VOLUME 11 ISSUE 1 2025

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 11, No. 1, 538-545 (2025)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Deep Ocean Waters Collected Off Western Noto Peninsula (Station Y-5) and Eastern Noto Peninsula (Station 3-2) Promote Osteoblastic Activity in Fish Scales

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Received: 2nd February, 2025; **Accepted:** 22nd March, 2025; **Published online:** 14th April, 2025

https://doi.org/10.33745/ijzi.2025.v11i01.057

Abstract: The Aquas Noto facility, located in Noto-cho, Ishikawa Prefecture, Japan, pumps deep ocean water (DOW) from a depth of 320 m. We previously reported that this 320 m DOW activates alkaline phosphatase (ALP), an osteoblastic marker enzyme, in goldfish scales. Additionally, we identified kynurenine, a compound absent in surface seawater of Tsukumo Bay but specifically present in 320 m DOW, which increased ALP activity at concentrations as low as 10^{-10} M. In the present study, we investigated whether DOW from greater depths also contains osteoblast-activating substances. During the May 2016 voyage of the Nagasaki Maru, DOW samples were collected at depths of 400, 1000, and 1746 m from Station Y-5 (37.25.41N, 135.38.50E) and at 520 m near methane hydrates from Station 3-2 (37.21.69N, 137.57.74E), using a Conductivity, Temperature and Depth water sampler. These samples were added to the culture medium at 20%, and ALP activity in goldfish scales was compared to that in a surface seawater control. DOW from 400 and 1000 m increased ALP activity in 2 of 3 individuals, while DOW from 1746 m had no effect. The 520 m DOW collected near methane hydrates significantly enhanced ALP activity. These findings suggest that DOW from depths of 400, 520, and 1000 m contains bioactive substances that promote osteoblastic activity. We conclude that the goldfish scale *in vitro* bioassay is a highly sensitive and effective tool for detecting bone-formative substances in seawater and can serve as a valuable system for evaluating the biological potential of DOW.

Keywords: Deep ocean water, Coast of the Noto Peninsula, Aquas Noto Facility, Goldfish scales, Osteoblastic activity, Alkaline phosphatase

Citation: Taira Kaori, Ikari Takahiro, Tsutsui Hideto, Senjyu Tomoharu, Kaneda Kouhei, Hirose Tasuku, Morii Yasuhiro, Yamawaki Nobuhiro, Yagi Mitsuharu, Srivastav Ajai K., Ohira Tsuyoshi, Urata Makoto, Hirayama Jun, Hattori Atsuhiko and Suzuki Nobuo: Deep ocean waters collected off Western Noto Peninsula (Station Y-5) and Eastern Noto Peninsula (Station 3-2) promote osteoblastic activity in fish scales. Intern. J. Zool. Invest. 11(1): 538-545, 2025.

https://doi.org/10.33745/ijzi.2025.v11i01.057

Introduction

The deep ocean water (DOW) is seawater found 200 m below the surface of the Earth's oceans and is characterized by abundant inorganic nutrients (nitrogen, phosphorus, and silicate) and clean water (containing few bacteria and low photosynthetic activity by phytoplankton) (Mohd Nani *et al.*, 2016). With these features, DOW has been applied for various uses. For aquaculture, pelagic shrimp, *Sergia lucens*, which live in the deep sea, can be kept for extended periods when reared in DOW (Okamoto, 2006). Namely, the shrimp could be kept for an average of only 17 days in surface seawater, while they could be kept for 58.8 days in DOW. The shrimp could be maintained for up to 185 days with DOW (Okamoto, 2006). We recently reported that weight loss in squid (*Todarodes pacificus*) is suppressed when reared in DOW (depth: 320 m) from the Aquas Noto facility (Noto-cho, Ishikawa Prefecture, Japan) compared to surface seawater (Hatano *et al.*, 2023). In Japanese flounder (*Paralichthys olivaceus*) reared under high-density conditions, we also found that fish reared in Noto DOW had lower plasma cortisol (a stress marker) levels compared to those reared in surface seawater (Ikari *et al.*, 2023). As a substance responsible for reducing stress, we identified kynurenine in Noto DOW, but not in surface seawater, demonstrating that kynurenine has a stress-reducing effect in Noto DOW (Ikari *et al.*, 2023).

On the other hand, fish scales contain osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells), which closely resemble those found in mammalian bone (Suzuki *et al.*, 2008, 2016a; Hirayama *et al.*, 2023). Using goldfish scales, Suzuki *et al.* (2000) and Suzuki and Hattori (2002) developed a unique bioassay system. DOW collected at a depth of 320 m by the Aquas Noto facility activated alkaline phosphatise (ALP), an osteoblastic marker enzyme, in goldfish scales. Furthermore, we demonstrated that kynurenine, even at a concentration of 10^{-10} M increased ALP activity in goldfish scales, similar to the effect observed with 320 m DOW from the Aquas Noto Facility (Ikari *et al.*, 2023). Therefore, in the present study, we aimed to investigate the presence of osteoblast-activating substances in DOW collected from depths greater than 320 m.

Materials and Methods

Animals

Male goldfish (*Carassius auratus*) were purchased from a commercial supplier (Higashikawa Fish Farm, Yamatokoriyama, Japan) and used for the *in vitro* scale bioassay (Suzuki *et al.*, 2000; Suzuki and Hattori, 2002).

DOW collection by a Conductivity, Temperature and Depth (CTD) water sampler

The scientific cruise for this study, designed NS16436, was conducted by the T/S Nagasaki Maru, operated by the Department of Fisheries, Nagasaki University, Japan. The ship departed from Mie-Shikimi, Nagasaki, at 08:45 am LST on May 7, 2016. Sampling began at Station Y-5 at 12:24 pm LST on May 9. This station is located off the western coast of the Noto Peninsula at lattitude 37° 25.41′N and longitude135° 38.50′E

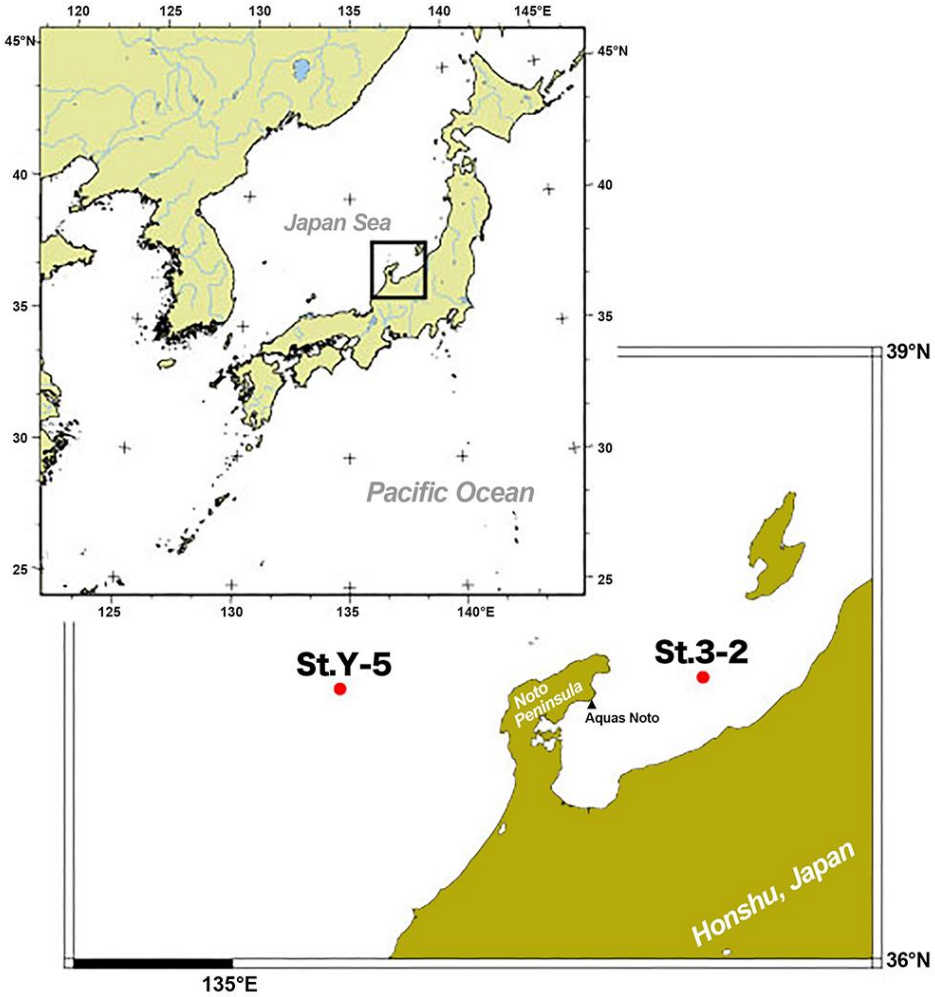

Fig. 1: Location of the sampling sites. The DOW samples were collected using a CTD water sampler aboard the T/S Nagasaki Maru, operated by Nagasaki University. Sampling was conducted at Station Y5 (135.6416667, 37.4235) at 12:24 pm on May 9, 2016, and Station 3-2 (137.9623333, 37.3615) at 11:24 am on May 14, 2016. DOW: deep ocean water; CTD: conductivity, temperature, and depth.

(Fig. 1). A CTD system (SEA-BIRD SCIENTIFIC SBE 9 plus with SBE Model 11 plus control units) and rosette multi-samplers (Ocean Test Equipment Co., Ltd.) were deployed approximately five meters above the seafloor (Fig. 1). DOW samples were collected at depths of 400, 1000, and 1746 m from Station Y-5 (37.25.41N, 135.38.50E) and at 520 m near methane hydrates from Station 3-2 (37.21.69N, 137.57.74E), using CTD water sampler. The collected water sampleswere stored in the ship's refrigerator and maintained at 4°C.

Subsequently, we arrived at Station 3-2, located at latitude 37° 21.69′N and longitude 137° 57.74′E, off the eastern coast of the Noto Peninsula, at 11:16 am LST on May 14, 2016. The CTD/RMS was deployed at 11:24 am LST, approximately five meters above the seafloor, to collect DOW from a depth of 520 m (Fig. 1).

Changes in ALP activities of goldfish scales by the addition of DOWs or surface seawater into the medium

Goldfish were first anesthetized using 0.03% ethyl 3-aminobenzoate-methanesulfonic acid salt (Sigma–Aldrich, Inc., St. Louis, MO, USA). Normally developed scales from both sides of the body were then removed to induce scale regeneration. Ten days later, the fish were re-anesthetized, and the

regenerating scales were collected for analysis. These scales were used to assess the effects of DOW samples on osteoblasts, with ALP activity serving as a marker enzyme (Suzuki *et al.*, 2000; Suzuki and Hattori, 2002). Collected regenerating scales were placed individually into wells of a culture plate containing Leibovitz's L-15 medium (phenolred-free; Invitrogen, Grand Island, NY, USA), supplemented with 1% penicillin–streptomycin (ICN Biomedicals, Inc., Aurora, OH, USA) and 20% DOW sample. The cultures were incubated for 6 h. The effect of DOW exposure on osteoblast activity was then compared to a control using surface seawater collected from a depth of 2 m in Tsukumo Bay (Noto Peninsula, Ishikawa Prefecture, Japan).

The method for measuring ALP activity was as follows. After aspirating the medium from each well using a eight channel pipettes, each scale was washed with phosphate-buffered saline. Then, 100 µl of alkaline buffer (100 mM Tris-HCl, pH 9.5, with 1 mM $MgCl_2$) was added to each well of a 96-well plate. The plate was immediately frozen at −80°C and stored at −20°C until analysis. For the assay, the plate was thawed, and 100 µl of 20 mM para-nitrophenyl phosphate (pNPP) in the corresponding alkaline buffer was added to each well. The plate was incubated at 23°C for 20 min under shaking conditions. The reaction was then stopped by adding 50 µl of 3N NaOH. Subsequently, 150 µl of the resulting colored solution was transferred to new wells, and absorbance was measured at 405 nm using a microplate reader. The absorbance values were converted to the amount of para-nitrophenol (pNP) produced, using a standard curve for pNP. After measuring ALP activity, the area of each scale was measured using NIH ImageJ software. Finally, ALP activity was normalized to the surface area (mm^2) and enzyme reaction time (h) of each scale (Suzuki *et al.*, 2009).

Statistical analysis

All results are expressed as means ± standard error (n = 5–7). Statistical significance between the control and experimental groups was assessed using an independent samples t-test, with a significance level set at $p< 0.05$.

Results

Effect of DOW (400, 1000, and 1700 m) on ALP activity of goldfish scales

Goldfish scales were cultured in medium supplemented with 20% DOW, and ALP activity was compared to that observed with surface seawater. DOW collected at depths of 400 m and 1000 m using a CTD water sampler was found to increase ALP activity in the scales of 2 out of 3 individuals. For 400 m DOW, the p-values were as follows: Fish No.1, p = 0.024; No.2, p = 0.247; No. 3, p = 0.0008 (Fig. 2). For 1000 m DOW: Fish No.1, p = 0.047; No. 2, p = 0.978; No. 3, p = 0.006 (Fig. 3). In contrast, DOW collected from 1746 m did not produce a significant change in ALP activity compared to the surface seawater control (Fig. 4).

Effect of DOW nearby methane hydrates on ALP activity of goldfish scales

In the case of DOW collected at 520 m near methane hydrates, ALP activity in goldfish scales increased compared to control surface seawater.The p-values for Fish No. 1, No. 2, and No. 3 were 0.0042, 0.077, and 0.023, respectively (Fig. 5).

Discussion

In the present study, we examined the presence of substances that activate osteoblasts in goldfish scales in DOW deeper than 320 m. We showed that a certain depth of DOW may increase ALP activity in goldfish scales. Analysis of 320 m DOW revealed that it contains kynurenine, a type of indole compound, and that kynurenine enhances osteoblast activity in goldfish scales (Ikari *et al.*, 2023). The component that activates osteoblasts in goldfish scales may be present in the layer from about 300 m to 1000 m. Additionally, it was found that DOW (520 m depth) near methane hydrates can activate osteoblasts in goldfish scales. It is possible that substances such as indole compounds have been eluted by methane hydrate eruptions. We are planning to proceed with the

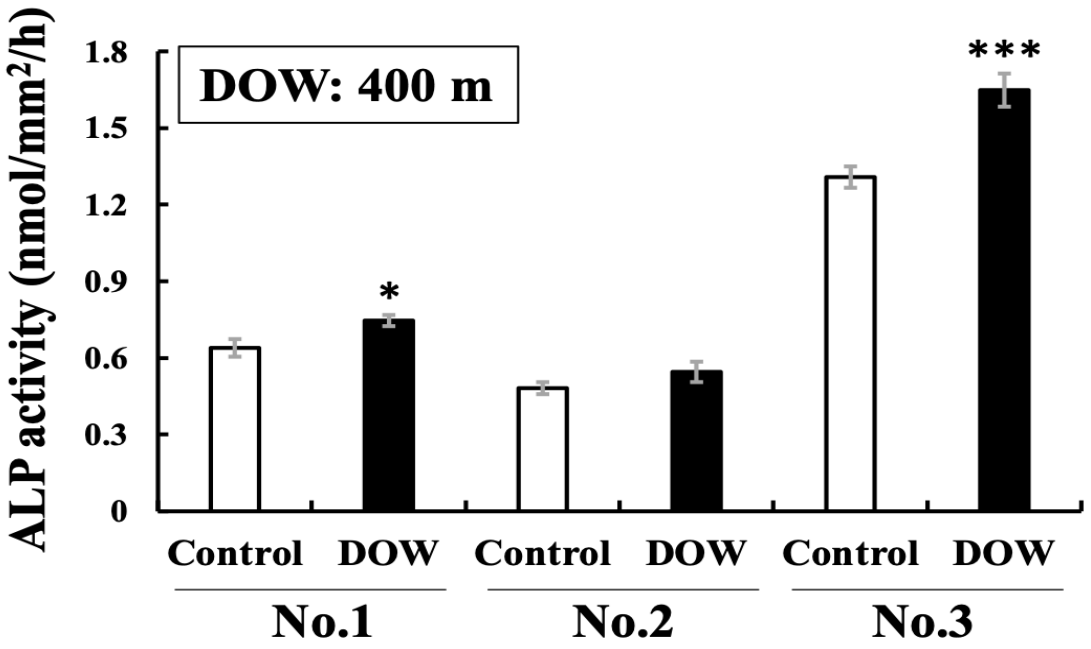

Fig. 2: Effect of 400 m DOW on ALP activity in goldfish scales. Asterisks (*) and (***) indicate statistically significant differences at p< 0.05 and p< 0.001, respectively, compared to the control (surface seawater). All results (n = 5–7) are presented as mean ± standard error. DOW: deep ocean water; ALP: alkaline phosphatase.

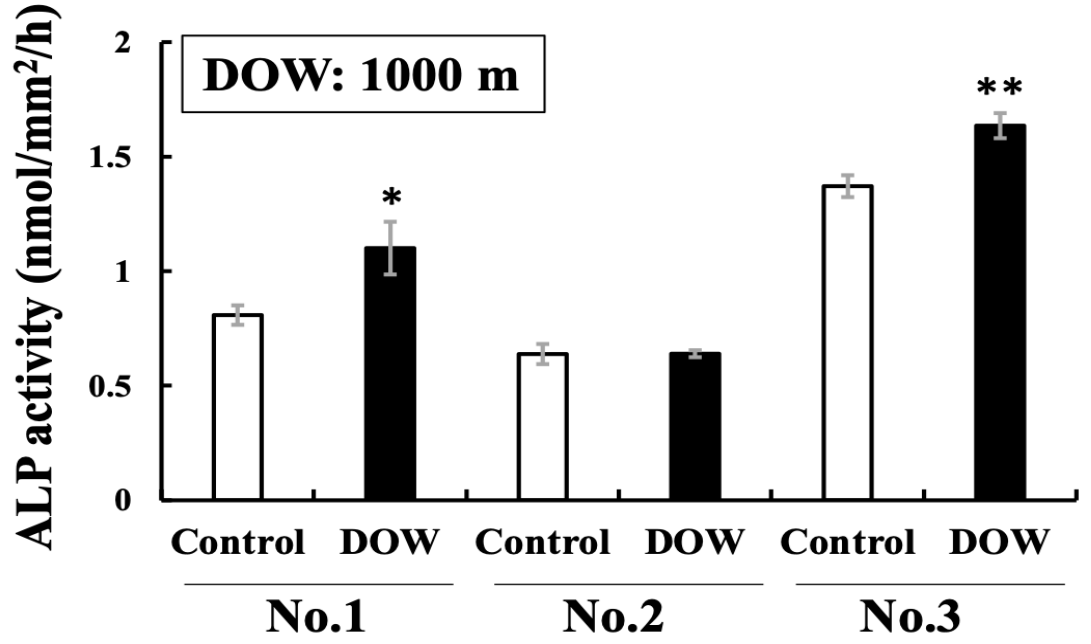

Fig. 3: Effect of 1000 m DOW on ALP activity in goldfish scales. Asterisks (*) and (**) indicate statistically significant differences at p< 0.05 and p< 0.01, respectively, compared to the control (surface seawater). All results (n = 5–7) are presented as mean ± standard error. DOW: deep ocean water; ALP: alkaline phosphatase.

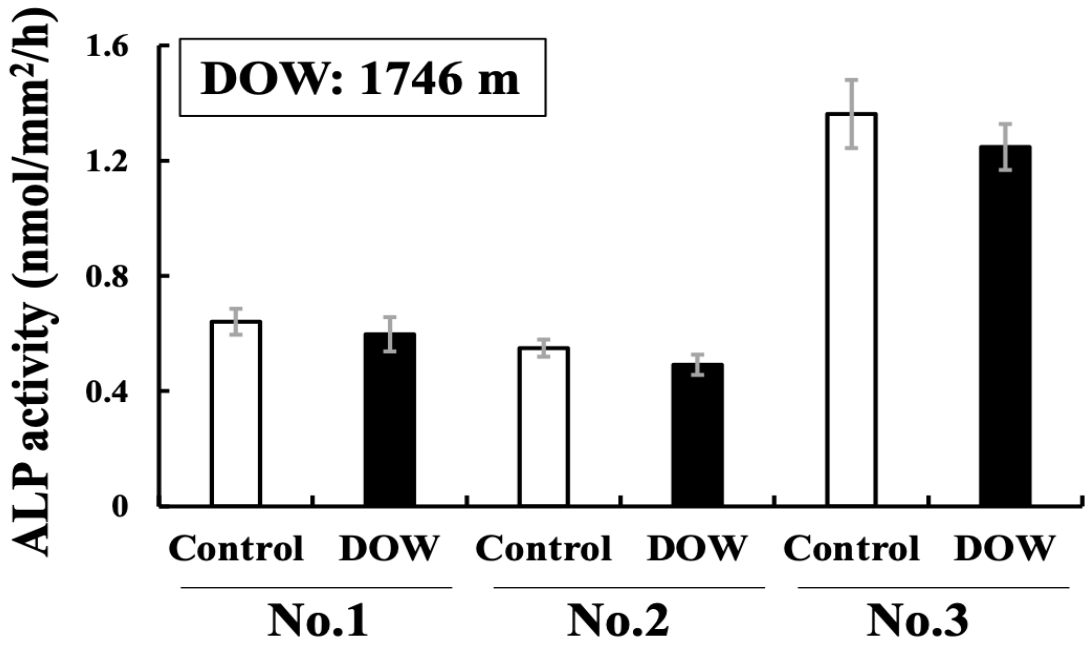

Fig. 4: Effect of 1746 m DOW on ALP activity in goldfish scales. No statistically significant differences were observed compared to the control (surface seawater). All results (n = 5–7) are presented as mean ± standard error. DOW: deep ocean water; ALP: alkaline phosphatase.

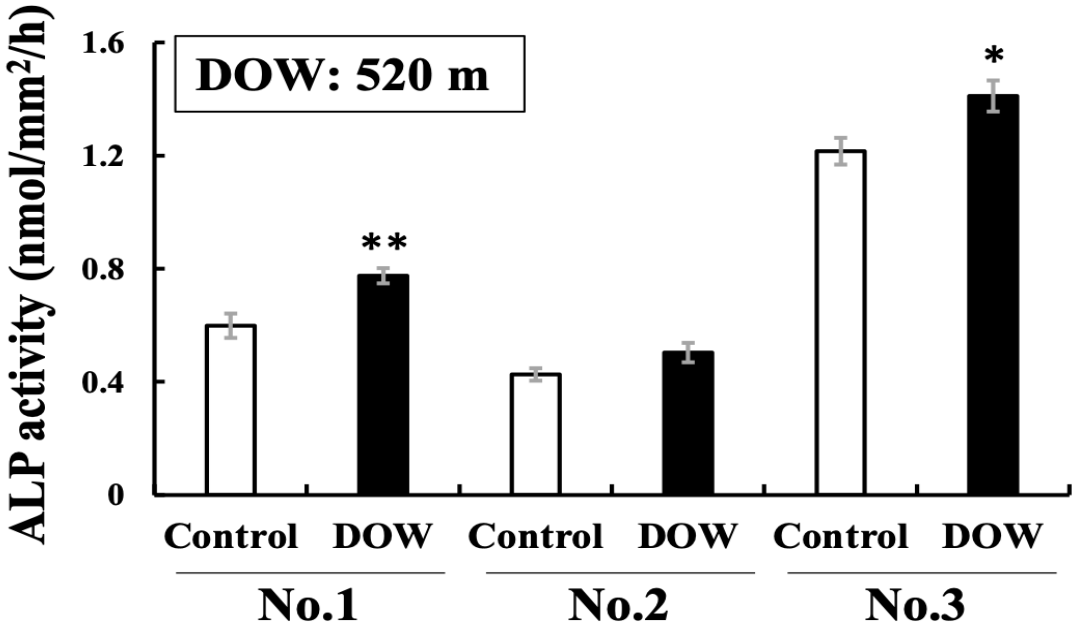

Fig. 5: Effect of 520 m DOW nearby methane hydrates on ALP activity in goldfish scales. Asterisks (*) and (**) indicate statistically significant differences at p< 0.05 and p< 0.01, respectively, compared to the control (surface seawater). All results (n = 5–7) are presented as mean ± standard error. DOW: deep ocean water; ALP: alkaline phosphatase.

analysis of the composition of seawater near methane hydrate.

Goldfish scales are recognized for their high sensitivity to environmental contaminants (Suzuki *et al.*, 2006, 2016b). Notably, they are also highly responsive to tributyltin (TBT), a marine pollutant originally used as a biocide in anti-fouling paint (Suzuki *et al.*, 2006). Our previous study demonstrated that ALP activity significantly declined compared to control levels following exposure to TBT at concentrations ranging from 10^{-10} to 10^{-5} M after 6 h of incubation (Suzuki *et al.*, 2006). Additionally, this system was employed to assess seawater from the Suez Canal contaminated with polycyclic aromatic hydrocarbons, environmental pollutants generated by the combustion of fossil fuels (Suzuki *et al.*, 2016b; Honda and Suzuki, 2020). Consequently, ALP activity decreased when the culture medium was supplemented with seawater diluted 500-fold (Suzuki *et al.*, 2016b). In the present study, osteoblasts in goldfish scales exhibited high sensitivity to substances present in DOW. Taken together, these findings support that our scale-based *in vitro* bioassay is a reliable and effective system for detecting bioactive substances or environmental pollutants.

Ethical Statement

This study was conducted in strict accordance with the ethical guidelines of Kanazawa University, Japan. The experiment was performed under anesthesia to minimize pain and discomfort to animal subjects.

Author Contributions

KT, TI, JH, AH and NS designed the experiments and wrote the manuscript. KT, TI, HT, TS, KK, TH, TO, MU, JH, AH and NS performed experiments. HT, TS, YM, NY, MY and NS sampling and analyzed the data. KT, TO, AKS, JH, AH and NS confirm the authenticity of all the raw data. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

References

Hatano K, Yoshida MA, Hirayama J, Kitani Y, Hattori A, Ogiso S, Watabe Y, Sekiguchi T, Tabuchi Y, Urata M, Matsumoto K, Sakatoku A, Srivastav Ajai K, Toyota K, Matsubara H and Suzuki N. (2023) Deep ocean water alters the cholesterol and mineral metabolism of squid *Todarodes pacificus* and suppresses its weight loss. Sci Rep. 13: 7591.

Hirayama J, Hattori A, Takahashi A, Furusawa Y, Tabuchi Y, Shibata M, Nagamatsu A, Yano S, Maruyama Y, Matsubara H, Sekiguchi T and Suzuki N. (2023) Physiological consequences of space flight, including abnormal bone metabolism, space radiation injury, and circadian clock dysregulation: Implications of melatonin use and regulation as a counter measure. J Pineal Res. 74: e12834.

Honda M and Suzuki N. (2020) Toxicities of polycyclic aromatic hydrocarbons for aquatic animals. Int J Environ Res Public Health 17: 1363.

Ikari T, Furusawa Y, Tabuchi Y, MaruyamaY, Hattori A, Kitani Y, Toyota K, Nagami A, Hirayama J, Watanabe K, Shigematsu A, Rafiuddin MA, Ogiso S, Fukushi K, Kuroda K, Hatano K, Sekiguchi T, Kawashima R, Srivastav Ajai K, Nishiuchi T, Sakatoku A, Yoshida MA, Matsubara H and Suzuki N. (2023) Kynurenine promotes calcitonin secretion and reduces cortisol in the Japanese flounder *Paralichthys olivaceus*. Sci Rep. 13: 8700.

Mohd Nani SZ, Majid FA, Jaafar AB, Mahdzir A and Musa MN. (2016) Potential health benefits of deep sea water: A review. Evid Based Complement Altern Med. 2016: 6520475.

Okamoto K. (2006) Comparison of survival and growth in adult pelagic shrimp *Sergia lucens* between deep and surface seawater cultures. Deep Ocean Water Res. 7: 1-7.

Suzuki N and Hattori A. (2002) Melatonin suppresses osteoclastic and osteoblastic activities in the scales of goldfish. J Pineal Res. 33: 253-258.

Suzuki N, Kitamura K and Hattori A (2016a) Fish scale is a suitable model for analyzing determinants of skeletal fragility in type 2 diabetes. Endocrine 54: 575-577.

Suzuki N, Kitamura K, Omori K, Nemoto T, Satoh Y, Tabata MJ, Ikegame M, Yamamoto T, Ijiri K, Furusawa Y, Kondo T, Takasaki I, Tabuchi Y, Wada S, Shimizu N, Sasayama Y, Endo M, Takeuchi T, Nara M, Somei M, Maruyama Y, Hayakawa K, Shimazu T, Shigeto Y, Yano S and Hattori A. (2009) Response of osteoblasts

and osteoclasts in regenerating scales to gravity loading. Biol Sci Space 23: 211-217.

Suzuki N, Sato M, Nassar HF, Abdel-Gawad FKh, Bassem SM, Yachiguchi K, Tabuchi Y, Endo M, Sekiguchi T, Urata M, Hattori A, Mishima H, Shimasaki Y, Oshima Y, Hong CS, Makino F, Tang N, Toriba A and Hayakawa K. (2016b) Seawater polluted with highly concentrated polycyclic aromatic hydrocarbons suppresses osteoblastic activity in the scales of goldfish, *Carassius auratus*. Zool Sci. 33: 407-413.

Suzuki N, Somei M, Seki A, Reiter RJ and Hattori A. (2008) Novel bromomelatonin derivatives as potentially effective drugs to treat bone diseases. J Pineal Res. 45: 229-234.

Suzuki N, Suzuki T and Kurokawa T. (2000) Suppression of osteoclastic activities by calcitonin in the scales of goldfish (freshwater teleost) and nibbler fish (seawater teleost). Peptides 21: 115-124.

Suzuki N, Tabata MJ, Kambegawa A, Srivastav Ajai K, Shimada A, Takeda H, Kobayashi M, Wada S, Katsumata T and Hattori A. (2006) Tributyltin inhibits osteoblastic activity and disrupts calcium metabolism through an increase in plasma calcium and calcitonin levels in teleosts. Life Sci. 78: 2533-2541.