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Paper:

Learning from the Eco-Toxicology of Fire-Fighting Foams in Aquatic Organisms: Altered Eco-Toxicity of Sodium Alkyl Sulfonates on Green Paramecia and Medaka Fish Maintained in Different Waters

Kaishi Goto[*1], Hiroshi Takaichi[*1], and Tomonori Kawano[*1,*2,*3,*4,*5,†]

[*1]Faculty and Graduate School of Environmental Engineering, The University of Kitakyushu
1-1 Hibikino, Wakamatsu, Kitakyushu, Fukuoka 808-0135, Japan
[†]Corresponding author, E-mail: kawanotom@kitakyu-u.ac.jp
[*2]Environmental and Fire-Fighting Technology Development Center, The University of Kitakyushu, Kitakyushu, Japan
[*3]International Photosynthesis Industrialization Research Center, The University of Kitakyushu, Kitakyushu, Japan
[*4]University of Florence LINV Kitakyushu Research Center (LINV@Kitakyushu), Kitakyushu, Japan
[*5]Université Paris Diderot, Sorbonne Paris Cité, Paris 7 Interdisciplinary Energy Research Institute (PIERI), Paris, France
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A variety of ciliated and flagellated protozoan species have been used as bio-indicators of the eco-toxic impacts of polluting chemicals, especially in aquatic environments such as rivers, ponds, lakes, and wetlands. To date, both the short-term and long-term impacts of fire-fighting foams (FFFs) in aquatic (freshwater environment) and semi-aquatic (wetland) ecosystems have been assessed in laboratory-scale model assays and in biotope-based assays. Little attention has been given to the fact that water qualities, such as hardness, drastically alter the toxic actions of various chemicals against living aquatic organisms including fishes, algae, and other microbes, suggesting that the laboratory water often employed in toxicity assays for fishes and microorganisms might not reflect the actual impact of chemicals in the ecosystem. Therefore, for examining the toxicity of certain chemicals (chiefly detergent-based and soap-based FFFs) in aquatic organisms, we have previously proposed that a series of simple eco-toxicity tests using natural waters sampled from the natural organism's habitats or blends of mineralcontaining water preparations mimicking the natural habitat waters be used in addition to tests in standard laboratory waters. Based on the knowledge of the eco-toxicity of FFFs obtained through past studies using model aquatic organisms such as green paramecia (*Paramecium bursaria*), we conducted a study aiming to uncover the toxic mechanism of sodium alkyl sulfonates, a series of synthetic detergents known as SAS, using a strain of *P. bursaria* originally sampled from a river, both in laboratory water and habitat river water (river water from where P. bursaria was collected; HRW). Here, we employed *P. bursaria* maintained in both a natural HRW-based assay medium and an ultrapure water-based low-mineral standard culturing medium for comparing the apparent toxicity of SAS. Data strongly suggested that the toxicities of most SAS detergents (alkyl chains shorter than 9 carbons or longer than 14 carbons) are minimized in the mineral-rich HRW compared to the commonly used UPW-based low-mineral ciliateculturing conditions. The toxicity of SAS members with moderate chain lengths, such as sodium dodecan sulfonate, tended to be minimized with elevated mineral content. A similar tendency was also observed in medaka fish, a tiny model fish.

Keywords: detergent, eco-toxicity, *Paramecium bursaria*, SAS, water hardness

1. Introduction

Several chemicals, including foaming agents, are used to fight and control urban fires and to aid in the protection of forest resources from wildland fires (including woodland and grassland fires). These fire-fighting foams (FFFs) are formulations composed principally of surfactants, and act by increasing water efficiency. These chemicals are gradually gaining acceptance as effective and efficient fire-fighting tools in several countries, such as the USA and Australia (Rawet et al., 1996; Adams and Simmons, 1999). Also, in Tokyo and Kitakyushu, Japan, followed by other cities nation-wide, local fire-fighting authorities have employed several FFFs in urban fire control since 1999 (Kitakyushu City Fire and Disaster Management Department, 2005). Compared to urban fire control, wildland fire management requires much greater amounts of fire-fighting chemicals to be emitted to the environment. Therefore, the possible eco-toxicities of these chemicals should be tested prior to designing chemical fire-fighting strategies in the field (Kawano et al., 2014).

As a part of a Japanese national project, environmentally inert FFFs have been developed (Mizuki et al., 2007, 2010). There has been an emerging need for testing the impacts of these FFFs on the living organisms composing the typical landscapes or ecosystems in Japan. In the course of the development of new generations of environ-

mentally friendly FFFs, we have proposed and conducted a series of bioassays for evaluating the hidden toxicities of commercially available and newly developed FFFs and their key components (chiefly synthetic and fatty acid-based detergents). The choices of biological materials employed in our recent bioassays include (1) a tiny freshwater model fish, *Oryzias latipes*, known as medaka fish (Lin et al., 2006; Kawano et al., 2007; Mizuki et al., 2007); (2) aquatic protozoa such as *Paramecium bursaria* (Kadono et al., 2006a; Mizuki et al., 2007; Goto et al., 2007) and *P. caudatum* (Kadono et al., 2006b); (3) germinating seeds of rice (*Oryza sativa* L.) plants (Kawano et al., 2006); and (4) microbes in activated sludge (Mizuki et al., 2010). These studies allowed us to assess the acute eco-toxicity and biodegradability of FFF-related chemicals in the surrounding environments. In fact, the model organisms listed above are key components in the semi-aquatic landscape in Japan. In addition to the laboratory-scale model assays, some outdoor biotope-based observations have been reported as part of our assessments of both the short-term and long-term impacts of FFFs in aquatic (freshwater) and semi-aquatic (wetland) ecosystems harboring diverse organisms such as plants, fishes, shells, insects, algae, and protozoa (Kawano et al., 2014). Briefly, in the biotope assays based on observations in 1 m^2 compact biotopes mimicking freshwater and wetland environments, both the acute and long-term eco-toxic impacts of 2FFFs (soap-based and synthetic detergent-based) were assessed. The spraying of synthetic detergent-based foam formula was shown to be more toxic than soap-based formula and mock water treatment.

Based on the knowledge of the eco-toxicity of FFFs obtained through the past studies using model aquatic organisms such as *P. bursaria*, we conducted a study to uncover the toxic mechanism of SAS derivatives, using a strain of *P. bursaria* originally sampled from a river, both in laboratory water and habitat river water (river water from where the *P. bursaria* was collected; HRW).

P. bursaria (common name, green paramecium) is a ciliate species that is capable of active swimming in response to various stimuli (Aonuma et al., 2007; Furukawa and Kawano, 2012), and is widely found in freshwater habitats such as rivers, lakes, and ponds (Kosaka, 1991). Previous studies have suggested that this organism acquired symbiotic green algae through an active evolutionary process (Kawano et al., 2004; Ohkawa et al., 2011). Interestingly, green algae can be removed from the hosting ciliate of *P. bursaria* (Tanaka et al., 2002) and replaced with any artificial particles of interest if the particle size matches the size of the aural cavity (up to 10 μm in diameter) (Irie at al., 2010; Furukawa and Kawano, 2012). Normally, the growth of the algae within symbiotic ciliates is highly regulated by the signals derived from the cells of hosting ciliates (Kadono et al., 2004), but some exceptions are also known (Irie et al., 2010).

The toxic impacts of chemicals to freshwater organisms, such as protozoan species, are usually assayed in simple model water supplemented with minimal minerals (such as an ISO-recommended water), or in media composed of laboratory waters such as distilled or ultrapure-filtered water (Duval et al., 2005). However, from an eco-toxicological point of view, such artificial water conditions hardly represent the actual environmental conditions surrounding living organisms. Therefore, we have recently proposed the use of a series of natural waters directly sampled from various natural basins or a series of water preparations mimicking these natural waters (by adding some key minerals at various concentrations) in addition to the tests in standard laboratory waters, when examining the toxicity of certain chemicals (such as detergents, commercial FFFs, and soap-based FFF formulae) in aquatic organisms such as fish (Lin et al., 2006; Kawano et al., 2007) and protozoan species (Kadono et al., 2006b, Goto et al., 2007, 2008).

Our previous studies have shown that the viability of 2 paramecium species (*P. bursaria* and *P. caudatum*) in the presence of 8 different natural fatty acid salts (soap components) can be drastically altered depending on the quality of the waters used (Kadono et al., 2006a,b). It is noteworthy that, both in the fish and protozoan models, the toxicity of soap components often observed under standard culturing conditions employing low-mineral media (such as UPW-based one) was likely lost in the natural HRW. Our intention at that time was to challenge the established belief that an eco-toxicity assay for aquatic organisms can be carried out only under artificial model water conditions. These studies were followed by a large-scale geographical eco-toxicity assessment using the cells of *P. bursaria* and 81 different water samples isolated from rivers, lakes, ponds, and springs in Japan, China, and Taiwan (Goto et al., 2007), and the obtained data strongly supported the earlier demonstration of a tight relationship between water hardness and soap toxicity. However, the soap components used in the previous study do not represent the behaviors of most widely used groups of household and industrial detergents, which are usually mixtures of synthetic surfactants.

In the present study, to verify whether the above result applies to the typical members of widely used detergents, 12 types of alkyl sulfonate salts (sodium alkyl sulfonates, SAS) that differed in their alkyl chain length were used as model detergents for toxicity tests using *P. bursaria* maintained in both a natural HRW-based culture medium and an ultrapure water (UPW)-based standard culture medium. Supplemental data were also obtained with medaka fish (*Oryzias latipes*), a widely used model fish, maintained in waters differing in salinity.

2. Materials and Methods

2.1. Organisms Used and Cultural Conditions

Using the methods of Kosaka (Kosaka, 1991), the INA-1 strain of green paramecia (*P. bursaria*, syngen 1, mating type I) was sampled from the Ongagawa River, Kama-city, Fukuoka Prefecture, Japan, at a limnological study station (**Fig. 1**; designated as the point INA as previously

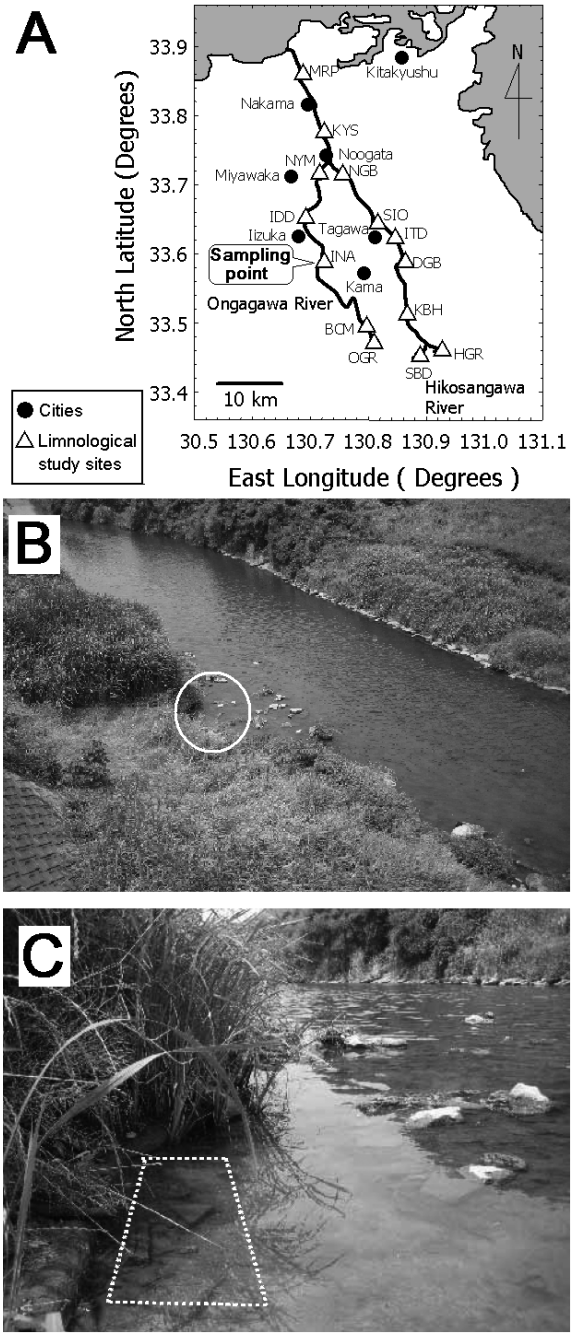

Fig. 1. INA water sampling point. (A) Location of limnological study sites on the Ongagawa River system (modified from Nishihama et al., 2008). (B) View of the INA sampling point from the bank. (C) Exact area (shown by the square) for sampling green paramecia and HRW.

described by Nishihama et al., 2008), through the sampling of 100 ml of water from the bottom of the basin using a transparent hollow plastic tube (length, 1 m; internal diameter, 1 cm). From the point INA (**Fig. 1**), running fresh river water was also collected with a bucket, filtered through Advantec filter papers (No.2 qualitative, Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and used as model HRW to maintain the INA-1 cells during assays.

The primary culture was propagated in a medium containing a yeast extract-based nutrition mixture (Kadono et al., 2006a), after inoculation with the food bacterium *Klebsiella pneumoniae*, under a light cycle of 12 h light and 12 h dark with ca. 3500 lux of natural-white fluorescent light at 23°C, as previously described (Furukawa et al., 2009).

As a model fish species inhabiting both fresh and brackish water, adult medaka (*O. latipes* red-orange variety) was chosen for the study. Adult fish brought up in tap water were purchased from a local animal supplier. Prior to the experiments, fishes were kept in de-chlorinated local tap water (Kitakyushu city tap water) that originated from the Ongagawa River. The assay waters for medaka fish were ultrapure water, Kitakyushu city tap water, and synthetic brackish water prepared by mixing artificial seawater (made of Daigo artificial seawater salt mixture, Nihonseiyaku Co. Ltd., Tokyo, Japan; purchased from Wako Pure Chemical Co. Ltd., Osaka, Japan) and ultrapure water (final salinity was equivalent to 25% seawater).

2.2. Detergents

The following SAS were used for toxicity assays. 1-Butanesulfonic acid sodium salt ($NaCH_3(CH_2)_3SO_3$), 1-pentanesulfonic acid sodium salt ($NaCH_3(CH_2)_4SO_3$), 1-hexanesulfonic acid sodium salt ($NaCH_3(CH_2)_5SO_3$), 1-heptanesulfonic acid sodium salt ($NaCH_3(CH_2)_6SO_3$), 1-octanesulfonic acid sodium salt ($NaCH_3(CH_2)_7SO_3$), 1-decanesulfonic acid sodium salt ($NaCH_3(CH_2)_9SO_3$), 1-dodecanesulfonic acid sodium salt ($NaCH_3(CH_2)_{11}SO_3$), and sodium linear-alkylbenzenesulfonate were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium 1-methanesulfonate ($NaCH_3SO_3$), sodium 1-nonanesulfonate ($NaCH_3(CH_2)_8SO_3$), and sodium 1-tetradecanesulfonate ($NaCH_3(CH_2)_{13}SO_3$) were obtained from Alfa Aesar (MA., USA). Sodium 1-hexadecanesulfonate ($NaCH_3(CH_2)_{15}SO_3$), and sodium 1-octadecanesulfonate ($NaCH_3(CH_2)_{17}SO_3$) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

2.3. Mineral Analyses

Minerals and ions in the water samples were detected with inductively coupled plasma-atomic emission spectroscopy (ICP-AES; Optima 4300 DV, Perkin Elmer) and an ionic chromatograph (DX-120, Dionex). Data on Mg and Ca contents were used to estimate the water hardness.

2.4. Toxicity Assay

SAS toxicity assays were carried out on 12-well plastic microplates, following Miyoshi et al. (Miyoshi et al., 2003). Briefly, the paramecium culture in the stationary phase was harvested and washed twice with the standard culture medium freshly prepared with UPW. Each well on the microplates was filled with 0.9 ml of UPW or HRW, with or without 0.05 ml of the SAS detergent solution, and 0.05 ml of the culture harboring 100 cells. The cells were incubated for 12 h at 23°C in the dark, and the number of

living cells was counted at the end of the incubation under a stereomicroscope (SMZ645; Nikon, Tokyo) and the median lethal concentration (LC_{50}) for each SAS member was determined.

Viability of medaka fish was tested in various waters and the survival rates at 96 h after transfer to various conditions were monitored, and LC_{50} for each chemical was worked out. Toxicity of $NaCH_3(CH_2)_{11}SO_3$ in medaka fish was tested essentially according to Lin et al. (2006), both in the freshwater conditions (UPW, TW) and BW condition (25% SW and 75% UPW, mixed). For each test, 5 fishes kept in 1 L of water at $23 \pm 2^{\circ}$C were used for single data point.

2.5. Surface Tension Score

Supplementation with detergent drastically alters the surface tension of water. As changes in surface tension and the availability of various agrochemicals have been documented (Nikolov et al., 2002), surface tension scores for SAS series at concentrations corresponding to LC_{50} and LC_{100} values were obtained by measuring the size of the spread of droplets of diluted SAS on the hydrophobic surface of Parafilm (Bemis Flexible Packaging, Neenah, WI, USA). As illustrated in **Fig. 6a**, the surface tension score "height (a)/diameter (b)" employed here is a simplified measure for surface tension which is conventionally expressed with the contact angle θ. According to a half-angle method for determining the contact angle θ (Williams et al., 2010), $\theta = 2\theta_{\text{demi}}$ and $\tan\theta_{\text{demi}} =$ height (a)/radius ($b/2$), thus, $\tan\theta_{\text{demi}}/2 = a/b$. Therefore, changes in the contact angle θ is function of the change in the ratio of a/b: $\theta = 2\arctan(2a/b)$.

3. Results and Discussion

In the present study, the toxicity of the SAS members representing 1 type of surface-active agent massively emitted from the urban area to the water environments was assessed in 2 different water models using *P. bursaria* as a model protozoan species inhabiting diverse freshwater ecosystems.

3.1. Mineral Composition and Water Hardness in HRW Collected from the Ongagawa River (Point INA)

Using ICP-AES analysis (also confirmed through ionic chromatography), the mineral composition of the HRW used here was found to be Na (25.5 ppm, w/v), Ca (23.8 ppm, w/v), Mg (7.95 ppm, w/v), Cd (0.096 ppm, w/v), Cr (0.092 ppm, w/v), Cu (0.07 ppm, w/v), Fe (0.401 ppm, w/v), P (0.127 ppm, w/v), and Zn (0.096 ppm, w/v). Co, K, Mn, and Ni were not at a detectable level.

Previously, we found that the water hardness drastically alters the viability of *P. bursaria* exposed to a series of soap components (such as sodium oleate) used as model detergents (Kadono et al., 2006a; Goto et al., 2007). Therefore, it is crucially important to define the hardness of the waters used. Based on the amount of Ca and Mg, the water hardness of the HRW sampled from the point INA was calculated to be 92.2 ppm (w/v), whereas the UPW that has been used for propagating ciliates in laboratory water conditions contained no detectable mineral elements, thus having 0 ppm (w/v) hardness.

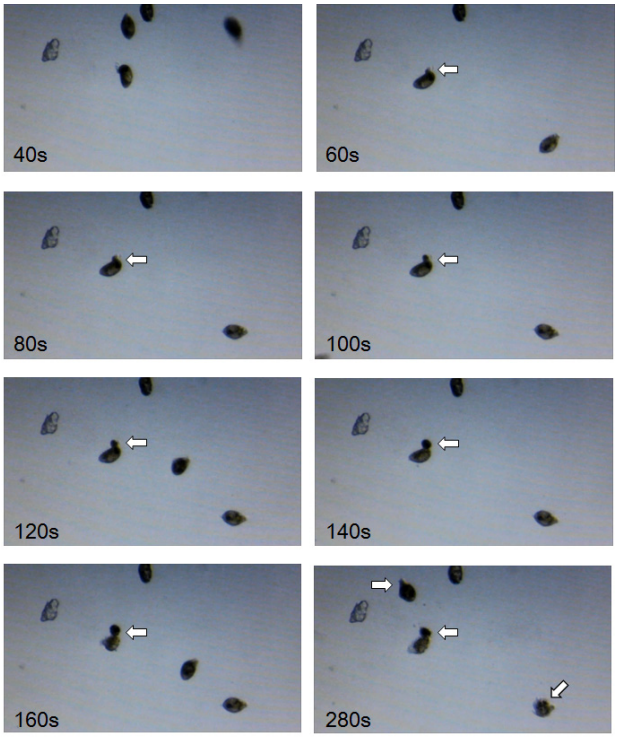

Fig. 2. Detergent-induced rupture of paramecium cells resulting in bulky leakage of cytosol and symbiotic algae from the cells. Time (s) after addition of $NaCH_3(CH_2)_6SO_3$ (8000 ppm).

3.2. Acute Cell Death and LC_{50} Values for SAS Detergents

The cellular membrane-targeted toxic actions of SAS-based detergents can be readily visualized by observing the acute punctuation of the ciliate cells that are simply surrounded by the plasma membrane without any supporting extracellular matrix. **Fig. 2** shows a typical microscopic observation of detergent-induced acute cell death accompanying the rapid and bulky leakage of cytosol and symbiotic green algae from the paramecium cells. The images were obtained immediately after adding a high dose (8000 ppm, w/v) of $NaCH_3(CH_2)_6SO_3$ to the cell suspension.

Depending on the types and concentrations of detergents added, the number of intact cells decreased during 12 hours of incubation, and the typical assay results with various SAS detergents are shown in **Fig. 3**. Focusing on the actions of SAS members with alkyl chains longer than $NaCH_3(CH_2)_6SO_3$, there was a tendency for detergents with longer alkyl chains to have higher toxicity in both water conditions.

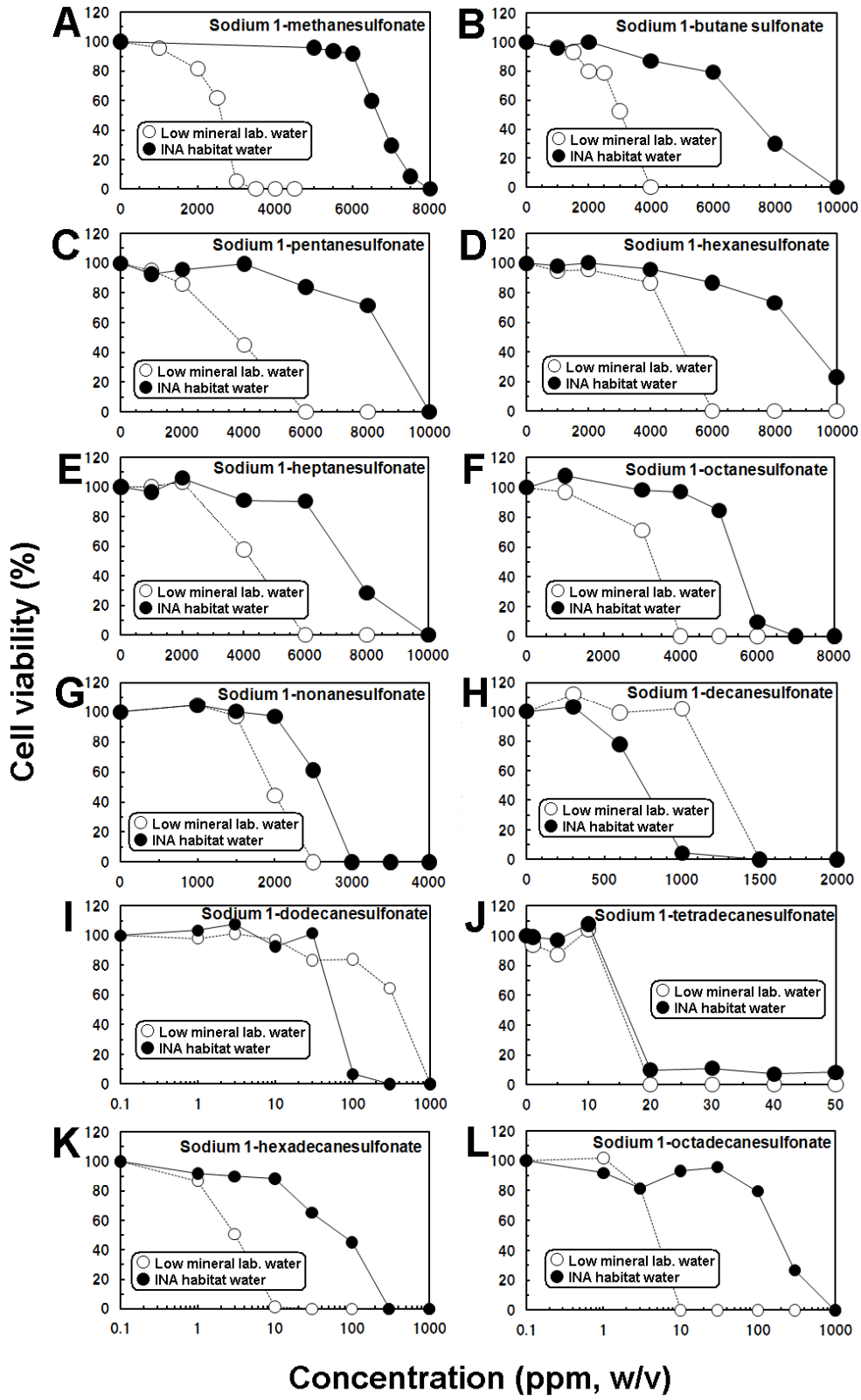

Fig. 3. Toxicities of SAS detergents in *P. bursaria* assessed in UPW-based laboratory low-mineral water and natural habitat river water (HRW). *Paramecium* cells were incubated with various concentrations of SAS detergents dissolved in HRW or UPW for up to 12 h and the survival rates were determined by counting live cells under a microscope. (A) $NaCH_3SO_3$, (B) $NaCH_3(CH_2)_3SO_3$, (C) $NaCH_3(CH_2)_4SO_3$, (D) $NaCH_3(CH_2)_5SO_3$, (E) $NaCH_3(CH_2)_6SO_3$, (F) $NaCH_3(CH_2)_7SO_3$, (G) $NaCH_3(CH_2)_8SO_3$, (H) $NaCH_3(CH_2)_9SO_3$, (I) $NaCH_3(CH_2)_{11}SO_3$, (J) $NaCH_3(CH_2)_{13}SO_3$, (K) $NaCH_3(CH_2)_{15}SO_3$, (L) $NaCH_3(CH_2)_{17}SO_3$.

Table 1. LC_{50} values for SAS detergents with various alkyl carbon chain lengths determined in UPW-based low-mineral assay medium.

	Carbon number in the alkyl chain of SAS											
	1	4	5	6	7	8	9	10	12	14	16	18
ppm (w/v)	3000	3100	3750	4900	4250	3400	1950	1250	300	15	12.5	7.5
mM	25.4	19.4	21.5	26	21	15.7	8.47	5.12	1.10	0.55	0.44	0.021

Table 2. LC_{50} values for SAS detergents with various alkyl carbon chain lengths determined in natural HRW-based assay medium.

	Carbon number in the alkyl chain of SAS											
	1	4	5	6	7	8	9	10	12	14	16	18
ppm (w/v)	6500	7200	8600	9000	7400	5500	2550	750	75	16	200	150
mM	55	45	49.4	47.8	36.6	25.4	11.1	3.07	0.28	0.05	0.61	0.421

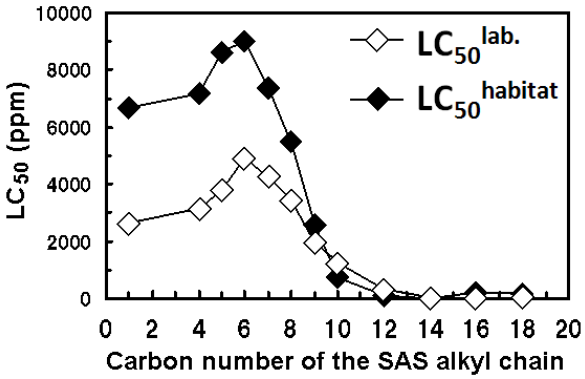

Fig. 4. Relationship between LC_{50} values and the size of alkyl chains on SAS detergents assessed in natural HRW and UPW-based low-mineral laboratory water.

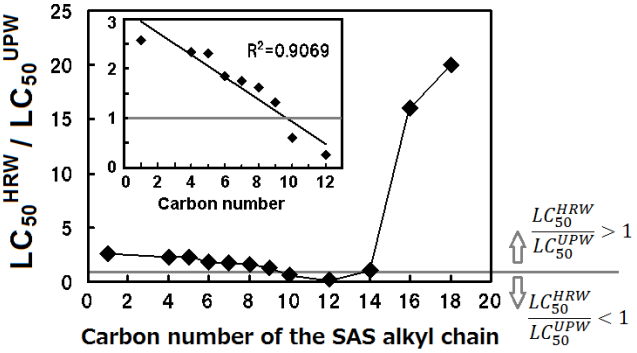

Fig. 5. Effect of alkyl chain length in SAS detergents on the ratio of LC_{50} values (s) recorded in HRW-based and UPW-based media. The ratio of the toxicity in 2 distinct water conditions ($LC_{50}^{HRW}/LC_{50}^{UPW}$) was calculated. Inset, relationship between the SAS alkyl chain length and $LC_{50}^{HRW}/LC_{50}^{UPW}$ ratio in a limited range of carbon numbers (between $NaCH_3SO_3$ and $NaCH_3(CH_2)_{11}SO_3$).

Finally, the LC_{50} values for all SAS members tested were worked out in two distinct assay media prepared with UPW (**Table 1**) or HRW (**Table 2**). LC_{50} values for each SAS detergent ranged from 15 to 4600 ppm (w/v) in UPW and from 16 to 9000 ppm (w/v) in HRW.

By plotting the LC_{50} values versus the length of alkyl chains in the SAS series, we can highlight the relationship between the effects of water conditions on the toxicity of detergents and the chemical structure (length of alkyl chains) (**Fig. 4**). We found a general rule that the toxicity values of the short-chained SAS detergents (alkyl chain length shorter than that of $NaCH_3(CH_2)_8SO_3$) were higher in UPW than in HRW (**Fig. 4**).

The above observation was further confirmed by plotting the ratio of LC_{50} values determined in HRW over those in UPW ($LC_{50}^{HRW}/LC_{50}^{UPW}$, **Fig. 5**). By highlighting the $LC_{50}^{HRW}/LC_{50}^{UPW}$ ratio, it became clear that not only the group of short-chained SAS detergents (molecules smaller than $NaCH_3(CH_2)_8SO_3$), but also the SAS members with the longest chains (i.e., $NaCH_3(CH_2)_{15}SO_3$ and $NaCH_3(CH_2)_{17}SO_3$) had lowered toxicity in the natural HRW-based culture compared to the UPW-based assay. However, $NaCH_3(CH_2)_9SO_3$ and $NaCH_3(CH_2)_{11}SO_3$ had $LC_{50}^{HRW}/LC_{50}^{UPW}$ ratios below 1.0, and $NaCH_3(CH_2)_{13}SO_3$ had almost identical LC_{50}values in the 2 distinct water conditions.

The results suggested that there could be at least 2 distinct phenomena contributing to the water condition-dependent changes in the toxicity of SAS members. Among the SAS members smaller than $NaCH_3(CH_2)_{11}SO_3$, a tight linear relationship between the alkyl chain length and the $LC_{50}^{HRW}/LC_{50}^{UPW}$ ratio was observed (**Fig. 5**, inset). However, the $LC_{50}^{HRW}/LC_{50}^{UPW}$ ratio drastically rose as alkyl chains in SAS increased above 14 (**Fig. 5**), for an unknown reason.

When a logarithmic scale was applied for $LC_{50}^{HRW}/LC_{50}^{UPW}$ scores, the relationship between the $LC_{50}^{HRW}/LC_{50}^{UPW}$ ratio and alkyl chain length in SAS members had a V-shape. Interestingly, similar V-shaped curves were obtained when plotting the surface tension scores of SAS members determined at concentrations corresponding to LC_{50} and LC_{100} values versus the number of carbons in SAS alkyl chains (**Fig. 6**), implying a relationship between the toxicity of SAS and changes in surface tension.

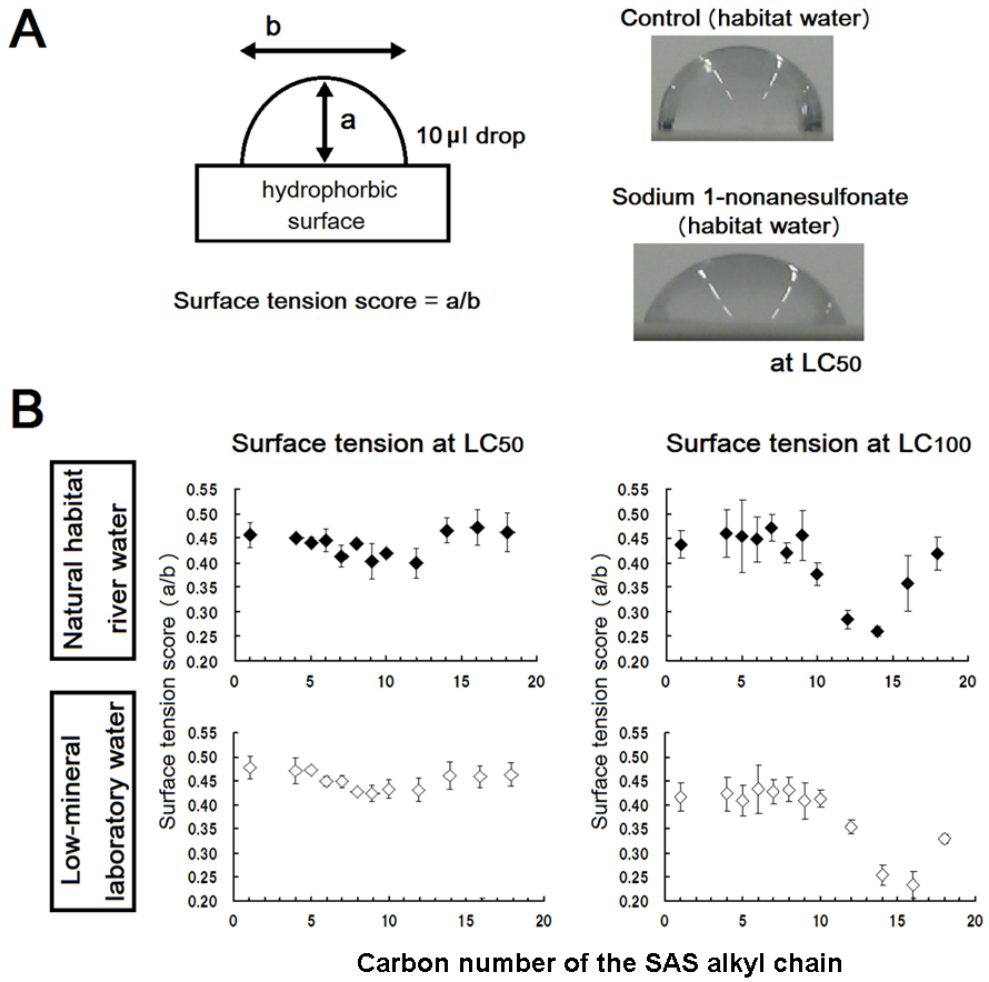

Fig. 6. Altered water surface tension in the presence of SAS detergents at concentrations corresponding to LC_{50} and LC_{100} values.

3.3. Elevated SAS Toxicity with Higher Salinity in a Fish Model

The data obtained from *P. bursaria* suggested that the toxicities of short and long SAS are likely minimized as the hardness of water is elevated, whereas the toxicities of SAS with alkyl chain lengths of 10–14 carbons (such as sodium dodecan sulfonate) are likely elevated. Previous studies on FFF toxicity suggested that the impact of water hardness on the alteration of detergent toxicity can be similarly observed in a wide range of aquatic organisms, from paramecium species to fish (Lin et al., 2006; Kadono et al., 2006a,b; Kawano et al., 2007; Mizuki et al., 2007). Therefore, we assumed that the mineral-dependent elevation of SAS toxicity observed in *P. bursaria* could be generalized and applicable to a fish model.

Simple comparisons of the toxicity of dodecan sulfonate in ultrapure water, tap water derived from the Ongagawa River, and model brackish water were performed (**Fig. 7**) using medaka fish, which are known to survive in and inhabit a wide range of water salinity from ultrapure water to seawater (Yokawa et al., 2009). As expected, the toxicity of dodecan sulfonate differed in different waters, confirming that higher mineral content elevates the ecotoxicity of SAS members.

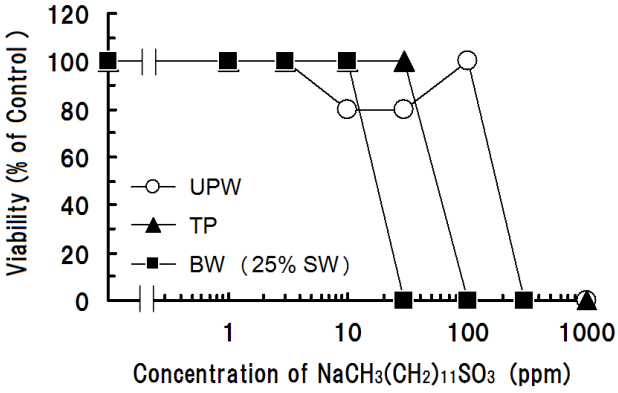

Fig. 7. Altered toxicity of dodecan sulfonate in medaka fish maintained in different waters. UPW, ultrapure water; TP, Kitakyushu city tap water; BW, brackish water; SW, seawater.

Aqueous vertebrates and invertebrates utilize gill structures for oxygen uptake and bicarbonate-chloride exchange through a plasma membrane (Lee and Pritchard, 1985). In fact, the phospholipid-based plasma membrane on the fish gill is a likely target of polluting detergents, thus contributing to the development of fish toxicity. Ac-

cordingly, the fate of seawater fishes in the presence of toxic gill-targeting detergents such as sodium dodecyl sulfate (SDS) can be predicted through the simplest model assay using phospholipid-based liposomes (Kalmanzon et al., 1992). The above study also suggested that microorganisms that expose their plasma membrane to the surrounding aqueous environment might be used as a simple alternative model for assessing the toxic actions of membrane-targeting detergents. As expected, detergents emitted in the aqueous environment reportedly target not only fishes but also living unicellular protozoans such as *Paramecium* species (Mizuki et al., 2007). As shown earlier, *P. bursaria* is suitable for the study of chemical toxicities in freshwater environments uniquely representing the behaviors of both ciliates and green algae (Takahashi et al., 2005; Kadono et al., 2006a; Goto et al., 2008).

The present study further supports the view that ciliate cells and fishes can be used as 2 typical models for assessing the behaviors of aquatic organisms exposed to membrane-targeting detergents. In order to formulate a novel FFF for wildland fires employing an SAS member as a synthetic detergent increasing foaming performance, the drastic differences, depending on water conditions, in the toxic nature of SAS members must be considered.

Abbreviations:

LC50, median lethal concentration; SAS, sodium alkyl sulfonates.

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Name:
Kaishi Goto

Affiliation:
Faculty and Graduate School of Environmental Engineering, The University of Kitakyushu
Address:
1-1 Hibikino, Wakamatsu-ku, Kitakyushu 808-0135, Japan

Name:
Hiroshi Takaichi

Affiliation:
Faculty and Graduate School of Environmental Engineering, The University of Kitakyushu
Address:
1-1 Hibikino, Wakamatsu-ku, Kitakyushu 808-0135, Japan

Name:
Tomonori Kawano

Affiliation:
Associate Professor, Faculty of Environmental Engineering, The University of Kitakyushu

Address:
1-1 Hibikino, Wakamatsu-ku, Kitakyushu 808-0135, Japan

Brief Career:
2000- Post-Doctoral Scientist, Institut National de la Recherche Agronomique (INRA)
2001- Research Associate, Graduate School of Science, Hiroshima University
2003- Associate Professor, Graduate School of Environmental Engineering, The University of Kitakyushu

Selected Publications:
• "Run-length encoding graphic rules, molecular biologically editable designs, and steganographical on-image numeric data embedment for DNA-based cryptographical coding system," Communicative and Integrative Biology, Vol.6, e23478.

Academic Societies & Scientific Organizations:
• Information Processing Society of Japan (IPSJ)
• Society of Plant Signaling and Behavior