25 ± 0.63	27.50 ± 0.96	26.63 ± 0.63
Dissolved O_2 (% saturation)	94.18 ± 4.55	93.78 ± 3.86	87.18 ± 2.14	86.85 ± 0.87
Dissolved O_2 (ppm)	7.51 ± 0.39	7.12 ± 0.32	6.59 ± 0.17	6.56 ± 0.10
pH	7.65 ± 0.11	7.73 ± 0.15	7.68 ± 0.12	7.69 ± 0.11



A. 113dB **B. 120dB** **C. 127dB**

Figure 2: Audiograms generated by the software Goldwave and mean sound intensity in decibels (dB) for low (A), medium (B) and high (C) underwater firecrackers explosions.

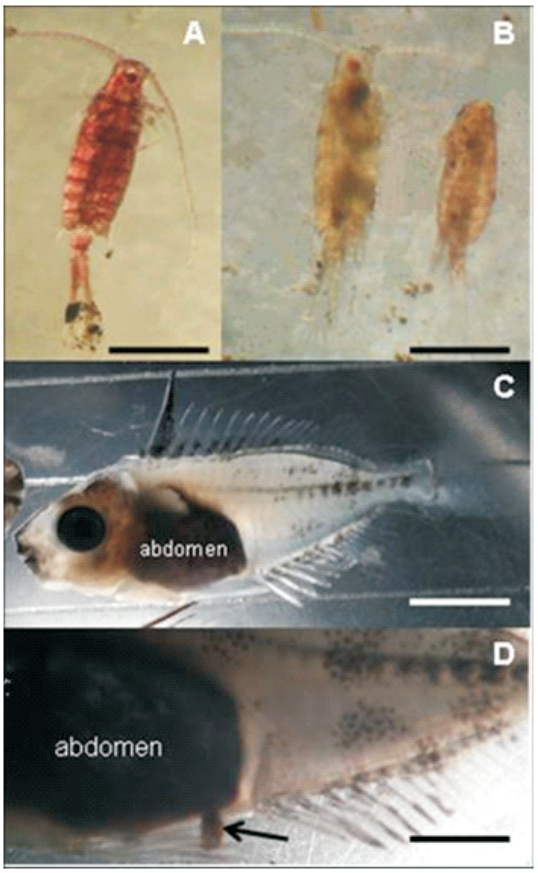


Figure 3: Effects of different intensities of *in situ* firecrackers explosion to copepod zooplankton and rabbitfish (*S. guttatus*) larvae. A. Copepods that remained alive stained red. B. Dead copepods were unstained. C. Control rabbitfish larva with showing intact abdomen. D. Rabbitfish larva showing distended abdomen and extruded intestine (black arrow). Horizontal scale bars: A, B = 0.5mm, C = 3.2 mm, D = 1.7 mm.

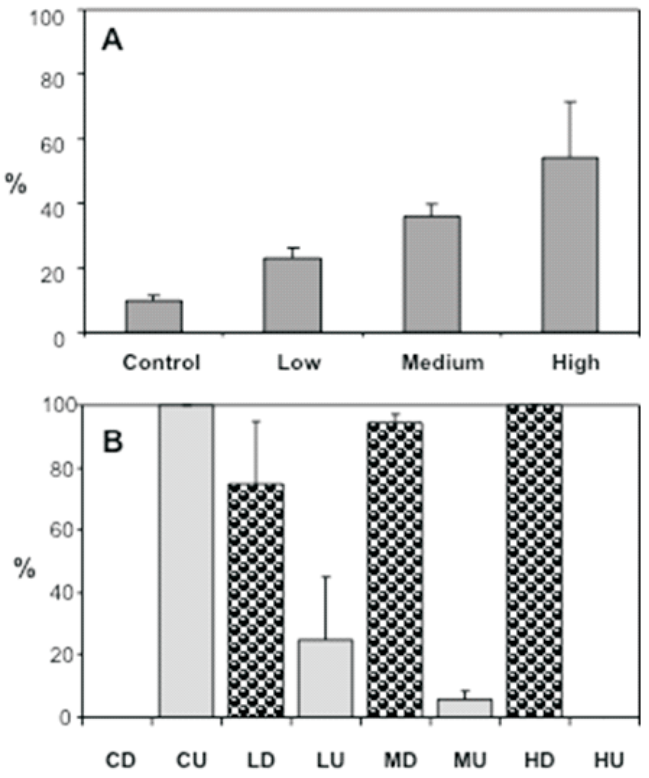


Figure 4: Effects of low (L), medium (M), and high (H) intensity of *in situ* firecracker explosion on zooplankton copepods and rabbitfish (*Siganus guttatus*) larvae. A. Percent mortality of copepods. B. Percent distended (D) and undistended (U) abdomen of *S. guttatus* larvae (B). C = control. Error bars = standard error.

Table 2: Comparison of charges and type of explosives between the present study and published studies.

Source	Weight of Charge (kg)	Number of Shots	Number Killed	Weight Killed (kg)
Aplin (1947):				
60% petrogel	18	1	-	64
Coker and Hollis (1950): HBX2	204	1	606	111
	545	1	262	79
Falk and Lawrence (1973): geogel	4.5	1	400+	-
Young and Willey (1977): (a) TNT	4.1	1	1000	-
(b) baratol	1.1	1	50	-
This Study KNO_3	<0.001	1	none	none

abdomen varied significantly (F = 8.37, df = 3, p< 0.01) with control individuals unaffected, few with undistended abdomens in low and medium blasts, and zero undistended (all distended) in the high blast treatment.

DISCUSSION

Copepods were ideal experimental animals in the present study as they dominated samples with 80-90% contribution to the total abundance of zooplankton, and they took up the neutral red stain. Other zooplankton taxa were excluded since the determination of the mortality of other zooplankton taxa using neutral red must be attempted only after testing that the targeted taxon take up the stain and retain it for an adequate period during preservation and subsequent microscopic analysis [23]. Indeed, uptake of neutral red is zooplankton taxon specific. For instance, we originally planned to use the brine shrimp, *Artemia* sp., nauplii but these animals did not take up the stain at all.

Our results on copepods concur with the report stating that intense sounds can heavily affect smaller animals as there is an inverse relationship between sound wave effects and body size which means that the smaller the body size of copepods, the greater the effect of sound [13]. However, our results contrast those reported by Bennett et al. (1994) [11] that 132-167 dB re 1μPa exposure had no effect on zooplankton biomass in Lake Pend Oreille, Idaho. Although it is difficult to compare their study with ours since sound properties differ between freshwater and marine waters [29], the findings of Bennett et al. (1994) [11] are still plausible since they studied the response to sublethal underwater explosion at the level of zooplankton community, and that the high population turnover rates of certain taxa would have rapidly replaced individuals killed by experimental blasts. We recommend in future studies a focus on the effects of long-term exposure to sublethal explosions on marine copepods and zooplankton communities.

This study adopted the scaled injury criteria of Hubbset. al. (1960) [30] to assess the level of sublethal injury on the external morphology of *S. guttatus* larvae. Distended abdomen and extruded intestine fall within the sublethal scale [30], and was observed across treatments, i.e. from low to high explosive charges. Lethal intensity for fishes would be blasts from dynamite and/or TNT that would produce pressures ranging between 239-234 dB re 1 μPa [11]. Although visual observations on fish larvae after blasting indicated no direct mortality, ensonified fish larvae may have damaged major internal organs (i.e., kidney, swim bladder, liver and intestines)[15,21]. These traumatized larvae are unlikely to survive in nature[19]. Studies showed that larvae and recently transformed small juvenile spot and pinfish were more vulnerable to underwater shock waves than large juveniles and adult fishes [30]. Furthermore, low intensity underwater explosion killed all larval anchovies (Engraulidae) and larval northern anchovy (*Engraulis mordax*) and smelt (Osmeridae) [31], but no pressure values were presented with either observation. In contrast, ensonification levels of 100 to 5,600 Hz at pressure levels (105-167 dB re 1μPa) similar to values in the present study did not affect survival and biomass of kokanee embryo in Lake Pend Oreille, Idaho [11]. The difference in results is best explained by species-specific variability in the response and tolerance of the younger stages to sublethal underwater explosion[11,32]. It is imperative to test a wide range of sublethal pressure levels to determine the lowest possible pressure value that can cause injury to fish larvae of different marine species.

The results of this study are compared with published data of Lewis (1996) [32] from blasting experiments (Table 2). The table shows various explosives with corresponding charges used to assess mortality on adult fishes. Among the explosives, the trinitrotoluene (TNT) had the highest number of mortality (1000 individuals) even at relatively low charge. On the other hand, this study showed no direct mortality after detonation. However, despite the use of relatively low charge (<1 g), the present study has shown that this can still inflict damage on fish larvae. In fact, a study of Kostyuchenko (1973) [33] demonstrating the impact of sublethal underwater explosions had shown that survival of eggs and larvae (with swim bladder) of engraulid (*Engraulisen-crasicholus*) and carangid (*Trachurus mediterraneus*) was 58% at 120 dB re 1 μPa intensity and 10-20 m explosion range within 24 hours. Notably, the results of the present study are based on a narrow blasting radius of 0.5 m but under a relatively low intensity (sublethal) explosion, and yet these conditions caused high numbers (75-100%) of injured larval fish. Dynamite or 'blast' fishing impacts a substrate radius of up to 4 m [35], but a decreasing pressure wave would still propagate much farther than 4m and we think the results we present here simulates sublethal pressure waves found at the periphery of a 'blast' fishing explosion.

CONCLUSION

This study provides evidence on the effects of sublethal sound from underwater fireworks explosion to copepod zooplankton and larvae of the rabbit fish *Siganusg uttatus*. We found that as the strength of the blast increases, the percent mortality of copepods also increases. This study reports for the first time tropical copepod mortality due to sublethal underwater explosions. This study also reinforces earlier findings that sublethal underwater explosions can cause injuries in young fish, particularly *S. guttatus* larvae. Furthermore, the type of explosives and charges employed inflicted injury to fish larvae.

ACKNOWLEDGEMENT

We are deeply grateful to the anonymous reviewers for the constructive suggestions, Dr. MTRD Sanchez-Metillo for copyediting the manuscript, and the Asian CORE-COMSEA Project of the Japan Society for the Promotion of Science (JSPS) for support.

REFERENCES

1. Popper AN, Hastings MC. The effects of human-generated sound on fish. Integrative Zoology 2009. 4:43-52.

2. Piercy JJB, Codling EA, Hill AJ, Smith DJ, Simpson SD. Habitat quality affects sound production and likely distance of detection on coral reefs. Marine Ecology Progress Series 2014:516:3547.

3. Keevin TM. A review of natural resource agency recommendations for mitigating the impacts of underwater blasting. Reviews in Fisheries Science 1998:6(4):281-313.

4. Stocks JR, Broad A, Radford C, Minchinton TE, Davis AR. Response of marine invertebrate larvae to natural and anthropogenic sound: a pilot study. The Open Marine Biology Journal 2012: 6: 57-61.

5. Falk MR, Lawrence MJ. Seismic exploration: its nature and effect on fish. Technical Report Series No.CEN T739.Department of the Environment, Fisheries and Marine Service, Central Region; Winnipeg, USA, 1973. p. 51.

6. Wright DG. A Discussion Paper on the Effects of Explosives on Fish and Marine Mammals in the Waters of the Northwest Territories. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1052, 1982. p.16.

7. Govoni JJ, West MA, Settle LR, Lynch RT, Greene MD. Effects of underwater explosions on larval fish: implications for a coastal engineering project. Journal of Coastal Research 2008:24 (2B): 228-233.

8. Garcia SM, Zerbi A, Aliaume C, Do Chi T, Lasserre G. The ecosystem approach to fisheries. Issues, terminology, principles, institutional foundations, implementation and outlook.FAO Fisheries Technical Paper. No. 443. Rome, FAO. 2003. 71 p.

9. Andalecio MN. Multi-criteria decision models for management of tropical coastal fisheries. A review. Agronomy and Sustainable Development 2010: 30: 557-580.

10. Raymundo LJ, Maypa AP, Gomez ED, Cadiz P. Can dynamite blasted reefs recover? A novel, low-tech approach to stimulating natural recovery in fish and coral populations.Marine Pollution Bulletin 2007: 54:10091019.

11. Bennett DH, Falter CM, Chipps SR, Niemela K, Kenney J. Efects of Underwater Sound Simulating the Intermediate Scale Measurement System on Fish and Zooplankton of Lake Pend Oreie, Idaho. Office of Naval Research, Arlington, VA, USA. 1994. p. 159

12. Moriyasu MR, Allain K, Benhalima, Clayton R. Effects of seismic and marine noise on invertebrates: a literature review. Research document 2004/126.Canada Science Advisory Secretariat. Fisheries and Oceans Canada; Ottawa, 2004. p. 44.

13. Yelverton JT, Richmond DR, Hicks W, Saunders K, Fletcher ER. The Relationship Between Fish Size and their Response to Underwater Blast, Report DNA 3677T, Director. Washington, DC: Defense Nuclear Agency. 1975.

14. Hubbs CL, Rechnitzer AB. Report on experiments designed to determine effects of underwater explosions on fish life. *California Fish and Game*1952:38:333-366.

15. Gaspin JB. Wiley ML, Peters CB, Experimental Investigations of the Effects of Underwater Explosions on Swim-bladder Fish, II: 1975 Chesapeake Bay Tests. Silver Spring, Mary-land: Naval Surface Weapons Center, Final Report NSWC/WOL/ 1976. pp. 76-61.

16. Gaspin JB, Experimental Investigations of the Effects of Underwater Explosions on Swimbladder Fish, I: 1973 Chesapeake Bay Tests. Silver Spring, Maryland: Naval Surface Weapons Cen-ter, Final Report NSWC/WOL/TR 1975. pp. 75-58.

17. Goertner JF, Dynamical Model for Explosion Injury to Fish. Naval Surface Weapons Center, White Oak Lab Tech. Rep. NSWC/WOL/TR 1978. pp. 76 -155.

18. O'Keefe DJ. Guidelines for Predicting the Effects of Underwater Explosions on Swimbladder Fish. Silver Spring, Maryland: Naval Surface Weapons Center, Final Report. NSWC TR 1984.pp. 82-326.

19. Wiley ML, Gaspin JB, Goertner JF. Effects of underwater explosions on fish with a dynamical model to predict fishkill. Ocean Science and Engineering 1981:6:223-284.

20. Munday DR, Ennis GL, Wright DG, Jefferies DC, McGreer ER, Mathers JS. Development and Eval-uation of a Model to Predict Effects of Buried Underwater Blasting Charges on Fish Populations in Shallow Water Areas.Canadian Technical Report of Fisheries and Aquatic Sciences No. 1418. 1986.

21. Wiley ML, Wilson JS. Environmental Effects of Explosive Testing.Chesapeake Bay Tests. Silver Spring, Maryland: Naval Surface Weapons Center, Final Report NSWC/WOL/TR 1975. pp. 75-58.

22. Gilmanov RA. Effect of shock waves during underwater borehole blasting. *Hydrotechnical Construction* 1984:18:221-225.

23. Elliot TD, Tang KW Simple staining method for differentiating live and dead marine zooplankton in field samples. Limnology and Oceanography 2009:7:585-590.

24. Cudina M, Prezelj J. Probability density prediction of peak sound pressure levels from firecracker explosions. Acoustical Physics 2006: 52(3): 351363.

25. GoldWave v6.21, www.goldwave.com/release.php, accessed 23 December 2013.

26. SPSS. SPSS for Windows version 11. SPSS Inc.Englewood Cliffs; New Jersey, USA, 2002.p. 637.

27. ASEAN [Association of Southeast Asian Nations]. ASEAN Marine Water Quality: Management Guidelines and Monitoring Manual. ASEAN Secretariat. New Millennium Pty Ltd. Australia. 2008.

28. DENR [Department of Environment and Natural Resources]. Administrative Order No. 34: Revised water usage and classification of water quality criteria amending section No. 68 and 69, Chapter III of the 1978 Rules and Regulations. DENR; Manila, Philippines. 1990.p. 6.

29. Medwin H, Clay CS. Fundamentals of Acoustical Oceanography. Academic Press; Massachusetts, USA. 1998.p. 712

30. Hubbs CL, Schultz EP, Wisner RL. Preliminary Report on Investigations of the Effects on Caged Fishes of Under-water Nitrocarbonitrate Explosions. Data Report, University of California, Scripps Institute of Oceanography, 1960. 21p.

31. Fitch JE, Young PH. Use and effect of explosives in California waters. California Fish and Game 1948:34:5370.

32. Lewis JH. Effects of Underwater Explosions on Life in the Sea.Aeronotical and Maritime Research Laboratory, Defence Science and Technology Organisation, Department of Defence, Canberra, Australia. 1996. 38 p.

33. Kostyuchenko LP. Effect of elastic waves generated in marine seismic prospecting on fish eggs in the Black Sea. Hydrobiologia 1973:9:45-48.

34. Slabbekoorn H, Bouton N, van Opzeeland I, Coers A, ten Cate C, Popper AN. A noisy spring: the impact of globally rising underwater sound levels on fish. Trends in Evolution and Ecology 2010:25(7):419-427.