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Effects of polluted and non-polluted suspended sediments on the oxygen consumption rate of olive flounder, *Paralichthys olivaceus*

Seung Oh Chu[a,1], Changkeun Lee[a,1], Junsung Noh[a], Sung Joon Song[a], Seongjin Hong[b], Jongseong Ryu[c], Jung-Suk Lee[d], Jungho Nam[e], Bong-Oh Kwon[f,*], Jong Seong Khim[a,*]

[a] *School of Earth and Environmental Sciences & Research Institute of Oceanography, Seoul National University, Seoul, Republic of Korea*
[b] *Department of Ocean Environmental Sciences, Chungnam National University, Daejeon, Republic of Korea*
[c] *Department of Marine Biotechnology, Anyang University, Ganghwa-gun, Incheon, Republic of Korea*
[d] *Neo Environmental Business Co. (NeoEnBiz), Bucheon 14523, Republic of Korea*
[e] *Korea Maritime Institute, Busan 49111, Republic of Korea*
[f] *Department of Marine Biotechnology, Kunsan National University, Kunsan 54150, Republic of Korea*

A R T I C L E I N F O

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A B S T R A C T

The potential ecological impacts of elevated suspended sediments (SS) in coastal areas due to human activities remain unclear. In particular, physiological response of benthic fish to SS exposure in polluted environment has not been documented. We determined sub-lethal toxicity of polluted and non-polluted SS to olive flounder. Test organism was exposed to varying concentrations of SS (0–4000 mg L^{-1}) and real-time oxygen consumption rate (OCR) was measured for 12 h. The early-juvenile was sensitive to SS, particularly at > 500 mg L^{-1}, but late-juvenile was tolerant up to 4000 mg SS L^{-1}. Metal polluted SS (HQ_{metal} > 1) increased OCR in general, particularly at > 1000 SS mg L^{-1}. Combined effect of copper and SS exposure on fish was either synergistic or antagonistic. Overall, potential adverse effect of polluted SS on fish greatly varied at different life stage and/or by metal pollution gradients.

1. Introduction

Suspended sediment (SS) often occurs at high concentrations in the water column as a result of human activities, such as agriculture, dredging, mining, and urban development (Doxaran et al., 2009; Newcombe and Macdonald, 1991). Normally small-sized particles, such as silt and clay, are highly resuspended although turbidity return to a background level within a short time. For example, the elevated SS in the central Chesapeake Bay was reported to reach 7200 mg SS L^{-1} and maintained about 2 h (Nichols et al., 1990). In Korea, there has been also significant concern on the sediment-induced turbidity because of increasing coastal dredging and offshore sand mining (Lee et al., 2003). Especially, conflicts on compensation become severe issue between stakeholders cross local fisherman, developers, and/or government (Jeong et al., 2017). Thus, scientific understanding on the fate and harmful effects of elevated SS is necessary.

Adverse (in)direct effects of high/short-term turbidity have been studied for some marine invertebrates, such as coral (Pollock et al., 2014), crayfish (Rosewarne et al., 2014), and several bivalves (Aldridge et al., 1987; Elfwing and Tedengre, 2002; Zheng et al., 2012). In addition to lesser mobile benthos, several pelagic fish species are documented to have adverse turbidity effects on immunity (Lake and Hinch, 1999), foraging efficiency or growth rate (Wenger et al., 2012), and survival rate (Palm, 2001). As elevated SS can acutely cause stress on fish and increase mortality even at the low concentrations (20 to 240 mg SS L^{-1}) (Gregory and Levings, 1996; Lowe et al., 2015), its impacts on lesser mobile benthic species are likely much high. However, turbidity toxicity study on marine benthic fish, particularly for the early life stage, is far limited (Gregory and Levings, 1996; Lowe et al., 2015). Thus, we tested juvenile olive flounder (*Paralichthys olivaceus*), a sedentary fish, that lives extensively in the Korean coastal waters, in the present study. This species accounts for > 50% of the Korea's aquaculture production (KOSIS, 2019), thus is likely to be exposed to the episodic increase of SS where dredging occurs in the ocean.

At times, the SS in polluted sediments with heavy metals etc. would cause synergistic and/or antagonistic mixture effects, but such study is seldom (Fig. S1). Indeed, essential heavy metals are required for the growth of organisms, facilitating key processes such as biological

* Corresponding authors.
E-mail addresses: bongkwon@kunsan.ac.kr (B.-O. Kwon), jskocean@snu.ac.kr (J.S. Khim).
[1] These authors contributed equally to this work.

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metabolism. Such metals include copper, iron, zinc, and manganese. In contrast, non-essential elements, such as lead, cadmium, mercury, arsenic, and chromium, inhibit biological metabolism. Thus, how heavy metals impact fish depends on the composition as well as their concentrations in the given environment (Xuan et al., 2013). Accordingly, mixtures of SS and heavy metals in the water column during the sediment resuspension might have larger impact on marine organisms (Hill et al., 2009). Thus, we investigated how the elevated concentrations and heavy metal pollution of SS adversely would impact marine benthic fish. We selected copper as a model compound to test mixture effects of heavy metal and SS as it is one of the most dominant and/or problematic marine pollutants in Korea for the past > 30 years (Khim et al., 2018).

The oxygen consumption rate (OCR) is widely used as a sensitive indicator to evaluate animal toxicity. This approach quantifies respiration and oxygen consumption continuously, which are fundamental characteristics for the maintenance of life (Reid et al., 2003; Larsson et al., 2013). The OCR can be measured continuously at shorter intervals during the exposure experiment, which allows the immediate detection of changes to biorhythms (Lefevre et al., 2015). In the present study, we aimed to address three core questions; 1) effect of elevated SS on small (early-juvenile) versus large fish (late-juvenile), 2) effect of in situ heavy metal polluted SS on fish, and finally 3) effect of dissolved copper with non-polluted SS mixture on fish. The study design encompassed four independent experiments; I) non-polluted SS, II) polluted SS, III) dissolved copper only, and IV) mixed copper and non-polluted SS (Table 1).

2. Materials and methods

2.1. Collection of sediment and analysis of heavy metals

Sediments were sampled from four sites along the Korean coast. Non-polluted sediments (Gwangyang harbor, Incheon harbor, and Busan harbor) were collected using the Van Veen grab in dredging areas (Fig. S2). Polluted sediments were collected from a stream near an industrial area of Ansan (Fig. S2). Collected sediments were transported to the laboratory, and were then 63 μm wet-sieved. After sieving, the supernatant was removed and stored at −15 °C to prevent changes to the biochemical properties of the sediments. The particle sizes and heavy metals (Cd, Cr, Cu, Ni, Pb, Zn, As, and Hg) of SS were analyzed. The heavy metals in the sediment were analyzed by ICP-MS (Inductively coupled plasma mass spectrometry: ELAN 6100, PerkinElmer) and ICP-OES (Inductively coupled plasma-optical emission spectrometry: Optima7300DV, PerkinElmer). Depending on the heavy metals detected in the sediments, the heavy metal pollution index and hazard quotients (HQ_{metal}) were used. HQ_{metal} was calculated as (Eq. (1)):

$$HQ_{metal} = \Sigma SHC/SQG \quad (1)$$

where SHC is the concentration of heavy metals in sediments, and SQG is sediment quality guideline based on the predicted effects level (PEL) (Macdonald et al., 1996).

2.2. Design of the SS exposure systems

The SS exposure system was implemented in a closed circulation chamber made of 8 mm thick acrylic, to prevent leakage due to pressure (Fig. 1a). The experimental system consisted of two sets with same structure, which contained three layers (Fig. 1a). The total volume of the exposure system was 14 L, and each chamber was the same size (L: 18, D: 18, H: 14.5 cm) for each layer. The upper layer (1) was used to measure dissolved oxygen (DO) before recording the respiration of test organisms. The fish were randomly placed into middle layer (2); respiration chamber. The lower layer (3) was used to measure DO after the respiration of test organisms in a temperature-controlled chamber (Fig. S3a).

Seawater was circulated in the order of layer (1) - (2) - (3), and was returned to layer (1) by submerged return motor. A 5 mm thick silicon tube was placed between the chamber, as a channel for seawater to circulate. The resuspension of SS was induced by a submerged motor, which was placed on the bottom of each chamber. A protection screen was placed to minimize water flow from the submerged motor causing stress to the test animals. Experimental seawater was filtered (GF/F, Whatman, Kent, UK), and the temperature was constantly controlled by a cooler (DAB-075, Daeil, Busan, Korea) during the experiment. DO and temperature in the experimental water bath were measured every minute using an optical sensor (Aqualabo, OPTOD, Champigny-sur-Marne, France). The measured data were transmitted to the module (Dongmoon ENT, RTU V2, Seoul, Korea) in real-time (Fig. S3b). To measure OCR, the flow rate was maintained at a minimum of 0.3 L min^{-1} (Fig. S3c).

2.3. QA/QC of the SS exposure system

QA/QC was performed to develop the SS exposure system. In particular, environmental variables (such as water temperature and DO) were maintained during the preliminary experiment. Temperature and DO were maintained at a stable level in the experimental system. Seawater temperature was 20.7 ± 0.01 and 20.8 ± 0.07 mg L^{-1} over

Table 1
Experimental design showing the suspended sediment toxicity test using olive flounder (*Paralichthys olivaceus*), including descriptions of test materials, test organisms, experimental conditions, and endpoints.

Experiment	I	II	III	IV
Test materials				
Spiked material	Non-polluted SS (< 63 μm)	Polluted SS (< 63 μm)	Copper	Copper and non-polluted SS (< 63 μm)
Concentration (mg L^{-1})	125, 250, 500, 1000, 2000, 4000	250, 500, 1000, 2000	0.1, 0.5, 1.0, 2.0	Copper (1) and SS (250, 2000)
Test organism				
Life stage	Early & late juvenile	Early juvenile	Early juvenile	Early juvenile
Fork length (cm)	14.1 ± 0.8 6.8 ± 0.8	6.8 ± 0.8 6.4 ± 0.7	8.0 ± 0.5	8.3 ± 0.5
Experimental condition				
Duration (h)	12	12	12	12
Temperature (°C)	20	20	20	20
Water volume (L)	14	14	14	14
Number of organisms	5	5	5	5
Number of replicates	3	3	2	2
End-point				
Sub-lethal effects		Oxygen consumption rate		
Data presented in	Figs. 2, 3, 4a	Fig. 3	Fig. 4b	Fig. 4c

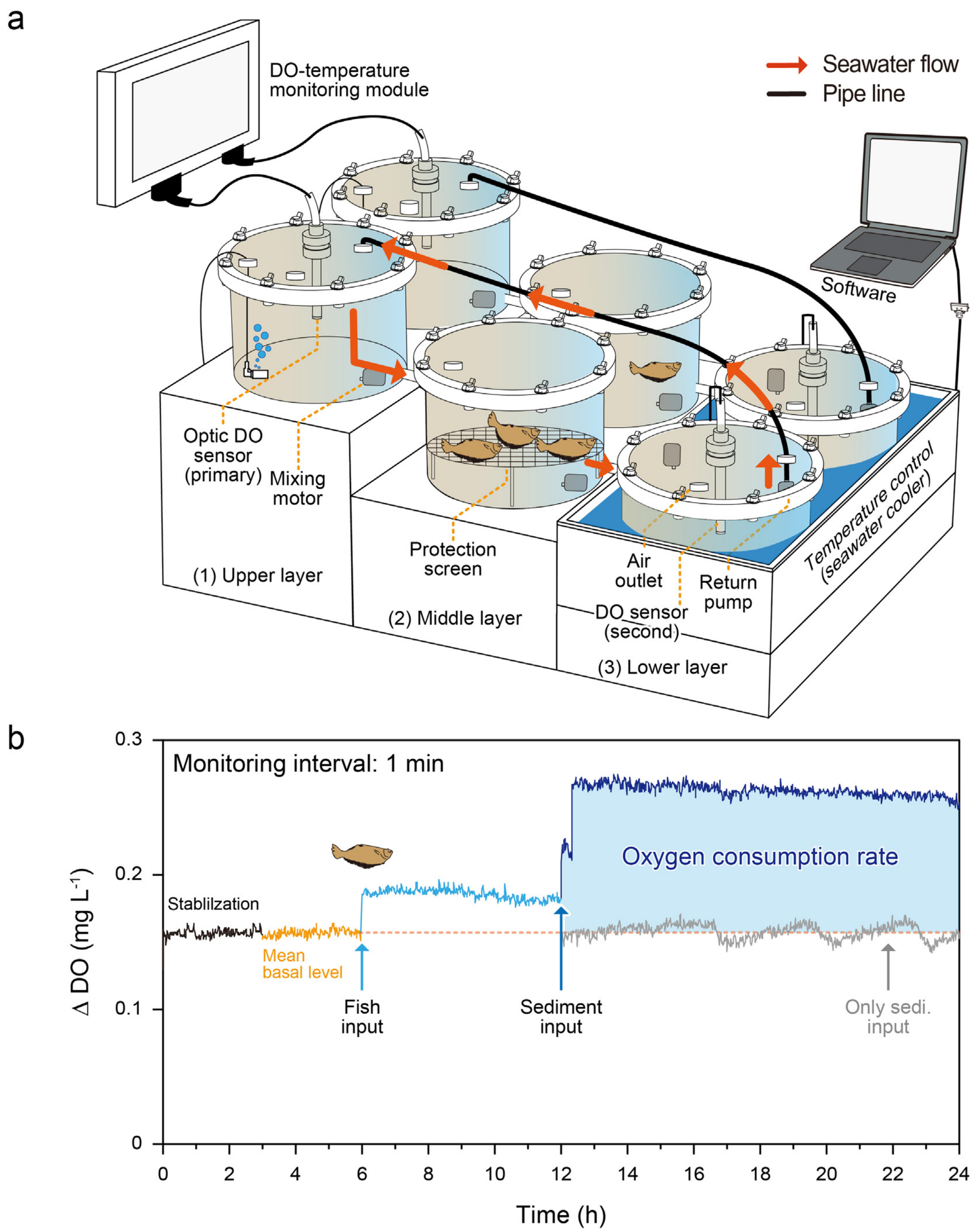

Fig. 1. Fish exposure system to suspended sediment. (a) closed-circulation system for measuring real-time oxygen consumption rate on fish; (b) measurement of dissolved oxygen (DO) during the experimental process. Experimental conditions are presented in Table 1.

12 h in two sets of exposure systems, respectively (Fig. S3a). During the experiment, DO concentrations were 7.50 ± 0.01 and 7.43 ± 0.02 mg L^{-1} over 12 h in two sets of exposure systems, respectively (Fig. S3b).

The flow rate of DO must be kept constant because it is used to calculate OCR (Jobling, 1982). To minimize organism's stress and variability of DO, we selected low flow rate (viz., DO = 0.17 mg L^{-1} at 0.4 L min^{-1}, on average, during 12 h) (Fig. S3c). DO concentrations in the following exposure to non-polluted sediments collected from Gwangyang, Busan, and Incheon were 0.22, 0.18, and 0.18 mg L^{-1}, respectively (Fig. S3d). In comparison, when using heavy metal polluted SS from Ansan, DO concentration was 0.20 mg L^{-1} (Fig. S3d). Because turbidity might change when the sediment is resuspended during the experiment period, the sediment resuspension capability in the exposure system was confirmed. The averaged sediment resuspension rate was confirmed as 75 ± 4% for four SS samples. Various preliminary experiments were conducted to detect potential environmental variations.

2.4. Handling of the test species

Experimental organisms of juvenile olive flounders were supplied by fish farms located near the sites where the sediments were collected. Following transfer to the laboratory, the fish were allowed to acclimate for at least 2 weeks in a 54 L volume (45 × 30 × 40 cm) glass chamber. Test chambers were placed in a temperature-controlled room that was maintained at 20 ± 1 °C. The light/dark photoperiod was 12/12 h, and salinity was 35 psu. Oxygen concentrations in all chambers were

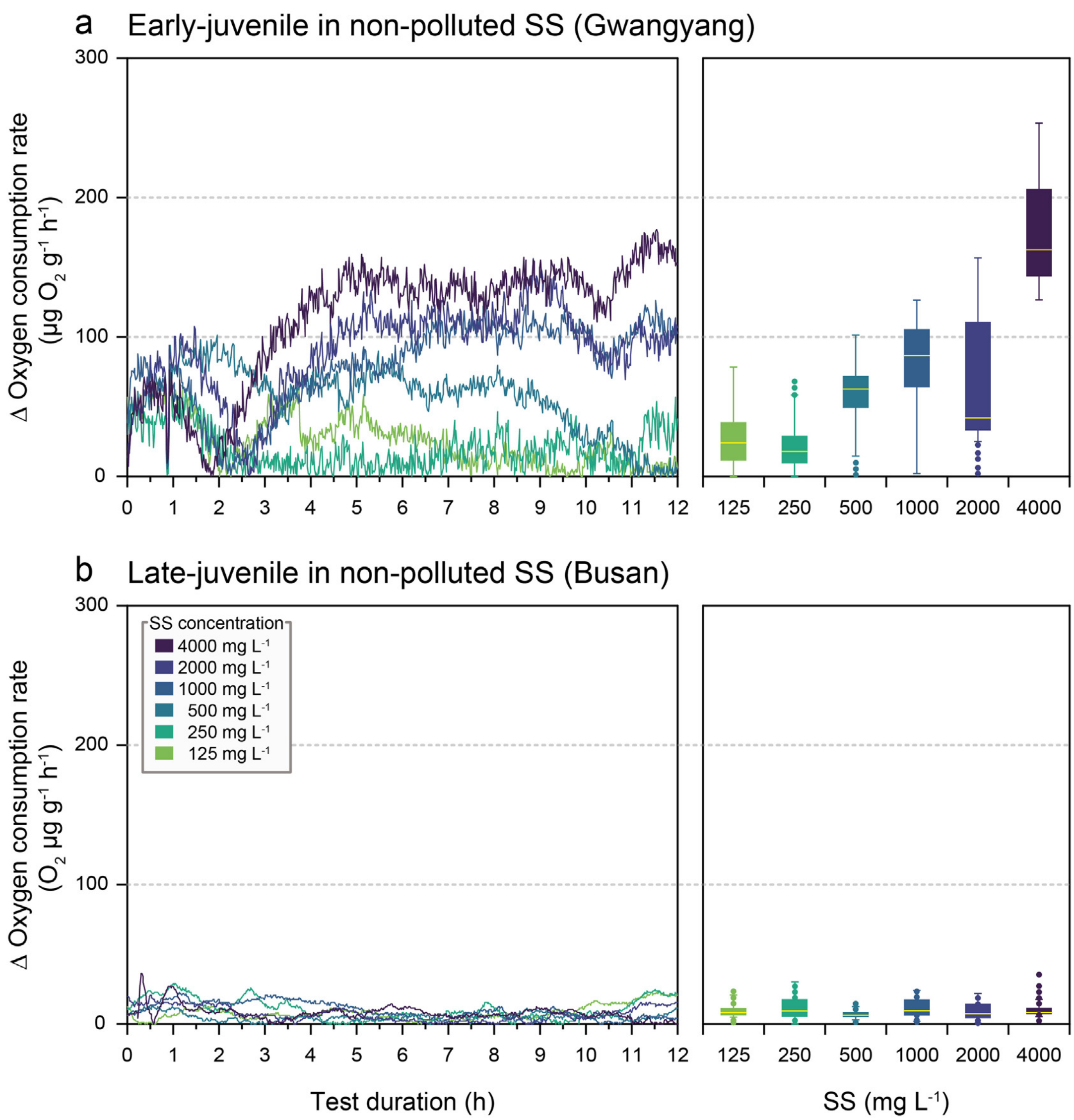

Fig. 2. Delta oxygen consumption rate (ΔOCR) of different life stages of olive flounder at increasing suspended sediment (SS) exposure for 12 h. (a) Early-juvenile ΔOCR recorded at 1 min intervals and boxplots for delta oxygen consumption rate by use of SS in Gwangyang. (b) Late-juvenile ΔOCR recorded at 1 min intervals and boxplots for delta oxygen consumption rate by use of SS in Busan. Each yellow line shows the median values of ΔOCR for 12 h. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

continuously maintained at levels of at least 7.5 mg L^{-1}. Fish were fed 3% of their body weight twice daily (09:00 and 21:00), and about 30% seawater was replaced every 3 days during the acclimation period. From 24 h before the experiment, fish were not fed. We used similar sized healthy fish for the experiment (ASTM, 2002).

2.5. SS exposure

A series of four independent experiments was performed using non-polluted SS, polluted SS, copper, and mixture of copper and non-polluted SS, depending on the specific purposes (Table 1). Non-polluted and polluted SS utilized in the experiments were from naturally dredged sediments. The copper and SS mixture was made of dissolved copper and non-polluted sediment. First, different life stage of olive flounder (average fork length of early-juvenile fish = 6.8 ± 0.8 cm, late-juvenile fish = 14.1 ± 0.8 cm) were exposed to non-polluted SS at 125, 250, 500, 1000, 2000, and 4000 mg L^{-1}. Second, similar-sized fish were exposed to polluted sediment. In the preliminary experiment, we set 4000 mg L^{-1} polluted SS as the highest SS, but SS decreased rapidly, preventing us from OCR recording. Thus, SS concentrations were tested at 250, 500, 1000, and 2000 mg L^{-1}. Third, as a preliminary test of mixture exposure experiment, dissolved copper was tested to determine single chemical toxicity at concentrations of 0.1, 0.5, 1.0, and 2.0 mg L^{-1}. Finally, mixture effects of copper and SS were tested with 1 mg Cu L^{-1} and 250 and 2000 mg SS L^{-1} of non-polluted SS.

Twelve-hour exposure time was used, which reflects the semidiurnal tide of the Yellow Sea. Before each experiment, the DO sensors were calibrated and monitored for 6 h to ensure that DO and temperature were maintained at a constantly stable level. Subsequently, the experimental organisms were placed in the middle chamber, and allowed to acclimate for at least 6 h before SS exposure. Then, SS was added to the top chamber and the DO concentrations of the top and bottom test chambers were measured. The OCR is calculated as (Eq. (2)) (Jobling, 1982):

$$\text{OCR } (\mu g\ O_2\ g^{-1}\ h^{-1}) = [(C_i - C_f) \times F]/W \tag{2}$$

where C_i and C_f are the DO concentrations before and after fish respiration, respectively (Fig. 1b). F is the flow rate (L h^{-1}) of the SS exposure system, and W is the fish weight in the experimental chamber.

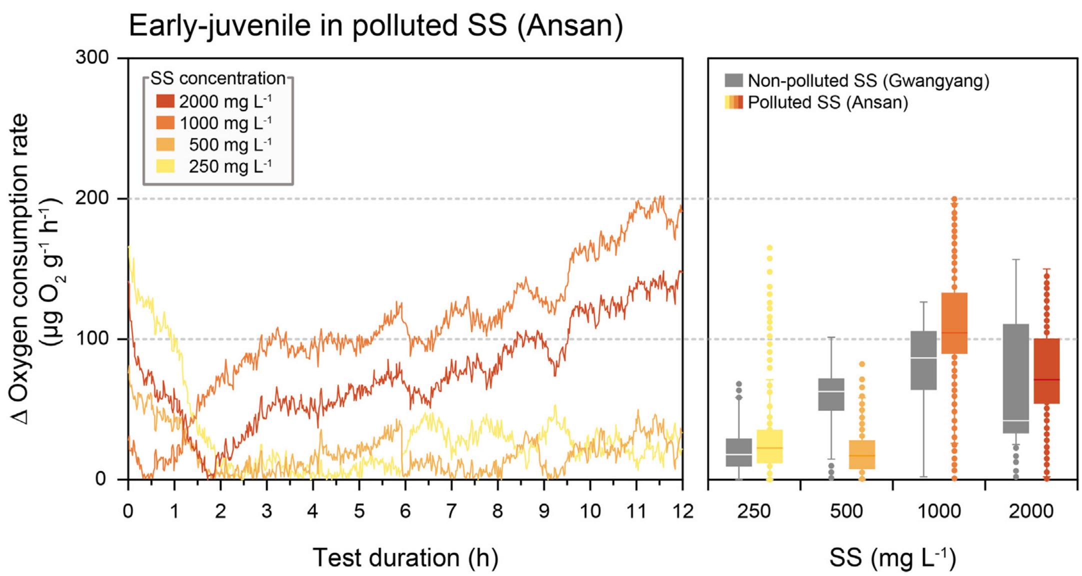

Fig. 3. Delta oxygen consumption rate (ΔOCR) of olive flounder exposed to heavy metal contaminated suspended sediment (SS) in Ansan. Gray boxplot shows ΔOCR of olive flounder exposed to non-polluted SS in Gwangyang.

2.6. Data analysis and statistics

To evaluate the delta OCR, we calculated the difference between negative control values (clean seawater) and treatments (non-polluted and polluted SS treatments) (Eq. (3)).

$$\Delta \mathrm{OCR}\ (\mu g\ O_2\ g^{-1}\ h^{-1}) = |(O_n - O_t)| \quad (3)$$

where O_n and O_t are the OCR values of negative control and SS treatment after fish respiration, respectively. All experimental data was analyzed by the Mann-Whitney *U* test method, comparing OCR after 30 min and after 12 h exposure. Sub-lethal and lethal trends of fish after SS exposure were assessed using polynomial model in SigmaPlot (Version 10.0, Systat Software GmbH, Erkrath, Germany).

3. Results and discussion

3.1. Characteristics of the sediments

The grain size and heavy metals of the sediments collected at four sites were analyzed (Figs. S2, S4, and Table S1). Grain size was the largest at Ansan, followed by Incheon, Gwangyang, and Busan. The concentrations of heavy metals in sediments from three sites were below the PEL-SQGs (Fig. S4). However, Cd, Cr, Cu, Ni, Pb, and Zn concentrations in sediment from Ansan exceeded the corresponding PELs. In particular, copper concentrations were 16 times greater than its PEL. HQ_{metal} determines the degree of pollution of each heavy metal, and highest at Ansan, followed by Incheon, Busan, and Gwangyang. Sediments from Ansan were extremely polluted with various heavy metals. When the HQ_{metal} value of a single heavy metal exceeds 1, it is considered harmful; thus, the extremely high concentrations of copper in the sediment at Ansan is of concern. Consequently, if these sediments are resuspended and flow into the marine ecosystem, they could be extremely dangerous to various marine organisms.

3.2. Effects of SS on different life stage of fish

OCR differed with fish life cycle when exposed to SS (Fig. 2). In the seawater control treatment, the 12 h averaged OCR was 334 ± 23 μg $O_2\ g^{-1}\ h^{-1}$ in early-juvenile stage of olive flounder, and 119 ± 6 μg $O_2\ g^{-1}\ h^{-1}$ in late-juvenile stage fish. The OCR significantly differed with respect to fish life cycle and/or body size in the seawater control without SS ($p < 0.01$). This result was consistent with the previous finding that earlier-life stage of fish (representing smaller fish) has a higher organ oxygen demand ratio (Buermann et al., 1997).

The OCR gradually declined in large flounder exposed to elevated SS over 12 h; there was significant difference between small and large individuals ($p < 0.01$). After exposure, the early-juvenile stage of olive flounder showed two patterrns in delta OCR, by combined aspects of exposure duration and SS concentrations (Fig. 2a). First, at the beginning of exposure, delta OCR tended to decrease over 2 h SS exposure, regardless of its concentrations. Next, a pattern of significant increase in delta OCR was characteristic, particularly under exposure of higher SS concentrations (1000–4000 mg SS L^{-1}) ($p < 0.01$, Table S2).

At 125 and 250 mg L^{-1} SS concentrations, the homeostasis of OCR appeared to adjust to stimulation (Lushchak, 2011). Previous studies reported increased coughing and oxygen consumption at relatively low concentrations of SS (Newcombe and Jensen, 1996). Delta OCR rapidly increased following exposure to > 500 mg L^{-1} SS in our study (Fig. 2). This decline was probably caused by SS damaged gill cells, which increased the distance needed for oxygen diffusion distance, reducing respiration efficiency (Hess et al., 2015). The green-lipped mussel (*Perna viridies* L.) showed no significant difference when exposed to suspended solids from 0 to 600 mg L^{-1} for 14 days; however, the scale of gill damage increased after 14 days of recovery from SS exposure in seawater (Shin et al., 2002). The SS likely impacts gill functioning following long-term exposure. Thus, early stage of fish was more sensitive to SS compared with late stage of fish.

3.3. Effects of polluted SS

The trend in the OCR responses of olive flounder differed for fish exposed to non-polluted and polluted SS ($p < 0.01$, Fig. 3 and Table S3). The OCR values of fish were 345 ± 20, 390 ± 34, 274 ± 47, and 271 ± 57 μg $O_2\ g^{-1}\ h^{-1}$ at non-polluted SS exposure of 250, 500, 1000, and 2000 mg L^{-1} for 12 h, respectively. The OCR when exposed to polluted SS at same concentration were 150 ± 33, 184 ± 24, 286 ± 52, and 239 ± 60 μg $O_2\ g^{-1}\ h^{-1}$, respectively. When exposed to polluted SS, the OCR showed two patterns, depending on SS concentrations. First, the delta OCR did not greatly vary between non-polluted and polluted SS (250 mg SS L^{-1}), statistically significant though ($p < 0.01$). In comparison, when fish were exposed to polluted SS of 500 to 2000 mg L^{-1}, delta OCR markedly differed for those exposed to non-polluted SS ($p < 0.01$, Fig. 3 and Table S3). This result was likely supported by the elevated heavy metals concentration measured in polluted sediment. Of note, Σ HQ_{metal} of polluted sediments was 27.7 times greater than those in non-polluted sediments (Fig. S4 and Table S1).

SS and various heavy metals showed a combined effect. Heavy metals are the most common source of marine pollutants, with different heavy metals affecting fish differently (Lee et al., 2016). The OCR of freshwater crab (*Sinopotamon henanense*) exposed to 28.6 mg Cd L^{-1} for 96 h was 28% higher than that of the control (Xuan et al., 2013). Exposure to 2.86 mg Cu L^{-1} for 21 days reduced the OCR by 65% compared to the control (Xuan et al., 2013). Exposure of the rohu (*Labeo rohita*) to 39.4 mg Cr L^{-1} for 96 h reduced the OCR by 56% compared to the control (Vutukuru, 2005). Compared to the control, the mortality of the surf clam (*Mactra veneriformis*) was 100% higher when exposed to 0.025–0.1 mg Cu L^{-1} for 4 weeks, while the respiratory rate was 75% lower than that of the control. In addition, exposure to heavy metals generates histological effects, causing an increase in gill mucous cells and epithelial necrosis (Shin et al., 2013). Chronic (24 weeks) exposure of the Venus clam (*Gomphina veneriformis*) to 0.64–1.79 mg Zn L^{-1} had sub-lethal effects, causing morphological changes, including the expansion of the hemolymph sinus system, the loss of the striated border of the inner epidermis, and an increase in the number of mucous cells in the mantle. Histological degeneration (including epithelial necrosis and the hyperplasia of mucous cells) was also detected in the gill and foot (Ju et al., 2006).

Metabolic rate appears to increase in response to physiological stress caused by polluted SS breaking down the energy balance (Boeck and Blust, 1997). In a previous study, freshwater shrimp (*Palaemonetes paludosus*) subjected to chronic exposure (8 months) to polluted sediment exhibited 51% higher OCR compared to shrimp exposed to non-polluted sediment, with more energy being required to maintain metabolism (Rowe, 1998). The OCR of various other taxa including snake, larval frog, and shrimp increased when exposed to pollution (Rowe et al., 2001). Exposure to certain stressors might also stimulate the hydromineral balance of nerves and liver glycogen to decrease, in parallel to increase plasma glucose, cardiac output, gill blood flow, and oxygen uptake and transfer (Bonga, 1997). Thus, respiratory impairment likely occurs through exposure to a combination of heavy metals and SS.

However, heavy metals exist with combined forms in natural environment, thus their combined effects on diverse organisms would vary. For example, exposure of the freshwater alga (*Chlorella* sp.) to Cu, Zn, and Cd for 72 h showed that Cu + Cd treatment had synergistic effects, whereas Cu + Zn and Cd + Cu + Zn treatments had no additive or antagonistic effects on growth rates (Franklin et al., 2002). Exposure of juvenile flatfish (*Solea senegalensis*) to three polluted SS (heavy metals, metalloids, and organic pollutants) for 28 days showed that organic polluted and heavy metal polluted SS treatments caused pathological damage (Costa et al., 2009). It was not possible to determine exactly which heavy metals influenced the OCR of fish at the moment; however, it is likely that the interaction between sediments and pollutants influences fish metabolism.

3.4. Combined effects of copper and non-polluted SS

In the preliminary experiment, the delta OCR of olive flounder showed dose-dependent responses when exposed to copper for 12 h (Fig. 4b). The OCR of fish were 187 ± 10, 160 ± 11, 124 ± 5, and 94 ± 44 μg O_2 g^{-1} h^{-1} at dissolved copper exposure of 0.1, 0.5, 1.0, and 2.0 mg L^{-1} for 12 h, respectively (Table S4). The OCR when exposed to mixture of dissolved copper (1 mg L^{-1}) and non-polluted SS (250 and 2000 mg L^{-1}) were 160 ± 5 and 109 ± 19 μg O_2 g^{-1} h^{-1}, respectively (Table S5). After 12 h exposure to 2 mg Cu L^{-1}, the delta OCR of fish significantly increased by 89% compared to the seawater control (Fig. 4b).

Copper naturally exists in the marine environment, and is an essential element for the growth of fish. However, high concentrations of copper negatively impact the physiology and behavior of marine organisms (Sutherland and Major, 1981). For instance, it changes reactive oxygen species levels, mucus secretion rates, oxygen consumption, and nitrogen excretion rates, and causes tissue damage (Vosloo et al., 2012). Exposure of the sea squirt (*Halocynthia roretzi*) to 1.6 mg Cu L^{-1} reduced infiltration rates by 80–88% and reduced OCR by 83–87%, in a size-dependent manner (Kang and Hur, 2012). Copper exposure negatively impacts the respiration efficiency in many species. It also impairs ion regulation and/or respiratory stress by causing changes to the gill structure and increasing ion osmosis in gill epithelia (Wilson and Taylor, 1993). Thus, copper toxicity impacts the mode of action of gills in marine organisms, including fish.

The delta OCR of olive flounder showed distinct dose-dependent response following exposure to copper combined with SS (Fig. 4c and Table S5). However, the delta OCR following exposure to the copper with 250 mg SS L^{-1} treatment was not higher compared to single copper exposure ($p < 0.01$, Fig. 4c and Table S5). Interestingly, the delta OCR of fish after exposed to the mixed copper and non-polluted SS was smaller than those exposed to copper only and non-polluted SS (Fig. 4c). These results indicate the OCR of fish exposed to non-polluted SS, copper, or copper with non-polluted SS showed no synergistic effect. It is indicated that dissolved free copper ions would decline after exposure to SS, thus its mixture effect becomes minimal (Fig. 4c).

The addition of SS to copper revealed a significant difference in OCR of fish, either negatively or positively, compared to copper only exposure, in our study. The LC_{50} of amphipods (*Melita plumulosa*) and bivalves (*Tellina deltoidal*) after 10-d exposure to dissolved copper was lower than that to particulate copper (Simpson, 2005). Dissolved copper has a stronger effect than particulate copper on benthic organisms (Simpson and King, 2005). High concentrations of dissolved copper might have absorbed to solid SS when resuspended, consequently decreased its impact on fish. Metal bioavailability can be different because of its high affinity for dissolved and particulate organic matter (Zhang et al., 2014). Fine particle with greater organic carbon contents of sediments have a relatively higher metal affinity than coarse sediments (Zhang et al., 2014). Overall, OCR changed the least in the presence of non-polluted SS, followed by copper, mixed copper and SS, and polluted SS at the bases of 1000–2000 mg SS L^{-1} and 1 mg Cu L^{-1} (Figs. 2–4). The results of this study generally indicate that SS is a key factor influencing the OCR of fish; however, its effect is strengthened it is mixed with various pollutants, including heavy metals. Therefore, it is necessary to investigate the background concentrations of various pollutants, including heavy metals, in areas where elevated SS might occur.

3.5. Toxicological effects of SS on various marine fishes: a mini-review

We collected toxicological data from published works to examine sub-lethal and lethal effects of SS exposure on various marine fish species, as part of study. Metadata generally included well-established test species of marine fish (Tables S6–S7). A previous study reported delayed reaction time in juvenile marine fish due to elevated SS at 180 mg L^{-1} (Wenger et al., 2012). But mortality at 180 mg SS L^{-1} was not significantly different from the control group. However, a slight increase of SS concentration (< 500 mg L^{-1}) might have caused a behavioral change (i.e., prey consumption) (Breitburg, 1988; Wenger et al., 2012). Overall, sub-lethal toxicities were found to be the least sensitive against SS exposure compared to lethal toxicities (Fig. 5). The present mini-review confirmed that the concentrations of SS exhibiting the deleterious effects on marine species would greatly vary across the diverse endpoints in lethal and sub-lethal toxicities.

4. Conclusions

The SS associated with sediment resuspension likely has sub-lethal effects on fish inhabiting coastal areas. The SS enters marine

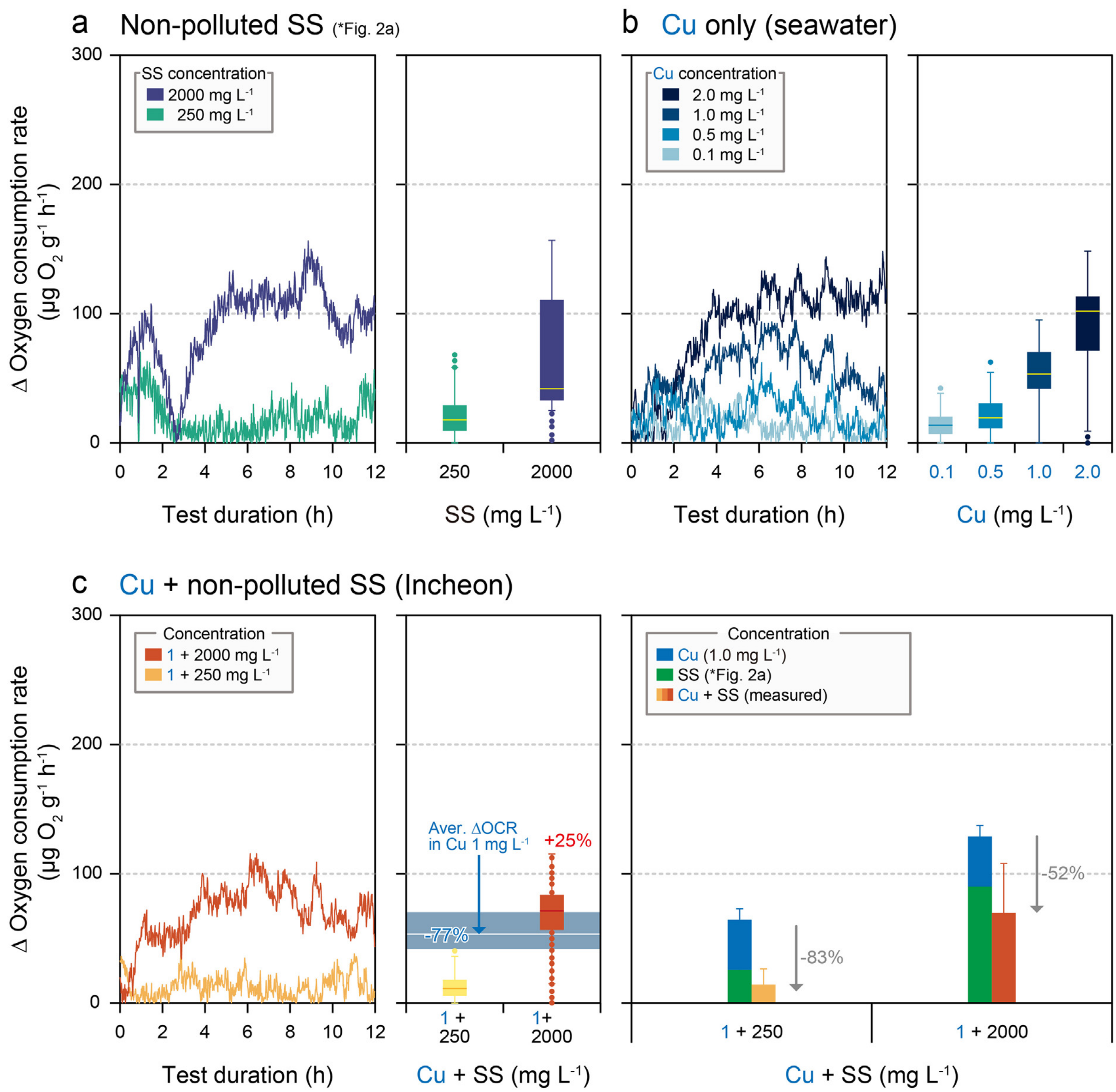

Fig. 4. Delta oxygen consumption rate (ΔOCR) of olive flounder exposed to (a) non-polluted suspended sediment (SS) (data from Fig. 2a), (b) dissolved copper, and (c) mixture copper and non-polluted SS in Incheon for 12 h. (c) comparison of measured ΔOCR for seawater only, dissolved copper only, and dissolved copper plus non-polluted SS mixture. The gray arrows explain the differences between single and mixture exposure results.

environment from various sources, leading to potentially higher concentrations than under natural condition, resulting in various sub-lethal and lethal effects on fish. In particular, such as net cage fish farm, it is likely to have a greater impact in the areas with densely populated and/or lesser moved species. Our results supported that short-term exposure (< 12 h) to SS had an adverse effect on the OCR of fish in size-dependent manner, with early life stage fish being impacted more severely than late life stage one. Altogether, early stage and/or sedentary fish can be under more dangerous situation when their ability to escape was limited. There was a clear dose-dependent response relationship between copper concentration and OCR of fish. In comparison, no synergistic effects were shown for delta OCR of olive flounder being exposed to the mixed copper and non-polluted SS. However, when the polluted sediment is resuspended with high copper concentration, it is likely more toxic to fish than single SS exposure. A mini-review of SS toxicities on fish indicates that great variations in sub-lethal and lethal toxicities exist cross species, life stage, and sediment concentration.

CRediT authorship contribution statement

Seung Oh Chu: Conceptualization, Formal analysis, Investigation, Visualization, Writing - original draft. **Changkeun Lee:** Conceptualization, Visualization, Writing - original draft, Writing - review & editing. **Junsung Noh:** Investigation, Visualization. **Sung Joon Song:** Project administration. **Seongjin Hong:** Project administration, Writing - review & editing. **Jongseong Ryu:** Resources, Writing - review & editing. **Jung-Suk Lee:** Resources, Writing - review & editing. **Jungho Nam:** Conceptualization, Project administration, Funding acquisition. **Bong-Oh Kwon:** Conceptualization, Project administration, Funding acquisition, Supervision. **Jong Seong Khim:** Conceptualization, Writing - review & editing, Project administration, Funding acquisition, Supervision.

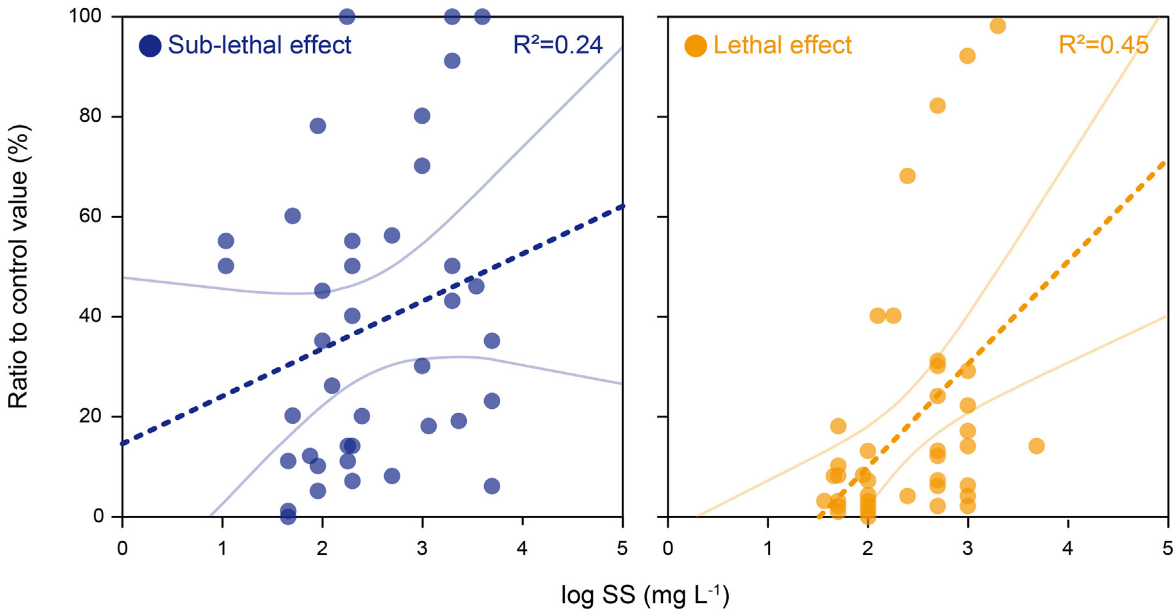

Fig. 5. Ratio to control values (%) showing sub-lethal and lethal effects on various marine fish species associated with elevated concentrations of suspended sediment (SS). Data collected from published studies and present work. Refer to the original meta-data provide in Tables S6–S7.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111113.

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<Supplementary Materials>

Effects of polluted and non-polluted suspended sediments on oxygen consumption rate of olive flounder, *Paralichthys olivaceus*

Seungoh Chu[1], Changkeun Lee[1], Junsung Noh, Sung Joon Song, Seongjin Hong, Jongseong Ryu, Jung-Suk Lee, Jungho Nam, Bong-Oh Kwon[*], Jong Seong Khim[*]

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[*]Corresponding authors.
E-mail addresses: bongkwon@snu.ac.kr (B.-O. Kwon); jskocean@snu.ac.kr (J.S. Khim).
[1] These authors contributed equally to this work.

Supplementary Figures

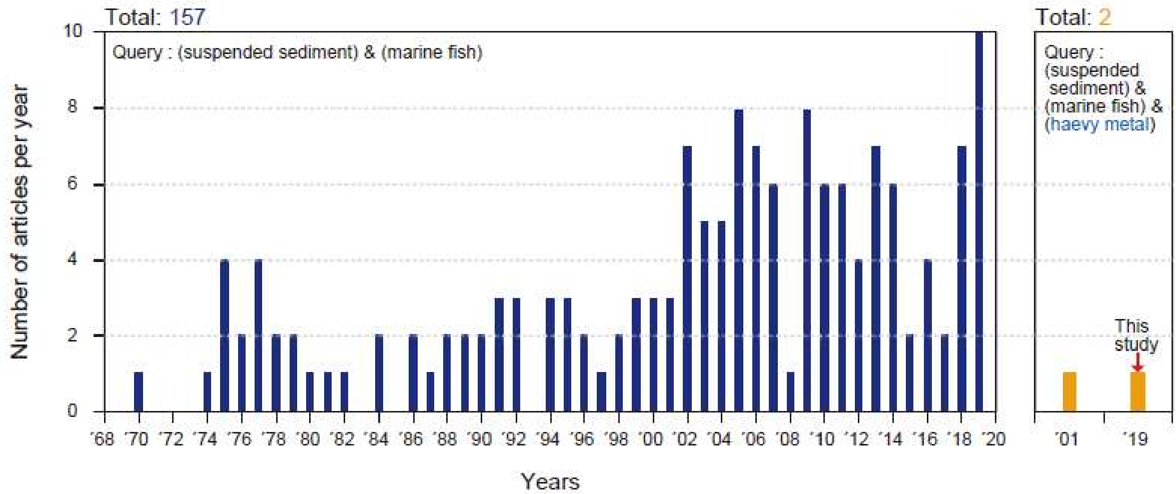

Fig. S1. Number of publication on suspended sediment effect for marine fish from 1968 to present.

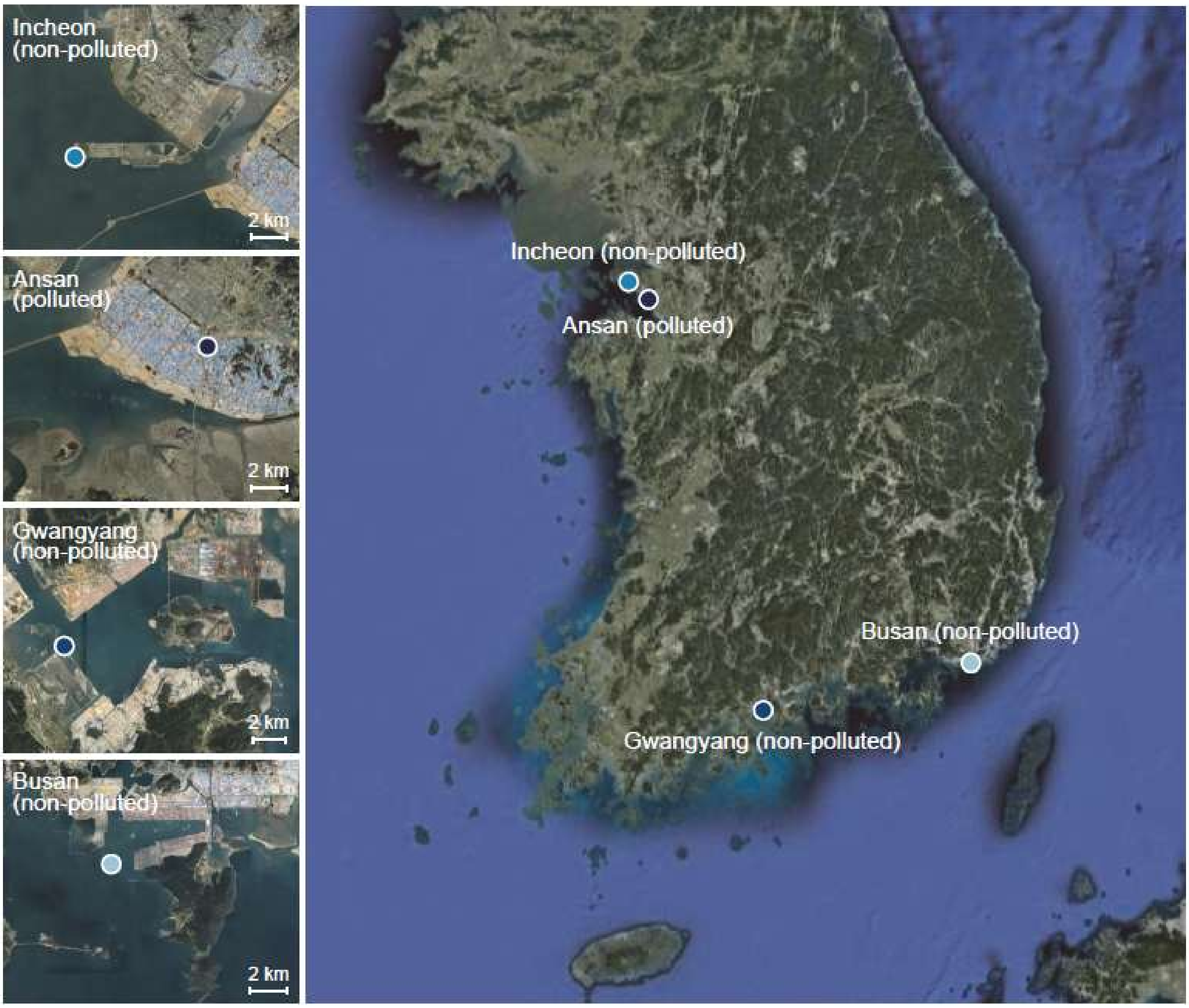

Fig. S2. Sampling locations for suspended sediment on the west-south coast of South Korea.

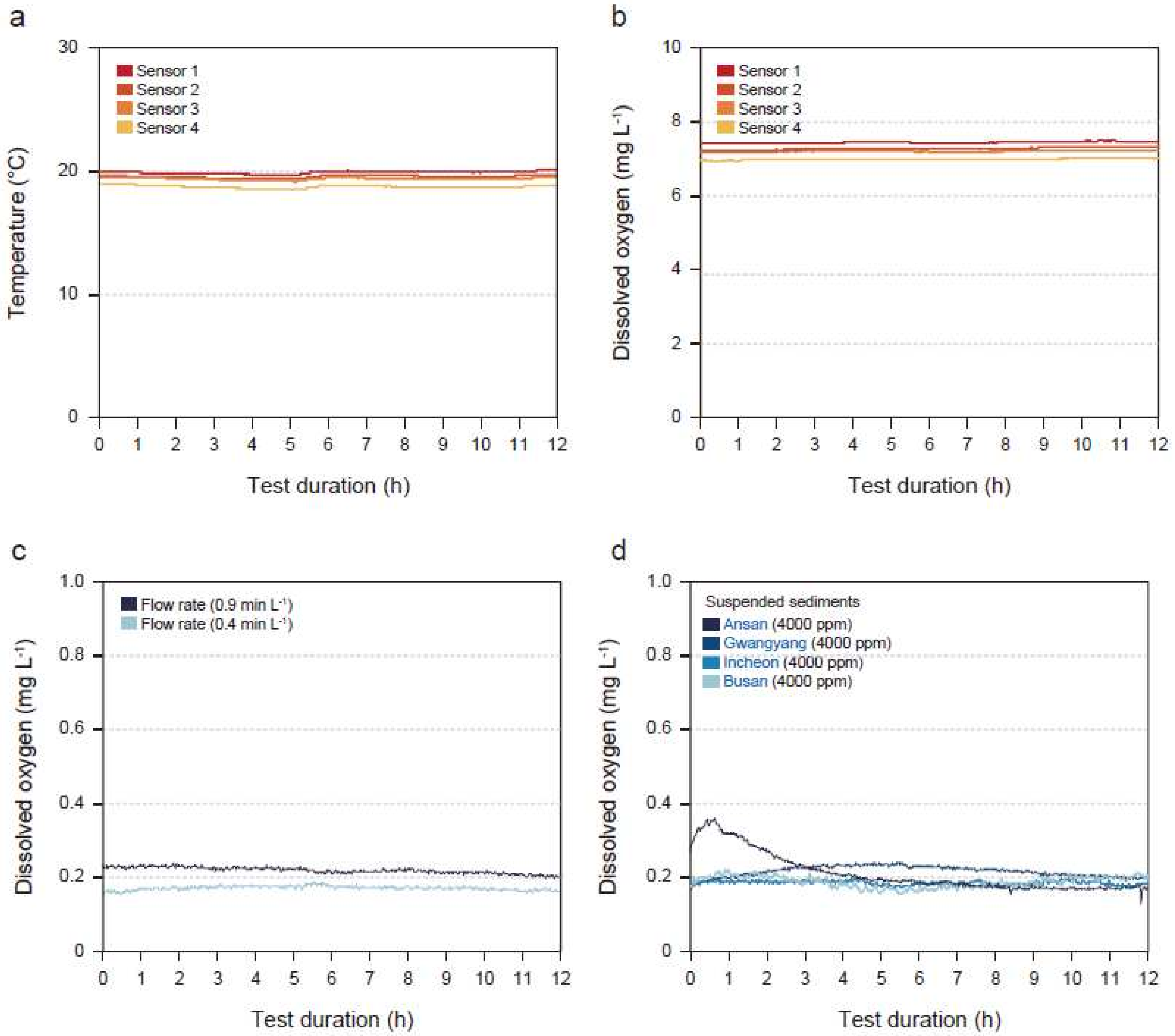

Fig. S3. QA/QC results for SS exposure system. During the experiment (a) temperature and (b-d) DO were maintained at a stable level in each experimental condition.

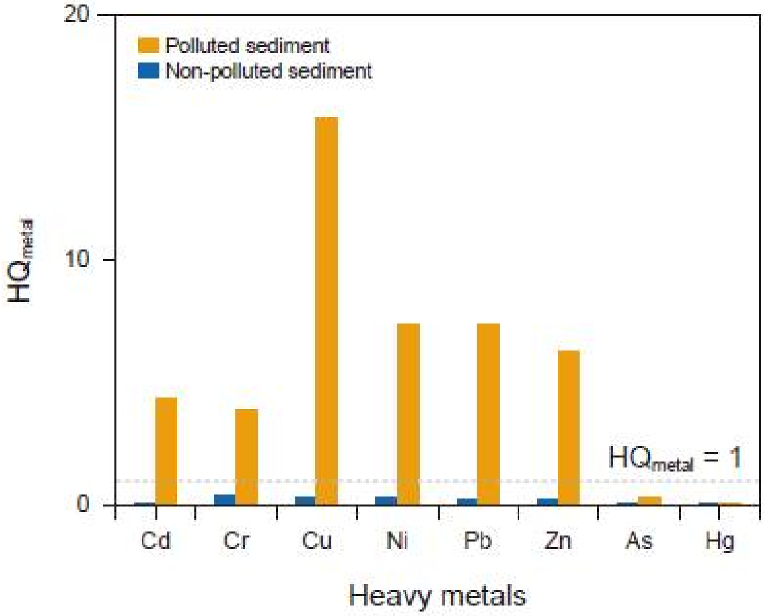

Fig. S4. Each HQ_{metal} values in polluted (Ansan) and non-polluted sediments (Gwangyang, Busan, and Incheon). The HQ_{metal} value of a single heavy metal exceeds 1, it is considered harmful.

Supplementary Tables

Table S1. Physicochemical properties and heavy metal concentrations of the sediments collected in Korean coastal waters.

Sampling site	**Grain size (%)**		**TOC**	**Cd**	**Cr**	**Cu**	**Ni**	**Pb**	**Zn**	**As**	**Hg**	**ΣHQ$_{metal}$**
	Silt	**Clay**	**(%)**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**	**(mg/kg)**	**(µg/kg)**	
Gwangyang	80.5	19.5	1.02	0.20	69.0	6.87	30.0	26.4	40.5	14.9	39.2	1.67
Busan	84.0	16.0	0.08	0.25	67.9	9.61	30.4	30.0	48.2	8.05	16.6	1.69
Ansan	71.3	28.7	1.48	12.6	740	1,060	627	926	1,030	33.5	0.01	47.7
Incheon	74.4	25.6	0.69	0.18	80.6	10.4	32.6	28.6	51.3	9.35	19.4	1.80
*PEL	-	-	-	2.72	181.0	64.4	80.5	119	157	75.5	0.62	

*Korean Probable Effects Level (PEL).

Table S2. T-test result comparing treatment and control (seawater only) on fish oxygen consumption rate (OCR) after non-polluted SS exposure for 12 h.

Experimental condition		OCR ($\mu g\ O_2\ g^{-1}\ h^{-1}$)	
Chamber	**SS ($mg\ L^{-1}$)**	**Early-juvenile**	**Late-juvenile**
Control	0	334 ± 23	119 ± 6.3
Treatment	125	328 ± 10*	120 ± 3.9*
	250	345 ± 20*	127 ± 12*
	500	390 ± 34*	118 ± 9.5
	1000	274 ± 47*	111 ± 4.6*
	2000	271 ± 57*	117 ± 4.2*
	4000	234 ± 53*	116 ± 12*

*Significantly correlated at the p< 0.01 level (2-tailed).

Table S3. T-test result comparing non-polluted SS and polluted SS treatment on fish oxygen consumption rate (OCR) after SS exposure for 12 h.

Experimental condition		OCR ($\mu g\ O_2\ g^{-1}\ h^{-1}$)	
Chamber	**SS ($mg\ L^{-1}$)**	**Non-polluted SS**	**Polluted SS**
Treatment	250	345 ± 20	150 ± 33*
	500	390 ± 34	184 ± 24*
	1000	274 ± 47	286 ± 52*
	2000	271 ± 57	239 ± 60*

*Significantly correlated at the $p < 0.01$ level (2-tailed).

Table S4. T-test result comparing treatment and control (seawater only) on fish oxygen consumption rate (OCR) after Cu exposure for 12 h.

Experimental condition		OCR ($\mu g\ O_2\ g^{-1}\ h^{-1}$)
Chamber	**Cu ($mg\ L^{-1}$)**	**Early-juvenile**
Control	0.0	179 ± 10
Treatment	0.1	187 ± 10*
	0.5	160 ± 11*
	1.0	124 ± 16*
	2.0	187 ± 10*

*Significantly correlated at the $p < 0.01$ level (2-tailed).

Table S5. T-test result comparing Cu and Cu + SS mixture treatment on fish oxygen consumption rate (OCR) after exposure for 12 h.

Experimental condition			OCR ($\mu g\ O_2\ g^{-1}\ h^{-1}$)
Chamber	**Cu ($mg\ L^{-1}$)**	**SS ($mg\ L^{-1}$)**	**Early-juvenile**
Cu only	1.0	-	179 ± 10
Cu + SS mixture	1.0	250	160 ± 5.2*
	1.0	2000	109 ± 19*

*Significantly correlated at the $p< 0.01$ level (2-tailed).

Table S6. A mini-review on sub-lethal effects of suspended sediment on juvenile marine fish.

Test organism Species	Experimental condition Con. (mg L^{-1})	Duration (d)	Endpoints	Ratio to negative control values	Reference
Epinephelus coioides	200	42	Plasma concentration	-0.55	Au et al., 2004
	2000	42	Gill chloride cell density	+1.00	
	200	42	Epithelium lifting	+0.07	
	50	42	Thickness of epithelium	-0.60	
	200	42		-0.50	
	2000	42		-0.43	
	50	42	Thickness of pillar system	+0.20	
	200	42		+0.40	
	2000	42		+0.50	
Oncorhynchus mykiss	5000	24	Condition factor	-0.06	Michel et al., 2013
	5000	24	Growth rate	-0.23	
	5000	24	Hepato-somatic index	+0.35	
Acanthochromis polyacanthus	45	42	Reaction time (seconds)	+0.11	Wenger et al., 2012
	90	42		+0.78	
	180	42		+1.00	
	45	42	Hepatocyte vacuolation	No effect	
	90	42		-0.10	
	180	42		-0.11	
	45	42	Growth rate	-0.01	
	90	42		-0.05	
	180	42		-0.14	
Lepomis macrochirus	1163	0.002	Feeding rate	-0.18	Gardner, 1981
	2326	0.002		-0.19	
	3488	0.002		-0.46	
Morone saxatilis	75	0.02	Prey consumption	-0.12	Breitburg, 1988
	200	0.02		-0.14	
	500	0.02		-0.08	
Salmo gairdneri	10	0.21	Ventilation	-0.55	Ross et al., 1985

	100	0.21		-0.45	
	1000	0.21		-0.70	
	10	0.21	Cough rate	-0.50	
	100	0.21		-0.35	
	1000	0.21		-0.30	
Paralithchys olivaceus	125	0.50	Oxygen consumption rate	+0.26	This study
	250	0.50		+0.20	
	500	0.50		+0.56	
	1000	0.50		+0.80	
	2000	0.50		+0.91	
	4000	0.50		+1.00	

Table S7. A mini-review on lethal effects of suspended sediment on juvenile marine fish.

Test organism	Experimental condition		Endpoints	Ratio to negative control values	Reference
Species	Con. (mg L^{-1})	Duration (d)			
Acanthochromis polyacanthus	45	42	Mortality	+0.08	Wenger et al., 2012
	90	42		+0.08	
	180	42		+0.40	
Salmo gairdneri	36	64	Mortality	+0.03	Goldes et al., 1988
	4887	64		+0.14	
Acipenser oxyrinchus oxyrinchus	100	3	Mortality	No effect	Wilkens, 2015
	250	3		+0.04	
	500	3		+0.12	
Alosa aestivalis	50	4	Hatching success	-0.01	Auld and Schubel, 1978
	100	4		-0.01	
	500	4		-0.13	
	1000	4		-0.06	
Alosa pseudoharengus	50	4	Hatching success	-0.02	
	100	4		-0.01	
	500	4		-0.07	
	1000	4		-0.06	
Alosa sapidissima	50	4	Hatching success	+0.10	
	100	4		-0.04	
	500	4		-0.06	
	1000	4		-0.14	
Morone americana	50	4	Hatching success	+0.03	
	100	4		No effect	
	500	4		-0.02	
	1000	4		-0.22	
Morone saxatilis	50	4	Hatching success	-0.18	
	100	4		-0.02	
	500	4		-0.02	

	1000	4		-0.04	
Perca flavescens	50	4	Hatching success	+0.08	
	100	4		+0.03	
	500	4		+0.06	
	1000	4		+0.02	
Alosa sapidissima	50	4	Mortality	-0.02	
	100	4		-0.13	
	500	4		-0.31	
	1000	4		-0.29	
Morone saxatilis	50	4	Mortality	-0.10	
	100	4		+0.01	
	500	4		-0.24	
	1000	4		-0.17	
Perca flavescens	50	4	Mortality	-0.03	
	100	4		-0.07	
	500	4		-0.30	
	1000	4		-0.29	
Paralichthys olivaceus	125	7	Mortality	+0.40	Yoon and Park, 2011
	250	7		+0.68	
	500	7		+0.82	
	1000	7		+0.92	
	2000	7		+0.98	
	2000	7		+0.98	

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