

Ecotoxicological effects of brine discharge on marine community by seawater desalination

Sung Jin Yoon, Gyung Soo Park*

Department of Marine Biotechnology, Anyang University, Incheon, 417-833, Korea
Tel. +82 32 930 6032; Fax +82 32 930 6036; e-mail: gspark@anyang.ac.kr

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ABSTRACT

To determine ecotoxicological effects of brine discharge from the desalination plant and the potential tolerance impact associated with various marine communities, marine ecotoxicological assessment was carried out using four phytoplankton species (*Skeletonema costatum*, *Chlorella vulgaris*, *Tetraselmis suecica* and *Isochrysis galbana*) and macroalgae (*Ulva pertusa*) as primary producer, and two zooplankton species (neonate for rotifer *Brachionus plicatilis*, adult of harpacticoid copepod *Tigriopus japonicus*) and one demersal fish (olive flounder, *Paralichthys olivaceus*) as consumers. Values of 72 h EC₅₀ of population growth inhibition (PGI) for *S. costatum*, *C. vulgaris*, *T. suecica* and *I. galbana* were estimated as 55.1, 61.7, 56.9 and 42.2 ppt, respectively, and *I. galbana* was the most sensitive to brine. No significant differences in brine sensitivity were found in the ranges of 30.0–45.0 ppt for *S. costatum* and *T. suecica*, and less than 40.0 ppt for *I. galbana*. Mortality rates of *B. plicatilis* neonate and adult of *T. japonicus* revealed that brine less than 60.0 ppt caused <50% mortality and over 65.0 ppt showed >50% mortality for the two species in terms of acute toxicity. 24 h LC₅₀ and LOEC value of rotifer neonate (68.1 ppt and 55.0 ppt, respectively) were higher than those of *T. japonicus* (63.6 ppt and 40.0 ppt, respectively) for 96 h test periods. Spoluration rate of green algae *U. pertusa* sharply decreased from 60.0 ppt and 0% between 65.0 and 80.0 ppt with a 96 h EC50 value of 53.3 ppt. Survival of olive flounder fry *P. olivaceus* significantly decreased with the increase of brine concentration and the 96 h LC₅₀ value was estimated as 48.6 ppt, lower than green algae, but NOEC (40.0 ppt) of olive flounder was not different from that of green algae. In general, 40 ppt of salinity seems to be a threshold concentration for acute toxicity of brine. Differences of brine effect on each test species has a connection with difference of eco-physiological characteristics such as tolerance of organism on brine, exposure times, and natural habitats of test species. Therefore, site-specific test strategy is highly recommendable to reflect the local ecosystems for the brine discharge and also site selection of desalination plants.

Keywords: Desalination; Brine; Acute toxicity; Phytoplankton; Macroalgae; Copepod; Rotifer; Olive flounder

* Corresponding author.

1. Introduction

Desalination plants have been considered from the point of view economic, social development, environmental sustainability which existing water allocation and possible reallocation, water supply and water demand management. From this tendency, desalination plants increasingly have been constructed in land as well as marine areas of many countries for supplying water for domestic purposes [1,2]. Reverse, little attention has been given to ecological damage with environment changes of water column due to various by-product induced by desalination plants. Several studies has cautioned ecotoxicological marine impacts from seawater desalination plants such as impacts of calcification, salinity and other chemical additives (biocides, scaling substances, antifoaming agents etc.) of marine organisms [3]. For example, various by-products from desalination plant can interfere directly on eco-physiological processes of the biotope, such as enzymatic activity, reproduction, breathing, photosynthesis and the changes of behaviour were observed on the level of the vertebrate [4–7]. Several studies have simulated various models and chemical measurement to forecast the dispersal of brines under various environmental conditions [8,9]. Especially, high brine can lead to disturb overall marine population's biotic factors such as succession/destruction of habitats (ex, flora and fauna), increase of mortality and the ecological role of each organism. Indeed, activity of seawater desalination induced on environmental impacts mainly generated from the discharge into the sea of the brine (ranged from 38 to 90psu) produced in front of the brine discharge outfalls [8,10,11].

Desalination plant discharges influence both the water quality and the organisms of impacted estuaries, directly or indirectly with most acute effects found in the vicinity of the discharge outfalls outlet. An increasing in salt concentration, which raises the density of waters, fosters stratification of receiving water bodies, can lead to an increase of turbidity, disrupt the photosynthesis process, and disturb the biogenesis, to an extinction of mainly sensitive benthic species, larvae and young individuals [7]. These impacts are more probable to develop in coastal area, closed or poorly mixed and also in vulnerable area. It may be possible to benthic organisms more sensitive to variations in salinity levels than pelagic individuals. However, survival and tolerance salinity levels of most marine species were not obviously determined in brine discharge condition. Of course, these phenomena can present from mixed results between chemical and brine responses of organisms. It has been reported that effects on effluent of desalination plants mostly evaluated using of chemical methods and distribution pattern of organism with salinity exposure. For example, the proposed environmental impact assessments (EIA) procedure follows guideline which consists of analysis of the source of

impacts, analysis of the impacted ecosystem, definition of links between source and targets, recommendation of mitigation measures and sustainability of the environmental protection measures [12]. In spite of these activities, ecotoxicological assessment considered ecological trophic levels (producer, consumer and decomposer) is shorthanded. As a single bioassay may not provide a full picture of the quality of the environment, a representative, cost effective, and quantitative battery test should be developed [13]. Recently, most ecotoxicological studies have introduced the battery test including at least three species such as decomposer, primary producer and consumers from the each trophic level for marine ecotoxicological test [14–16]. Although the number of scientific publications dealing with issue was limited within changes of chemical and ecological distribution by modelling study, the discharge of brine into the sea requires particular attention and scientific assessment of possible impacts on the marine organisms [6,17,18].

The main objective of the present study is to estimate the ecotoxicological effects of brine discharge from the desalination plant and to determine the potential tolerance impact associated with various marine communities on each trophic level as producers and consumers of marine ecosystem.

2. Experimental design for marine ecotoxicological assessments

To establish ecotoxicological sensitivity of brine exposure, three trophic levels of marine species, phytoplankton/macroalgae as marine produces, and rotifer/copepod/fish as consumers, were selected to test toxic effect of brine using marine ecotoxicological standard methods [16,19,20]. These test species represent the major primary producer and consumer in marine trophic levels in coastal waters of Korea, and acute toxicity tests of brine were carried out covering all the trophic levels as battery test. Standard test species and endpoints for brine toxicity test were summarized in Table 1.

Specific population growth inhibition tests of four phytoplankton species, *Skeletonema costatum*, *Chlorella vulgaris*, *Tetraselmis suecica* and *Isochrysis galbana*, were conducted during 72 h according to protocol for the phytoplankton toxicity test [19,21,22]. Specific growth rate, r , for specific period was calculated as the logarithmic increase in cell density (cells mL⁻¹), as follows:

$$r = (\ln N_t - \ln N_0) / t \quad (1)$$

where r is the specific growth rate from initial to final moment time (h), N_0 and N_t are the initial and final cell density, respectively, and t is the final moment time (h). Brine effects on phytoplankton population growth inhibition was presented as EC₅₀ (50% population growth

Table 1
Standard test species and their endpoints for brine acute toxicity test

Tropic levels	Groups	Test species	Life stages	Endpoints
Primary producers	Phytoplankton	<i>Skeletonema costatum</i> , <i>Chlorella vulgaris</i> , <i>Tetraselmis suecica</i> , <i>Isochrysis galbana</i>	—	Population growth inhibition (EC ₅₀)
	Macroalgae	<i>Ulva pertusa</i>	Adult	Sporulation (EC ₅₀)
Consumers	Zooplankton	<i>Brachinonus plicatilis</i>	Neonate	Mortality (LC ₅₀)
	Benthic copepod	<i>Tigriopus japonicus</i>	Adult	Mortality (LC ₅₀)
	Demersal fish	<i>Paralichthys olivaceus</i>	Fry	Mortality (LC ₅₀)

Table 2
Summary of test conditions and test acceptability criteria of four species groups for brine toxicity. Numbers in parentheses are test conditions for rotifer toxicity test

Parameter	Test conditions			
	Phytoplankton	Macroalgae	Copepod (Rotifer)	Demersal fish
Test type	Static non-renewal	Static non-renewal	Static non-renewal	Static non-renewal
Test duration, h	72	96	96 (24)	96
Test temperature, °C	20.0 ± 1.0	15.0 ± 1.0	20.0 ± 1.0	20.0 ± 1.0
pH	7.9 ± 0.3	7.8 ± 0.5	7.9 ± 0.7	7 ± 0.7
DO, mg/L	> 6.0	> 6.0	> 6.8	> 7.0
Light intensity, μmol m ⁻² s ⁻¹	80–100	80–100	Lab condition	Lab condition
Photoperiod (L : D)	Continuous lightness	12 : 12	12 : 12	12 : 12
Test volume, mL	30	20	50 (20)	1,800
Renewal periods	None	None	None	None
Feeding regime	None	None	None	None
Test concentration	Control + nine	Control + nine	Control + five (nine)	Control + five
Number of replicates	3	3	3	3
Test acceptability criteria (in control)	$r > 0.04/\text{h}$ in specific growth rates	>80.0% in sporulation rate	>90.0% in survival rates	>80.0% in survival rates

inhibition concentration) and compared to sensitivities between phytoplankton test species.

96-h brine exposure test of macroalgae *Ulva pertusa* was conducted to estimate sporulation rates using commercial algal toxicity test kit (U-kit, Greenpioneer, Incheon, Korea) [19].

Two primary consumers such as rotifer neonate (after 24 h birth) of *Brachinonus plicatilis* and adult of harpacticoid copepod *Tigriopus japonicus* were used to test static acute toxicity for brine exposure during 24 h and 96 h, respectively [16,19]. Mortalities of test species were monitored with 24 h interval. The exposed individuals without swimming or appendage movements during 10 s were considered as dead [23].

The fry (8 months old) of olive flounder, *Paralichthys olivaceus* were obtained from commercial hatchery and maintained in the lab for 7 days for acclimatization. Test

methods for fish acute toxicity were followed by US EPA (United States Environmental Protection Agency) and domestic standard methods [16,20].

Test conditions of four test groups are summarized in Table 2. The 50% lethal or effective concentrations (LC₅₀ or EC₅₀), NOEC (no observed effective concentration), LOEC (lowest observed effective concentration) and 95% confidence limits were calculated by Toxicol 5.0 software (Toxicalc 5.0, Tidepool Scientific Software, USA).

3. Ecotoxicological responses of marine organisms by brine exposure

Brine tolerance ranges of phytoplankton species were different from test species. However, there was a significant relationship between brine concentration and population growth inhibition (PGI) of the four phytoplankton

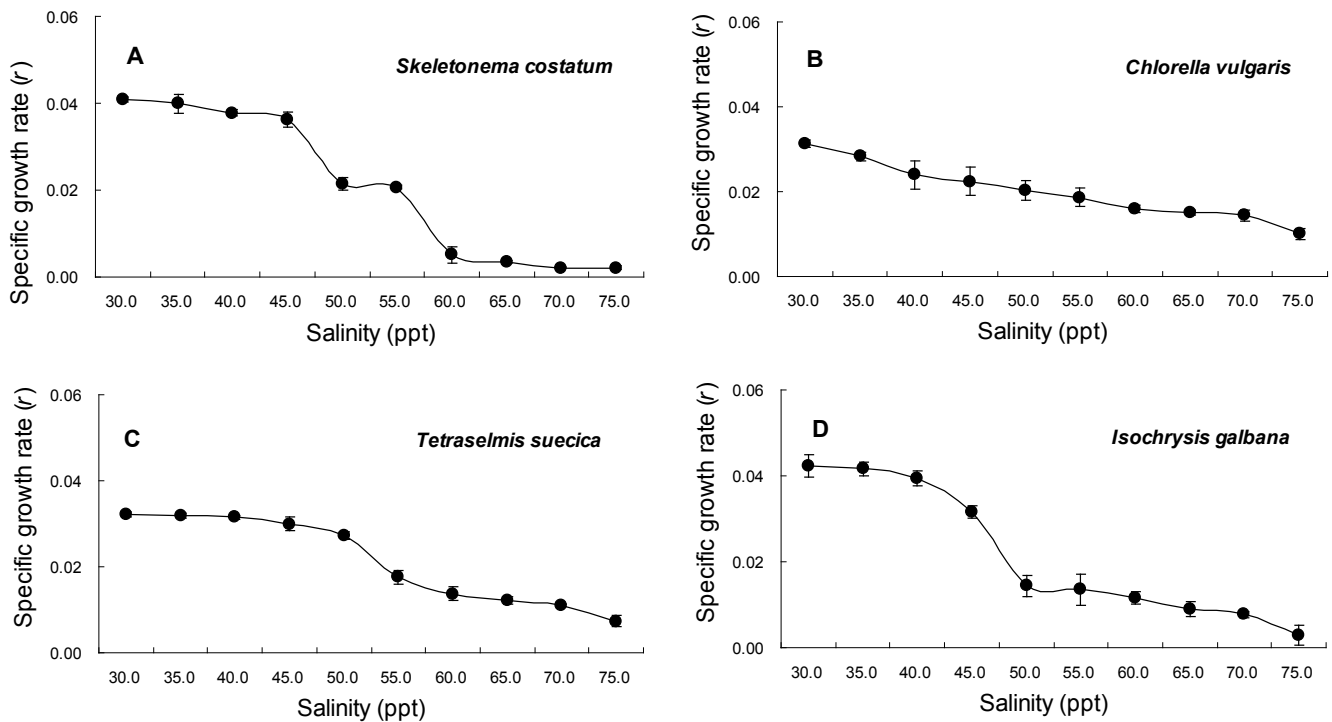


Fig. 1. Specific population growth inhibitions by increased salinity for four marine phytoplankton species.

species (Fig. 1). Population growth of *S. costatum*, *T. suecica* and *I. galbana* was sharply decreased over 45.0 ppt, rather smooth decrease of *C. vulgaris* population growth with the increased salinity. 72 h EC_{50} values of *S. costatum*, *C. vulgaris*, *T. suecica* and *I. galbana* were estimated as 55.1, 61.7, 56.9 and 42.2 ppt, respectively. *I. galbana* was the most sensitive to brine and showed lowest LOEC as <35.0 ppt. Highest LOEC was found at *T. suecica* as 50.0 ppt (Table 3). No significant differences between test species in brine sensitivity were found within the ranges of 30.0–45.0 ppt.

Inhibition of photosynthesis of the green alga *Chlamydomonas* and feasibility was verified by comparative studies with standard phytotoxicity tests using both solutions with distinct pure brine and complex mixtures in which additionally various toxic heavy metals were added [5]. In their results, photosynthesis of *Chlamydomonas* was not affected in 150.0 mL L⁻¹ (i.e. 7 g L⁻¹) brine concentration and these could not survive at concentrations higher than 100 mL L⁻¹. In contrast, Growth of alga *C. pulsatilla* (euryhaline flagellate) observed optimal at low salinity (about 10% artificial seawater, ASW), but it can maintain high division rates with normal cell carbon, protein, nitrogen, and phosphate contents at salinities as high as 200% ASW. The alga could osmoregulate without major water loss over the salinity range of 10–200% ASW. It maintained cell water content of 65–70% over this salinity range [24]. Moreover, Fe³⁺ iron uptake by halotolerant alga *Dunaliella* is mediated by a plasma membrane

transferring. Following the result, salinities as high as 3.5 M (NaCl) did not inhibit iron uptake or decrease the apparent affinity for Fe³⁺ ions for *Dunaliella*. And they concluded that transferrin-like protein (Ttf) activity is not affected by high salt. On the contrary, biomass increment was occurred by the mixing of between brine and adjacent seawater [25]. These results can interpret that the tolerance of brine differs from inhabit condition and capacity of osmoregulation with species. NOECs of test species used in this study were lower than 35.0 ppt for *S. costatum* and *I. galbana*, and the other two species were higher than 40.0 ppt. These may be due to the acclimation to high levels of salinity.

Mortalities of rotifer neonates and harpacticoid copepod adults (benthic meiofauna) exposed to brine were presented in Fig. 2. Mortality rates of the two species at control (30.0 ppt) were less than 2% as an average of 0 and 1.7% at control for rotifer and copepod, respectively. Within the tested ranges of brine concentration, susceptibility of invertebrates, mainly crustaceans, may not be varied. However, brine concentration lower than 60.0 ppt induced <50% mortality and over 65.0 ppt resulted in >50% mortality. 24 h LC_{50} and LOEC values of rotifer (68.1 and 55.0 ppt, respectively) were higher than those of copepod test (63.6 and 40.0 ppt, respectively). However, sensitivity of the two species on brine was lower than phytoplankton (Table 3). The tolerance limits of the test species to brine were observed within 35.0 ppt although

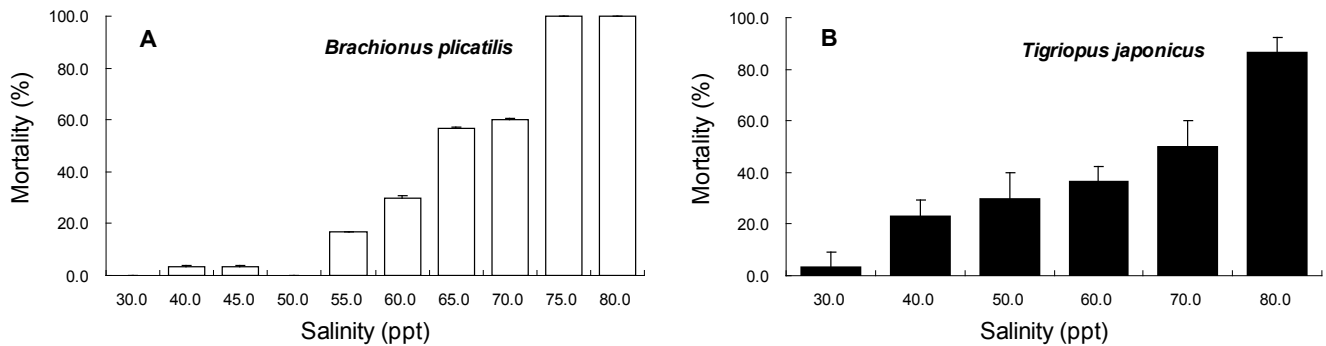


Fig. 2. Mortality of marine rotifer neonate (A) and benthic copepod adult (B) with increased salinity.

Table 3

Summary of ecotoxicological parameters of phytoplankton, macroalgae, rotifer, copepod and demersal fish exposed to high saline water

Groups	Species names	EC ₅₀ /LC ₅₀ (psu)	95% CI	NOEC	LOEC
Phytoplankton	<i>Skeletonema costatum</i>	55.1	44.3–55.4	35.0	40.0
	<i>Chlorella vulgaris</i>	61.7	55.9–68.3	40.0	40.0
	<i>Tetraselmis suecica</i>	56.9	53.8–60.7	50.0	55.0
	<i>Isochrysis galbana</i>	42.2	40.2–44.2	< 35.0	35.0
Macroalgae	<i>Ulva pertusa</i>	53.3	48.0–56.8	40.0	45.0
Rotifer	<i>Brachionus plicatilis</i>	68.1	63.2–78.7	50.0	55.0
Benthic copepod	<i>Tigriopus japonicus</i>	63.6	56.8–71.6	< 40.0	40.0
Demersal fish	<i>Paralichthys olivaceus</i>	48.6	46.4–50.0	40.0	45.0

toxic concentration ranges were different from test organisms. It has been fully reported that the information of eco-physiological effects of low salinity exposure of marine zooplankton, but those of high brine exposure were a little because most studies has focused osmoregulation adaptation of freshwater discharge into the marine environment [26,27]. For example about high salinity concentration, Zakaria et al. studied zooplankton abundance of four water types in EI-Mex Bay, namely; mixed land drainage (<10.0 ppt), mixed water (10.0–30.0 ppt), diluted seawater (30.0–38.5 ppt) and Mediterranean water (>38.5 ppt) [28]. As a result from their study, components of zooplankton of marine form increased with salinity increment, especially 95.1% to the total number recorded species presented in Mediterranean type water although low abundance (5.9×10^3 inds. m^{-3}), and copepod and their larval stage were the most dominant group in high salinity water and only two species of rotifer represented that these two species could tolerate the high salinity. These results can apply to our study. Following the values of NOEC, rotifer and harpacticoid copepod can survive lower than 40.0 ppt and it is fully possible to support our study that most of species were endemic species same as those used in this study [28]. Generally, crustaceans and

other invertebrate larvae had been accepted more sensitive to variations in salinity levels than fully developed individuals [7]. However, these considerations do not support our results due to difference of development stages and life cycles. As a result of this study, most of the test species may not be resistant (such as behaviour and metabolism) to brine waters higher than 40.0 ppt (for species acclimated at 30.0 ppt) during prolonged test period, although test organisms are euryhaline. Therefore, sensitivity of marine organisms on brine in the field may be related with changes of endogenous condition adapted by temperature, salinity, light intensity and water movement in the habitat [29].

Spoluration of macroalgae *U. pertusa* was sharply decreased at 60.0 ppt, 0% spoluration between 65.0 and 80.0 ppt (Fig. 3A). 96 h EC₅₀ and LOEC values of the green algae were 53.3 ppt and 45.0 ppt, respectively. Mortality of olive flounder fry at control was less than 10%. However, survival rates of the fish fry in treatment groups significantly decreased with increase of brine concentration. 96 h LC₅₀ value (48.6 ppt) for olive flounder fry was lower than those of macroalgae, but NOEC (40.0 ppt) and LOEC (45.0 ppt) of the fish were not different from macroalgae (Fig. 3B, Table 3).

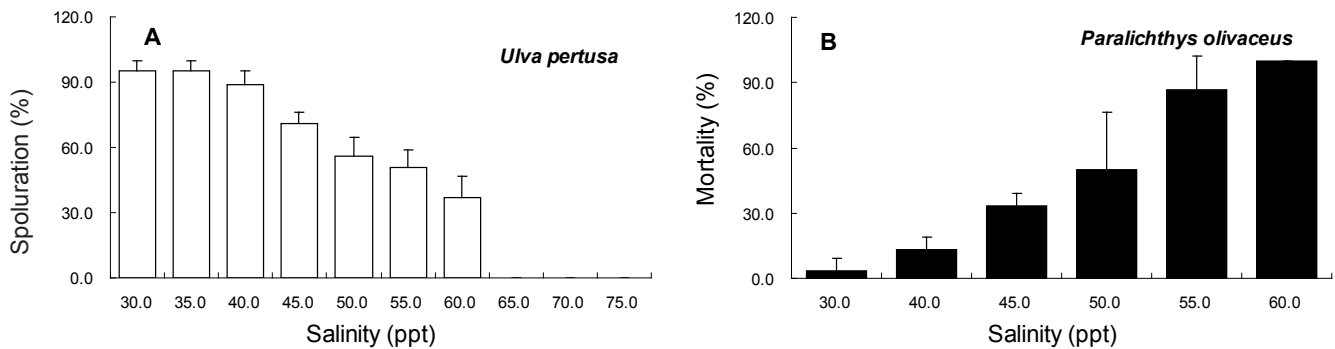


Fig. 3. Spoluration of macroalgae (A) and mortality of olive flounder fry (B) with increased salinity.

In the case of several aquatic macrophytes *Lepidium* and *Lemna*, their growths were strongly inhibited when the brine concentration was higher than 0.9 g L^{-1} [5]. Also, *Lemna minor* had toxic effect from 16.7 ppt or higher, but growth of *Lemna* was increased by salt concentrations until 3.3 ppt as compared to other *Lemna* plants grown in fertilized pond water [10]. Additionally, growth inhibition (EC50) of *Lemna* exhibited very low value of salinity ranged from 4.9 to 5.5 g L^{-1} and salt removal efficiency was up to 25% of initial salinity [30]. Genus of *Lemna* and *Lepidium*, although it has certain areas and climates where it is particularly well-adapted are found in marshy area and reported that they can habit under low salinity concentration as well as freshwater [10,31]. These results can use useful information for monitoring of ecological effects of brine discharge at low salinity area such as closed bay, tidal flat and marshy area, but not to open marine ecosystem. In the monitoring of seawater desalination by reverse osmosis (SWRO), significant increases of salinity at the bottom (about 38.5 ppt) were observed several kilometres away from the discharge point [11]. Distributions of echinoderm and seagrass *Posidonia oceanica* seasonally differed from between discharge point and control point. Two organisms had disappeared from the meadow in front of the desalination plant and southern locality currently affected from brine, however, important increment was observed at the northern locality, which compared with the dispersion of the brine since the northern locality is the less affected by the brine. Regression of *P. oceanica*, which has been demonstrated the low tolerance to salinity increments, may be related with their movement away from the brine in northern locality [32].

Fish that can tolerate a wide range of salinity at same in their life-cycle are called euryhaline species. This fish which includes salmon, eels, striped bass and flounder, can live or survive in wide ranges of salinity, varying from fresh to brackish to marine waters [33]. In some case of effects of sudden changes in salinity, juvenile spotted sea bass *Lateolabrax* sp. can withstand sudden drops in salinity from 31.5 ppt to freshwater, and yet maintain a regular though some what dampened endogenous rhythms of

oxygen consumption [34]. Also sea bass *Dicentrarchus labrax* is known to have a remarkable ability to regulate plasma electrolytes over a wide range of salinities [35]. In this study, survival of *P. olivaceus* in treatment groups significantly decreased with increment of brine concentration and this species can survive lower than 40.0 ppt (Fig. 3B, Table 3). Although tolerance concentration of salinity change of the fish was different from other studies, it may be possible that a period of gradual acclimation, though, may be needed for euryhaline fish to tolerate large changes in salinity under 40.0 ppt. However, if salinity concentration would maintain higher than 40.0 ppt without dilution process during prolonged period, their kidney function may be extremely damaged and fish will die by energy consumption for osmoregulation or avoid from habitat. Finally, an increase in the brine concentration can reduce reproduction, behaviour and metabolism to extinction of marine primary producer and consumer such as phytoplankton, crustacean, others invertebrates and fish larvae floating in the pelagic as well as benthic ecosystem.

In order to minimize the impact of desalination plants it is important to select a correct site-specific location of the plants and to maximize the dilution of its brine [4]. The environmental impact of the desalination plant discharge have been considered by site-specific natural hydrodynamic changes in the ocean (current, wind, tidal movements, temperature differences, etc) as well as the mixing energy introduced with the desalination plant discharge diffuser system. As occasion demands consideration, presently, most plant effluent is designed to discharge away from shoreline in sea with diffuser area and environmental sensitivity of marine organisms. These efforts were identified that establishing the site-specific maximum level of salinity concentration at which marine organisms not only survive, but can also grow and reproduce normally at the projects of Carlsbad and Huntington Beach desalination plants in California (salinity discharge threshold was determined to be over 40.0 ppt) [36]. This could be achieved using a previous dilution of the brine with seawater and helpful for preventing pollution, the

protection of inhabitant and marine species inhabiting the discharge area.

Correlation coefficients (r^2) between ecotoxicological response of test species and brine concentration were estimated from 0.71 to 0.97. There was a significant relationship between concentration and response for three phytoplankton species (*C. vulgaris*, *T. suecica* and *I. galbana*), rotifer neonates *B. plicatilis*, macroalgae *U. pertusa* and demersal fish fry *P. olivaceus* ($>r^2 = 0.9$), except for *S. costatum* and *T. japonicus* (Fig. 4).

4. Summary and conclusions

This study tested ecotoxicological effects of brine exposure on various trophic levels of marine organisms such as phytoplankton, zooplankton, benthic copepod, macroalgae, and fish. The effects of brine on marine organisms were very various in terms of the test species. Phytoplankton *I. galbana* and olive flounder *P. olivaceus* fry were the most vulnerable and rotifer *B. plicatilis* and benthic copepod *T. japonicus* were the most tolerable to brine discharge. These results can suggest that difference of brine effect on each test species must be related to eco-physiological characteristics of the test organisms such as adaptation capacity of organism, exposure time, environments of inhabitation and origin of species etc.

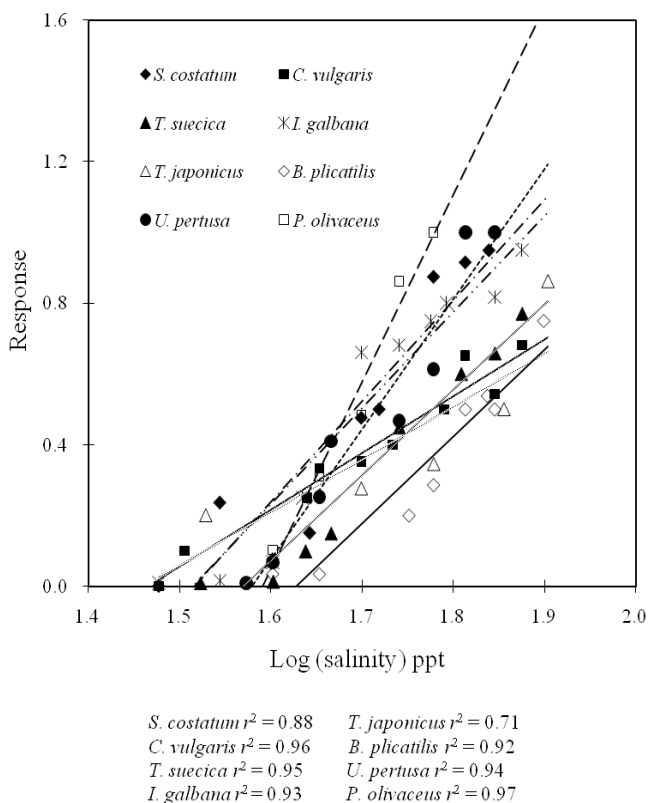


Fig. 4. Concentration-response curves between brine concentration and EC/LC of eight test species.

In conclusion, because the significant biotic factors are connected to abundance, survival, the ecological roles and the reproductive strategies of the affected organisms, it is necessary to establish continuous monitoring plans before and after the construction of the desalination plant and must be carried out evaluation and development of strategies to prevent the environmental impacts of the desalination effluents into the marine ecosystems. Site-specific test strategy is highly recommendable to reflect the local ecosystems for the site selection of desalination plants.

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