

A putative research model for sustainable fisheries driven by noninvasive diagnostic imaging

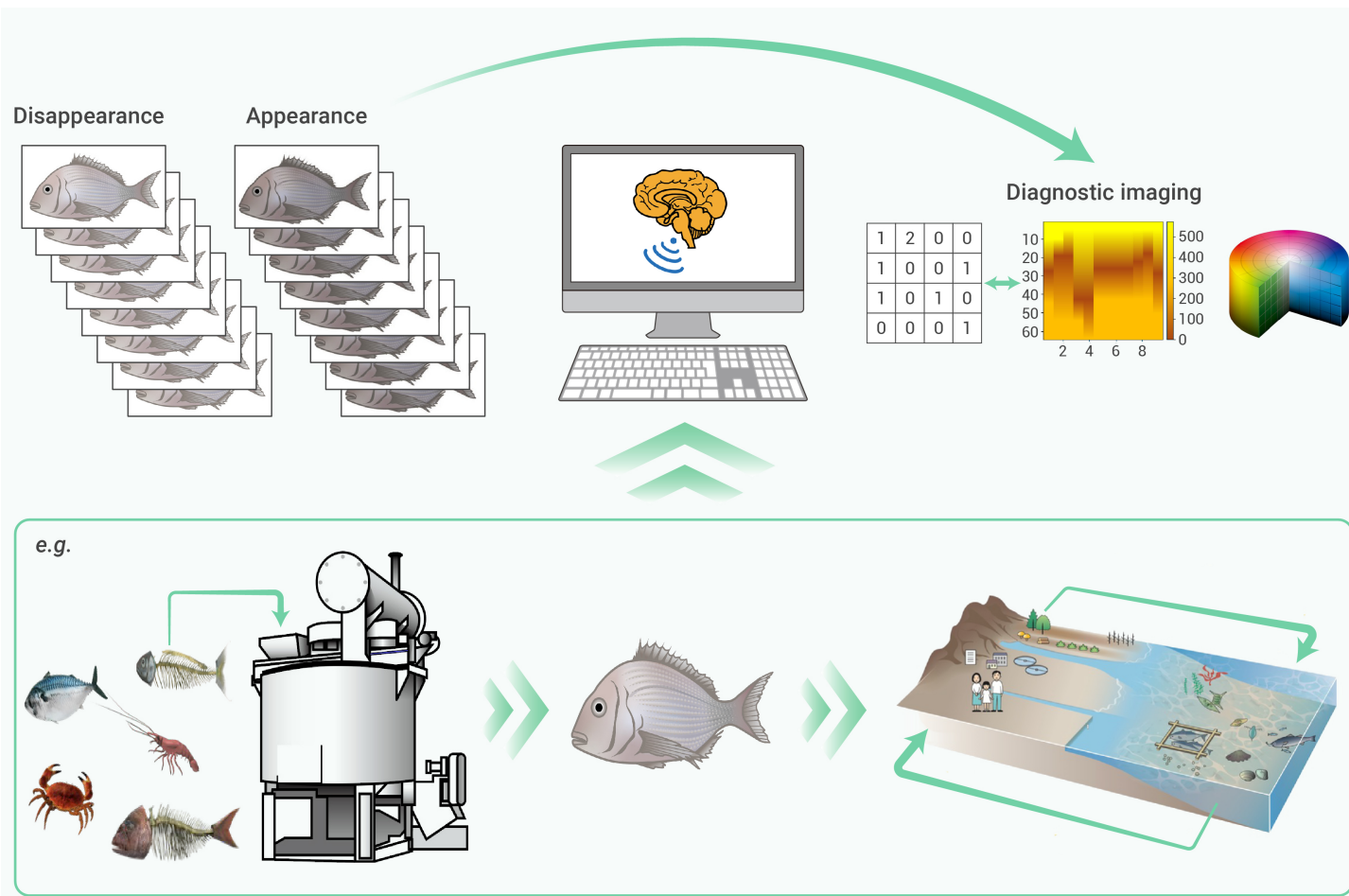
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Received: April 23, 2025; Accepted: August 18, 2025; Published Online: August 20, 2025; <https://doi.org/10.59717/j.xinn-life.2025.100155>

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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Anti-pathogenic property of thermophile-fermented compost as a feed additive for fish.
- Noninvasive diagnosis of the compost functional properties using field photographs.
- A novel research model for efficient and rapid sustainable fisheries dissemination.

A putative research model for sustainable fisheries driven by noninvasive diagnostic imaging

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Citation: Miyamoto H., Ito S., Suzuki K., et al. (2025). A putative research model for sustainable fisheries driven by noninvasive diagnostic imaging. *The Innovation Life* 3:100155.

Diagnostic imaging technology has been developing rapidly in recent years, and the demand for its application is mainly in the medical field. Here, we examined the scope of application that enables rapid functional evaluation using disease-resistant fermented feed for fish as a model for evaluation in the primary industry. A survival test against the fish pathogen *Edwardsiella tarda* revealed the antipathogenic properties of a compost-type feed additive fermented by thermophilic *Bacillaceae* using nonedible marine resources. After prior administration of the compost extract to seabream as a fish model for 70 days, the mortality rate after 28 days of exposure to the fish pathogen *Edwardsiella* peaked at 20%, although the rate was 60% without prior administration. Under such conditions, the serum complement activity of seabream tend to increase. Furthermore, the differences in the degrees of body length, smoothness and glossiness of the fish body surface depending on the administration conditions were computationally analyzed using the field photographs. The texture analysis for the grey level co-occurrence matrix (GLCM) and local binary pattern (LBP), and color extraction analysis for colour tone (hue), colourfulness (saturation), and brightness (value) revealed quantifiable change in color balance of fish body surface after the compost administration. These results suggest that the antipathogenic property of thermophile-fermented compost as a functional feed additive can be efficiently estimated by an external diagnostic technique. This study provides a new perspective for the utilization of noninvasive diagnosis to estimate quantitatively the quality of its production activities in the food and environmental industry.

INTRODUCTION

Planetary boundaries,¹ a concept that states the limits of human survival on Earth, were defined in 2009, and thereafter, the strength of the limits has increased. The excessive influx of nitrogen, phosphorus, new chemicals, and artificially synthesized antimicrobials into the biosphere, followed by the loss of biodiversity, has already reached dangerous levels.² In the light of these issues, a global goal should be to quickly move towards a recovery trend from biodiversity loss, that is, a "nature positive" trend.³

To address these issues, it is necessary to adopt a comprehensive approach that integrates science and technology. For example, organic matter recycling technology is important for the effective use of nitrogen and phosphorus in environmental science technology.⁴ However, to promote the use of compost, evaluating its functionality is necessary. The most popular example of the effective use of compost made from recycled organic matter is agricultural use,⁵⁻⁷ as well as its use as feed.⁸⁻¹⁰ The throughline among

these applications is the efficient recycling of fermented product resources. Among new chemical substances, antibiotic use can lead to the spread of resistant bacteria; therefore, alternative technologies are needed, along with appropriate restrictions on their use. Furthermore, even if new alternative technologies are developed, it will take time to disseminate them because the evaluation of the functionality of animals and plants requires detailed analysis, which is generally expensive and time-consuming. Therefore, a simple and fast combined evaluation technique is necessary at an early stage for the move towards a sustainable society.¹¹

In recent years, the multiphasic function of fermented compost, which is produced from nonedible marine animal resources with thermophilic *Bacillaceae* in closed fed-batched bioreactors under high-temperature conditions (70-80°C) (hereinafter, thermophile-fermented compost, but not faecal fermented compost) and used as an organic fertilizer or feed additive,¹²⁻²⁰ has been investigated. As a feed additive, compost has been shown to alter the gut microbiota of insects,^{21,22} rodents,²³ and livestock,^{18,20} in addition to improving the productivity of fishes,^{15,16} chickens and pigs,¹⁷ reducing still-birth rates in pigs,¹⁸ and improving the immune system and antioxidant activity in rodents and in carp used as animal models.^{16,19,24} In fishes, improvements in the muscle quality (with increased free amino acid levels) of carp and flounder have been observed,¹⁵⁻¹⁶ and seagrass overgrowth has been confirmed in and around the drainage channels of the aquaculture facilities for flounder.²⁵ In these facilities, the mortality rate has also improved.

In this study, we assessed the effects of thermophile-fermented compost on infectious diseases using seabream as a fish model. The assessment was performed by evaluating the inside and outside the body (*i.e.*, via an *in vivo* internal and external evaluation). In particular, since the surface of the fish body has long been known to change depending on environmental conditions,²⁶⁻³² the surface was statistically analyzed by examining photographs via newly created commands using diagnostic imaging techniques that have attracted attention in recent years.³³⁻⁴⁶ Thus, the *in vivo* external evaluation of the fish body surface allowed estimation of the physiological responses observed in the *in vivo* internal evaluation, and the utilization of the thermophile-fermented compost for studying fish infectious diseases was demonstrated. The findings of this study highlight the possibility of efficiently evaluating the antipathogenic effects of the thermophile-fermented compost with diagnostic imaging using field photographs. These results provide a potential model in which diagnostic imaging can be applied to increase the efficiency of the realization of sustainable primary industries.

MATERIALS AND METHODS

Fish breeding conditions

Fish tests were conducted using seabream at the Oigawa proving grounds in Japan (N34.80, E138.31) (Chubushiryo Co., Ltd., Japan) between February and June. In brief, a round tank (0.5 t capacity) was circulated in one direction with seawater, and the temperature of the round tank water was maintained at approximately 22°C. The seabreams (at most 15 per tank) were fed commercially available extruded pellet (EP) feed (Tai Next EP; Chubushiryo Co., Ltd., Japan) with 0% (group name: Gr1), 1% (group name: Gr2), or 5% (group name: Gr3) (weight/weight) aqueous solutions of thermophile-fermented compost (compost extract, explained below). The contents of the following compost powder in Gr1, G2, and G3 were 0% (0% compost extract, the conditions without water as vehicle), 0.01% (1% compost extract, aqueous solutions of thermophile-fermented compost), and 0.05% (1% compost extract, aqueous solutions of thermophile-fermented compost), respectively. The thermophile-fermented compost was prepared as follows. Fermented compost from marine animal resources (MAR compost) was produced by an aerobic repeated fed-batch fermentation system at high temperatures (approximately 70–80°C) via fermentation-associated self-heating (Miroku Co., Ltd. and Keiyo Gas Energy Solution Co., Ltd., Japan). As previously described,¹² this system that decomposes MAR using three fermentation bioreactor tanks (p2 m x 1.3 m height/tank) and transfers 1/3 the volume of the fermented MAR within 24 hours on the process of the self-heating (from 50 to 80°C). The powdered compost discharged from the fermenter was diluted to 1/100 with water (vol./vol.) and incubated under aerobic conditions at 60°C for at least 10 h (this diluted solution was used as the compost extract).^{15–19,23} All experimental procedures involving fish were conducted according to the strict animal care guidelines of Chubushiryo Co., Ltd., which belong to the Japan Feed Manufacturers Association (<https://www.jafma.or.jp/en/top/>).

In vivo internal detection

Blood urea nitrogen (BUN), glucose (GLU), triacylglyceride (TG), total cholesterol (T-cho), and total protein (T-Pro) levels in blood samples were determined via a SPOTCHEM™EZ SP-4430 automated biochemical analyzer (ARKRAY Co., Ltd., Japan). The free amino acid content was analyzed in the muscle of the occipital part of the fish obtained after the test. These muscle samples were homogenized with phosphate buffer, and the supernatant (1.5 ml) was poured into a Microcon 3000 column (Millipore Co., Ltd., USA). The filtrates were analyzed using a capillary electrophoresis system (CE system G1600A) equipped with a fused silica capillary with a basic anion buffer, as previously reported. Serum complement activity was determined by assessing the haemolysis rate ACH50 of an alternative complement pathway haemolytic activity.⁴⁷ Recovery tests were performed in seawater supplemented with 2-methylquinoline (Wako Pure Chemical Co., Ltd., Japan) at the manufacturer's recommended amount per body weight, and the time to awaking from anaesthesia was measured to assess the health status of the fish body.

Artificial infection test

After the seabreams were fed 0% (Gr1), 1% (Gr2), or 5% (Gr3) (weight/weight) aqueous solutions of the compost extract for 70 days, the three groups of the fish (n=10 per group) were intraperitoneally (*i.p.*) infected with 0.3 mL (conc. 4.3×10^6 CFU/mL) of *Edwardsiella tarda* (E05-92; Chubushiryo Co., Ltd., Japan). Subsequent breeding was performed for 28 days and evaluated for the survival tests. The detection of the pathogen was confirmed as previously reported.⁴⁸ The obtained survival data were visualized as survival curves via the function 'ggsurvplot' of the R software libraries "survival" and "survminer" using the Kaplan-Meier method. These difference between the groups were evaluated by the log-rank test and likelihood ratio test via the function 'coxph' of the library "survival". These data were represented via the library "broom", and a forest plot based on the Cox regression was generated via the function 'ggforest'.

Photography conditions

Individuals different from those in the infection test (Gr1, n=10; Gr2, n=10; and Gr3, n=8) were filmed in May before the end of the test. Photographs

were taken immediately after sedation for filming. The filming conditions were set as follows: filming equipment, Canon IXY DIGITAL 50; colour profile, sRGB IEC61966-2.1; size, 1600 × 1200; focus distance, 5.8 mm; alpha channel, no; metering mode, pattern; F value, f/2.8; and exposure time, 1/50. The original and modified photographs were stored at the figshare site (<https://doi.org/10.6084/m9.figshare.28646039>), and the other information was also recorded on each photograph stored the site. The colour change of the background and the dotting of the photographs for the *in vivo* external evaluation (body length evaluation, texture analysis, and HSV analysis) were performed using the Adobe Express (<https://www.adobe.com/jp/express/feature/image/remove-background>) and Adobe Photoshop 2025 (version 26.4.1). Gaussian treated images were modified on the basis of the commands described in the GitHub site (https://github.com/kecosz/cv2_jpg_preprocessing) and DOI (<https://doi.org/10.5281/zenodo.15081995>).

Body length evaluation

To avoid being affected by the accuracy of the shooting in the field, the dimensional information of the fish body was evaluated by ratio calculation based on the pixel data of the photograph. Open CV was performed using Python (version 3.10.8) in Mac OS Sequoia (version 15.3) on arm64. Libraries and modules such as "os", "cv2", "numpy", "math", and "pandas", were imported, and the body length ratio was determined according to the original commands. The procedure is shown in Figures 3A–B, and in GitHub (https://github.com/hmiyamoto2000/program_tai3-main/tree/v0.1.1; https://github.com/hmiyamoto2000/program_tai1_com) and in DOI (<https://doi.org/10.5281/zenodo.15080985>).

Body surface texture analysis

The grey level co-occurrence matrix (GLCM) and/or local binary pattern (LBP) for texture analysis has been developed mainly for the medical engineering and for construction structure analysis via the evaluation of smoothness of image surfaces.^{33–46} These analyses are one of the image analysis techniques that are not easily affected by illuminance. Here, on the basis of the following URL (<https://github.com/AIM-Harvard/pyradiomics/tree/master/radiomics>), newly developed commands were performed using Python (version 3.7.16) in Mac OS Ventura (version 13.6.7) on Intel (x86_64), and Python (version 3.10.8) in Mac OS Sequoia (version 15.3) on arm64. Libraries and modules such as "os", "cv2", "numpy", "math", "pandas", "affine", "tiffle", "csv", "itertools", "SimpleITK", "scipy", "Image", "ImageDraw", "tqdm", "glszm", "feature", "my_makedirs", "calc_glcmm_mean", "make_img_mask", "calculation", and "glszm_t" were imported. The procedure is shown in Figures 4A–B and was performed at the GitHub site (https://github.com/hmiyamoto2000/program_tai1_Texture_main/tree/v1.0.6) and DOI (<https://doi.org/10.5281/zenodo.15081663>).

Feature selection

To extract feature texture factors using machine learning algorithms, random forest models,⁴⁹ one of machine learning method with bagging (bootstrap aggregation), and XGBoost (eXtreme Gradient Boost),⁵⁰ machine learning with extreme gradient boosting,¹¹ and LightGBM (light gradient boosting machine) for a highly efficient gradient boosting decision tree,⁵¹ were used. These machine learning algorithms can be used not only for predicting test data based on training data, but also for selecting features to classify between groups (feature selection).^{11,52,53} The following libraries and modules were used via Python (version 3.10.8) in Mac OS Sequoia (version 15.3) on arm64: "xgboost", "RandomForestClassifier", "lightgbm", "train_test_split", "accuracy_score", "pyplot", "pandas", "yaml", and "csv". These selected feature factors were visualized as a bubble chart using the "matplotlib" (<https://matplotlib.org/stable/index.html>).

ROC analysis

To evaluate the diagnostic accuracy of the feature texture factors, receiver operating characteristic (ROC) curve analysis was performed. The area under the curve (AUC) was calculated via the R library "Epi".

Body surface HSV analysis

The colour tone, colorfulness, and brightness of the fish body surface were

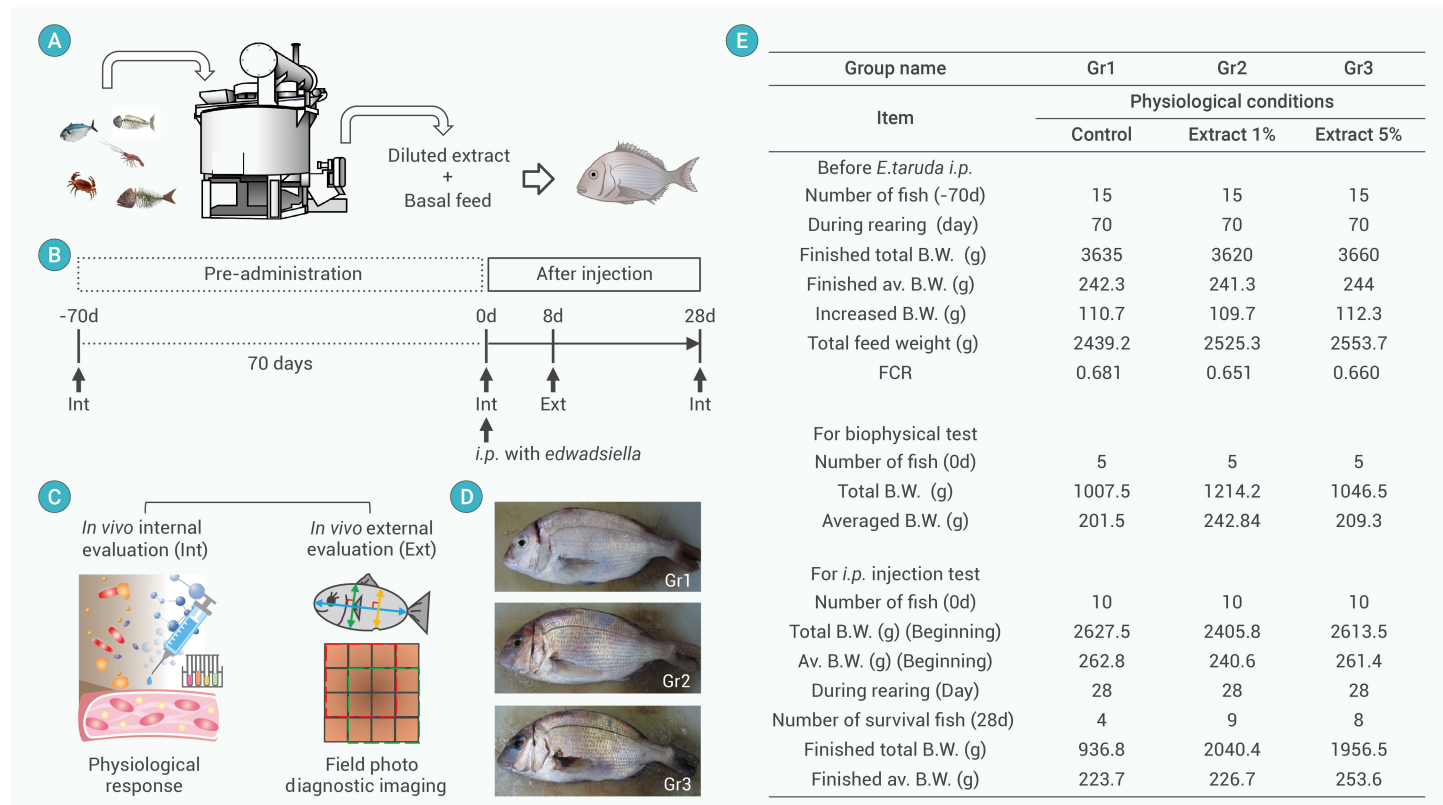


Figure 1. Outline of this research (A) Concept for the use of recycled fermented feed. In this study, the antipathogenic effects of the addition of 0%, 1%, and 5% of compost extract as a feed additive to basic feed were evaluated. (B) Plan for this study. A 70-day breeding test was conducted in advance; no compost extract (0%) was administered to the control group (Gr1), and 1% or 5% compost extract was administered to the test groups (Gr2 and Gr3, respectively). After preliminary feeding, *Edwardsiella tarda* was injected intraperitoneally (0.3 ml of the solution with the pathogen, and the content was 4.3×10^5 CFU/ml), and the subsequent survival rate was evaluated. In addition, the physiological response and diagnostic imaging of the body surface for three groups (Gr1, Gr2, and Gr3) were investigated via *in vivo* internal evaluation ("Int" at -70d, 0d, and 28d in the plan of Figure 1B) and *in vivo* external evaluation ("Ext" in 8d in the plan). (C) Conceptual image of *in vivo* internal evaluation and *in vivo* external evaluation. *In vivo* internal evaluation (Int) involves the evaluation of physiological responses in fish. *In vivo* external evaluation (Ext) refers to field photo diagnostic imaging, which is a quantitative diagnosis of images on the basis of photographs from the field. (D) The three photographs are representative pictures of Gr1, Gr2, and Gr3, respectively. (E) Breeding results for the preparatory and *i.p.* injection test. Abbreviations are as follows: *i.p.*, intraperitoneal injection; Gr1 (Control), the control group without compost extract and water as vehicle; Gr2 (Extract 1%), the group administered 1% compost extract (0.01% compost powder diluted with water); Gr3 (Extract 5%), the group administered 5% compost extract (0.05% compost powder diluted with water); BW, body weight; FCR, feed conversion ratio (increased body weight per unit feed volume during the breeding period).

evaluated via the analysis of HSV (hue, saturation, and value) analysis. To obtain the levels of HSV, open CV was performed using Python (version 3.7.16) in Mac OS Ventura (version 13.6.7) on Intel (x86_64), and Python (version 3.10.8) in Mac OS Sequoia (version 15.3) on arm64. Libraries and modules such as "cv2", "numpy", "matplotlib", "tqdm", "scipy.stats", "pandas", "scikit_posthocs", and "pairwise_turkeyhsd" were imported. The procedure is shown in Figure 6A-B for the GitHub site (https://github.com/hmiyamoto2000/program_tai1_HSV_main/tree/v1.0.5) and DOI (<https://doi.org/10.5281/zenodo.15081661>).

Statistical analyses

The following statistical tests were performed for frequentist equivalence testing: *P* values determined via the Shapiro-Wilk test, *F* test, and Bartlett test were used to select parametric and nonparametric analyses. In addition, ANOVA followed by Tukey's HSD and Kruskal-Wallis one-way analysis of variance followed by the Steel-Dwass test were performed as appropriate methods depending on the dataset obtained via texture and HSV analysis. For the comparison between the Gr1 group (reference) and the combined compost extract treated group (Gr2 and Gr3), an unpaired *t* test or the Welch's *t* test was used for parametric analysis, or the Wilcoxon rank-sum test was used for nonparametric analysis. Significance was declared when $P < 0.05$, and a tendency was assumed at $0.05 \leq P < 0.20$. These calculation data were prepared by using the Python (version 3.10.8) and R software (version 4.4.2). Prism software (version 10.3.1) was used for graphical visualization. The data are presented as the means $\pm$ SDs. Data collection and analysis were not blinded to the experimental conditions.

RESULTS

Fish assessment overview

In this study, the effects of administering a diluted extract of thermophile-fermented compost (compost extract) to seabream were examined (Figure 1A). As shown in Figure 1B, the schedule consisted of 70 days of pretreatment followed by injection of the pathogen *Edwardsiella tarda*; subsequently, the survival test was performed for 28 days. In addition, the physiological response and body surface were examined by two types of evaluation (an *in vivo* internal evaluation and field photo diagnostic imaging for *in vivo* external evaluation, as shown in Figure 1C). Notably, the administration of the compost extract improved the surface gloss of the fish (as observed by comparing Gr2 and Gr3 with Gr1) (Figure 1D). Although no significant differences were observed in these breeding outcomes (Figures 1E & S1), a reduction in the number of deaths was confirmed in the survival test.

Evaluation of physiological responses in a fish model

The Kaplan-Meier survival curve (Figure 2A) revealed a significant difference among the three groups (log-rank test, $P = 0.02$). In particular, a significant difference ($P = 0.049$) was observed between Gr1 (0% compost extract) and Gr2 (1% compost extract), and the survival rate of Gr2 was greater than that of Gr1. The survival rate of Gr3 (5% compost extract) tended to be greater than that of Gr1 ($P = 0.11$). The forest plot based on the Cox regression (Figure S2A) also revealed marked differences (likelihood ratio test, $P = 0.03$; Wald test, $P = 0.06$) and differences for the following interventions: intervention Gr2, $P = 0.0453$; and intervention Gr3, $P = 0.0931$. In addition, the detection rate of the pathogen in the surviving fish was significantly lower in

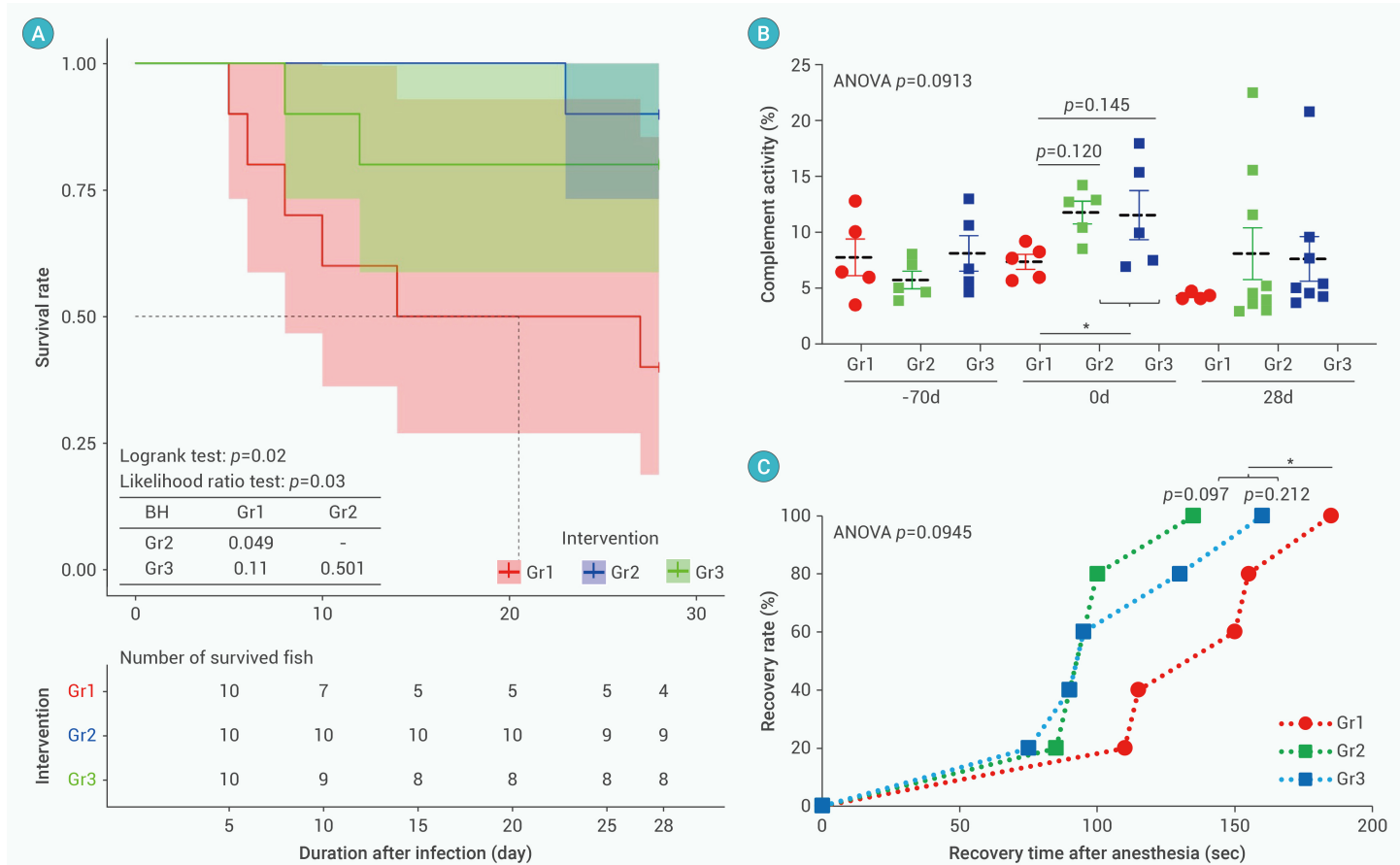


Figure 2. Evaluation of survival rates and physiological indicators after compost administration (A) Survival curves obtained using the Kaplan-Meier method. Statistical analyses were performed using the Cox regression function to determine the values from the log-rank test and likelihood ratio test in the graph. The table below shows the number of survivors on each of the five test days. (B) Serum complement activity before and after test initiation. The abbreviations are as follows: -70 d, starting point in the prior breeding period; 0 d, just before the *Edwardsiella* injection; and 28 d, 28 days after the *Edwardsiella* injection. (C) Recovery time after anaesthesia administration. 2-Methylquinoline, an anaesthetic that can be generally administered without the use of a syringe, was used.

Gr2 and Gr3 than in Gr1 (Figure S2B). These observations indicated that the survival data were independent of the dose of compost extract. The average body weights of all the groups decreased after the test, but the differences were not observed (Figure S2C). The fork length and the condition factor did not change. In the serum and muscle samples collected immediately before the survival test (0 d), the levels of serum BUN, Glu, TG, T-Chol, and T-Pro did not significantly differ among the three groups (Figure S3). The levels of free amino acids (glutamate and glycine) and lactate in the muscle were also not significantly different. Differences between Gr1 and G2_G3 exhibited significant differences in complement activity, which is an immune indicator (Student's t test $P = 0.0255$ as $n = 10$) (Figure 2B), were observed between Gr1 and G2_G3; the activity levels of Gr2_Gr3 were greater. This trend was also observed between the individual groups (G1 and Gr2; and Gr1 and Gr3), although the difference was not always significant (ANOVA $P = 0.0913$; G1 and Gr2, Tukey HSD $P = 0.120$ as $n = 5$; and Tukey HSD $P = 0.145$ as $n = 5$). However, these trends were not always maintained until the end of the trial. Furthermore, a verification of the effect of waking up from anaesthesia, although not statistically significant (ANOVA $P = 0.0945$; Tukey HSD $P = 0.097$ as $n = 5$; and Tukey HSD $P = 0.212$ as $n = 5$) (Figure 2C), confirmed that individuals in the compost extract groups (Gr2 and Gr3) could significantly awaken quickly from anaesthesia (Student's t test $P = 0.0306$ as $n = 10$), although significant differences were not necessarily observed between the individual groups (G1 and Gr2; and Gr1 and Gr3). Thus, the physiological changes associated with the effects of compost extract administration were slightly confirmed by *in vivo* internal evaluation, although the statistical values were obscured, and these changes were not always dose dependent.

Evaluation of fish body length

Assessing the dimensions of the fish body is important for understanding

the condition of the fish, but performing accurate quantification in field operations is difficult. However, it is relatively easy to take photographs and evaluate them at a later date. Here, a command was developed to assess images by calculating the ratio for each part in the absence of standard dimensions, such as a yardstick, and the operation was performed. As shown in Figures 3A-B, it was possible to calculate the ratio from the mouth to the caudal fin (X1-X2) after setting a reference line and the related points in different RGB colours. The ratio of the orthogonal line of the pectoral fin to X1-X2 and the ratio of the orthogonal line of the anus were calculated. These commands were deposited at the GitHub site as described in the Data Availability section. The results confirmed that the target fish bodies significantly differed in the ratio of R1 to R2 (Figure 3C). Specifically, Gr2, which had the lowest mortality rate, presented increased ratios of R1 ($P = 0.0418925$) and R2 ($P = 0.0503825$). The ratios of Gr3 were significantly different from those of Gr2 (R1, $P = 0.0247454$; R2, $P = 0.0376840$). No statistically significant differences were observed for R3.

Evaluation of fish body surface texture

As shown in Figure 1D, the fish body surface area appeared to differ among the groups. However, a quantitative evaluation method for such differences has rarely been established in the fishery industry, and performing accurate quantification in field operations, even when possible, is difficult. Therefore, the quality of the fish body surface was evaluated via the grey level co-occurrence matrix (GLCM), which is mainly used in medical engineering and construction structure analysis, as a texture analysis tool.³³⁻⁴⁶ As shown in Figures 4A-B, to evaluate the fish according to the same criteria, we developed a command to set reference points at the tip of the fish's mouth and caudal fin and adjust the points so that they were horizontal. In addition, the background-modified images were also prepared (Figure S4). Next, 1/3 of the

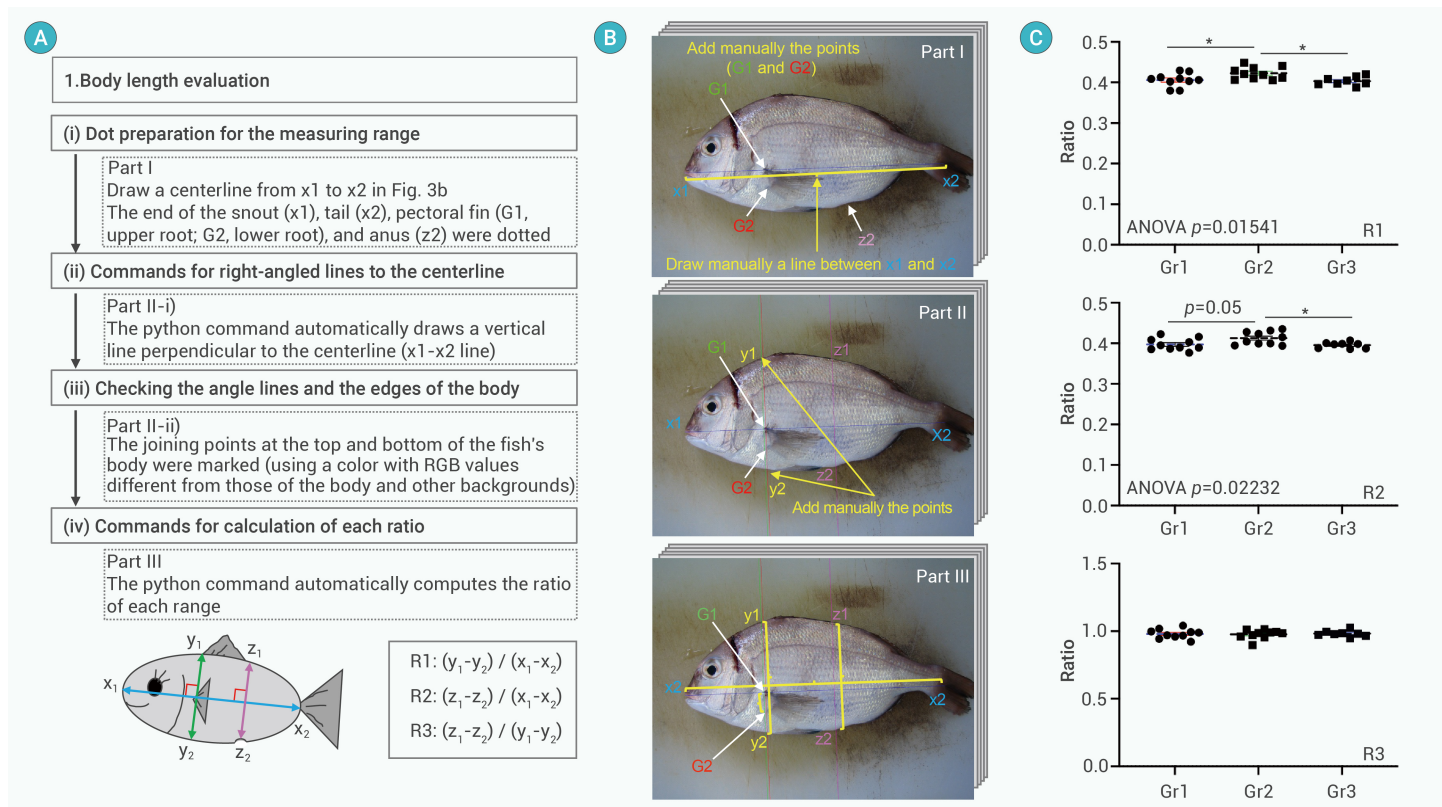


Figure 3. Body length evaluation (A) Workflow of the analytical steps for body length measurements used in this study. (B) Photographs for each measurement step. The photographs are shown for the description of each part of the workflow in Figure 3A. Step (i) in Figure 3A is performed to determine the measurement range. That is, the blue line, rgb (0, 0, 255), as the RGB value, connects x_1 and x_2 (standard body length, the centerline in this study), as shown in Part I of Figure 3B. Then, dots with different RGB values are placed at the base of the pectoral fins and at the front of the anus. The colours of these dots are set as follows: G_1 in Part I, rgb (0, 255, 0); G_2 , 255:0:0 as the RGB value; and z_2 , rgb (254, 254, 0). Next, a dedicated command is used to draw lines perpendicular to the blue line, as shown in (ii) in Figure 3A. In Part II of Figure 3B, the orthogonal lines G_1 , G_2 , and z_2 are shown. Thereafter, dots with different RGB values are manually placed at the intersection of the upper and lower surfaces of the fish body as shown in (iii) in Figure 3A. The colours of these dots are set as follows: y_1 and y_2 in Part II, rgb (255, 0, 255); z_1 in Part II, rgb (254, 254, 0); and x_1 and x_2 , rgb (2, 0, 255). Finally, a developed command automatically calculates the ratio of body length to height in Part III of Figure 3B, as shown in (iv). (C) Evaluation of the body length ratio for R1, R2, and R3. The asterisks indicate $P < 0.05$. The abbreviations were as follows: x_1 - x_2 , the distance between x_1 and x_2 ; y_1 - y_2 , the distance between y_1 and y_2 ; z_1 - z_2 , the distance between z_1 and z_2 ; R1, the ratio $(y_1 - y_2) / (x_1 - x_2)$; R2, the ratio $(z_1 - z_2) / (x_1 - x_2)$; and R3, the ratio $(z_1 - z_2) / (y_1 - y_2)$.

central part of the fish body was automatically evaluated as the site to be measured via the newly developed commands. These commands were deposited in GitHub sites and the DOIs as described in the Data Availability section. In addition, because the photographs of the site were not always of high quality, the image data were edited using the Gaussian model, allowing the evaluation of blurry images to be carried out. Three machine learning algorithms were used to select the feature metrics for the texture analysis data. The algorithm described here was used not to predict the accuracy of the test data on the basis of the training data, but to improve the selection accuracy of the feature metrics. By analyzing the original photographs, eight feature metrics were selected for LightGBM (Figure S5). The highest feature metrics are shown in Figure 4C. Among these metric groups, the metric with higher values for both Gr2 and Gr3 than for Gr1 was GLSZM_ZoneEntropy (ANOVA $P = 3.205e-06$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0124425$; Gr1 vs. Gr3, Tukey's HSD $P = 0.0480842$; and Gr2 vs. Gr3, Tukey's HSD $P = 0.0030646$). However, GLSZM_SmallAreaEmphasis (ANOVA $P = 3.131e-05$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0435837$; Gr1 vs. Gr3, Tukey's HSD $P = 0.0000187$; and Gr2 vs. Gr3, Tukey's HSD $P = 0.0083970$), GLSZM_SizeZoneNonUniformityNormalized (ANOVA $P = 8.293e-06$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0249181$; Gr1 vs. Gr3, Tukey's HSD $P = 0.0000048$; and Gr2 vs. Gr3, Tukey's HSD $P = 0.0040308$), and LBP_R1_P8_ave (Gr1 vs. Gr2, Tukey's HSD $P = 0.0754322$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.0325014$) were metrics that presented lower values (Figures 5A & S6A). In addition, the other metrics did not significantly differ. Next, as a result of the analysis based on the Gaussian image, GLSZM_ZoneEntropy, GLSZM_SmallAreaEmphasis, and LBP_R1_P8_ave were measured among these important metrics, and the trend was similar to that in the original image (Figure 4D). GLSZM_ZoneEntropy for Gr2 and Gr3 was significantly greater than that for Gr1 (ANOVA $P = 0.001929$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0015341$; Gr1 vs. Gr3, Tukey's HSD $P = 0.0000018$;

and GLSZM_SmallAreaEmphasis for Gr2 and Gr3 was significantly lower than that for Gr1 (ANOVA $P = 0.003665$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0188099$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.0053235$) (Figure 5B), and LBP_R1_P8_ave showed statistically significant differences in some areas (ANOVA $P = 0.005641$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0235563$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.2313297$) (Figure S6B). In the Gaussian image, GLSZM_LargeAreaLowGrayLevelEmphasis was a newly important metric candidate, but it did not show statistically significant differences (Figure S6B). There were no statistically significant differences in the other factors.

On the basis of the above points, the performance of the selected feature metrics was evaluated by receiver operating characteristic (ROC) curve analysis. As described in Figure S7, the area under the curve (AUC) for GLSZM_ZoneEntropy was calculated as a value close to 1 (high true positive rate) (0.944 for the AUC of the original image; and 0.839 for the AUC of the Gaussian image). In contrast, AUC values for GLSZM_SmallAreaEmphasis and GLSZM_SizeZoneNonUniformityNormalized were low levels (AUC < 0.2). These commonality were found in the original image and in the degraded image model using the Gaussian image (blurred images) by texture analysis. Thus, a significant increase in GLSZM_ZoneEntropy, and a significant decrease in GLSZM_SmallAreaEmphasis and GLSZM_SizeZoneNonUniformityNormalized were deemed essential metrics under these experimental conditions.

Evaluation of colour tone, colourfulness, and brightness of the fish body surface

In onsite photography, it is difficult to adjust the light. However, regardless of the degree of illumination, differences can be recognized by human vision, and these differences may be visible to those who have abundant experience in the field. Assessing this difference is important for understanding the

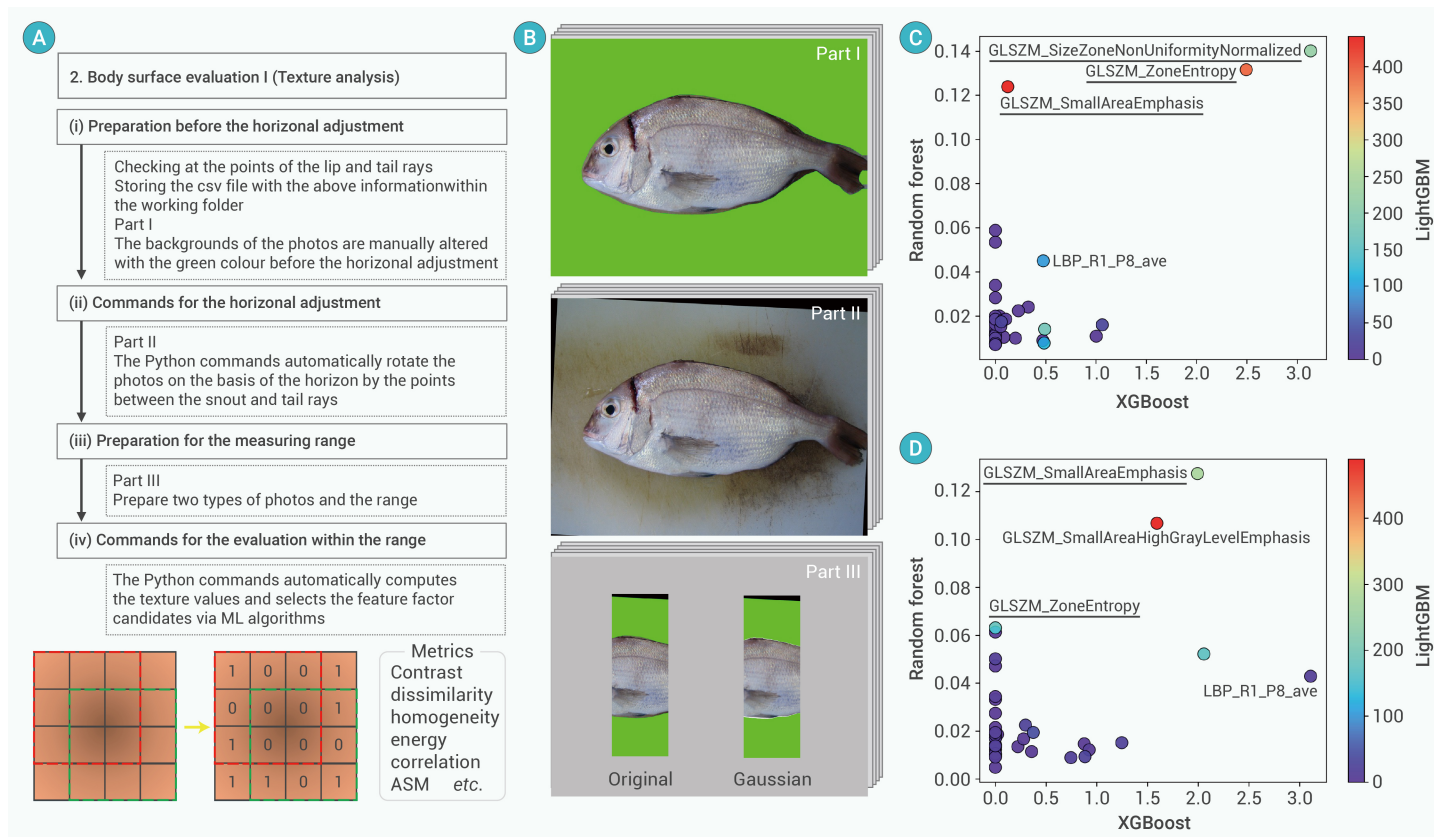


Figure 4. Evaluation by texture analysis of the body surface (A) Workflow of the analytical steps for texture analysis used in this study. (B) Photograph of each texture measurement step. First, the positions of the mouth and tail are summarized in a CSV file based on the numerical values of ImageJ as the information to adjust the horizon level of the photo, as shown in (i). The background is changed to green as rgb (0,255,0), as shown in Part I. Next, a special command rotates the photo, and the fish bodies are placed in parallel, as shown in Part II of step (ii). To clarify the measurement range for the fish body as shown in step (iii), the above horizon adjustment is performed. The measurement range around the pectoral fins is automatically cut out as 1/3 of the area from the lip to the tail of the fish via special commands, as shown in Part III, and the texture analysis of this part is performed. The texture metrics include the contrast, dissimilarity, homogeneity, energy, correlation, and ASM and so forth. Furthermore, the analytical procedure is also used for Gaussian-treated photographs (gaussian_gauss2.00 as a Gaussian level). The conditions for the texture analysis and Gaussian treatment are described in the Materials and Methods section. (C) Texture feature metric candidates from the original image and (D) those from the Gaussian-treated images. The candidates are selected via three types of machine learning (ML) algorithms. The abbreviations were as follows: GLCM, the grey level co-occurrence matrix; LBP, local binary pattern; XGBoost, eXtreme Gradient Boost; and LightGBM, light gradient boosting machine.

condition of fish, but performing accurate quantification in field operations is difficult. Therefore, the surface of the fish body was evaluated via HSV (hue, saturation, and value) analysis. Among other things, the levels of hue were assumed to be less susceptible to light. As shown in Figure 6A, a survey was conducted on the part examined for texture analysis. The analyzed data were statistically evaluated at every 20 pixels. These commands were deposited at the GitHub site as described in the Data Availability section. As a result, the saturation (colourfulness) and value (brightness) profiles did not always differ among Gr1, Gr2, and Gr3 (Figures S8-S9). However, the hue (colour tone) profiles for the original image were significantly different among Gr1, Gr2, and Gr3 (Figure 6B) for the following pixel values: the pixel [2:22] (ANOVA $P = 0.0154$; Gr1 vs. Gr2, Tukey's HSD $P = 0.2624$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.0114$); [12:32] (ANOVA $P = 0.0010$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0354$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.0008$); [22:42] (ANOVA $P = 0.0319$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0504$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.0686$); [106:126] (Kruskal-Wallis $P = 0.0431$; Gr1 vs. Gr2, Steel-Dwass $P = 0.164876$; and Gr1 vs. Gr3, Steel-Dwass $P = 0.067624$); [116:136] (Kruskal-Wallis $P = 0.0082$; Gr1 vs. Gr2, Steel-Dwass $P = 0.041206$; and Gr1 vs. Gr3, Steel-Dwass $P = 0.012415$); [126:146] (Kruskal-Wallis $P = 0.0009$; Gr1 vs. Gr3, Steel-Dwass $P = 0.00183$); [136:156] (Kruskal-Wallis $P = 0.0009$; Gr1 vs. Gr3, Steel-Dwass $P = 0.00183$); [146:166] (Kruskal-Wallis $P = 0.0009$; Gr1 vs. Gr3, Steel-Dwass $P = 0.00183$); and [156:176] (Kruskal-Wallis $P = 0.0142$; Gr1 vs. Gr3, Steel-Dwass $P = 0.021453$). Similar trends were also observed in the Gaussian-treated photographs (Figure 6C) as follows: the pixels [2:22] (ANOVA $P = 0.0129$; Gr1 vs. Gr2, Tukey's HSD $P = 0.2016$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.0097$); [12:32] (ANOVA $P = 0.0005$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0157$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.0004$); [22:42] (ANOVA $P = 0.0319$; Gr1 vs. Gr2, Tukey's HSD $P = 0.0483$; and Gr1 vs. Gr3, Tukey's HSD $P = 0.0721$);

[106:126] (Kruskal-Wallis $P = 0.0340$; Gr1 vs. Gr2, Steel-Dwass $P = 0.141586$; and Gr1 vs. Gr3, Steel-Dwass $P = 0.054397$); [116:136] (Kruskal-Wallis $P = 0.0074$; Gr1 vs. Gr2, Steel-Dwass $P = 0.033731$; and Gr1 vs. Gr3, Steel-Dwass $P = 0.012415$); [126:146] (Kruskal-Wallis $P = 0.0009$; Gr1 vs. Gr3, Steel-Dwass $P = 0.00183$); [136:156] (Kruskal-Wallis $P = 0.0009$; Gr1 vs. Gr3, Steel-Dwass $P = 0.00183$); [146:166] (Kruskal-Wallis $P = 0.0009$; Gr1 vs. Gr3, Steel-Dwass $P = 0.00183$); and [156:176] (Kruskal-Wallis $P = 0.0142$; Gr1 vs. Gr3, Steel-Dwass $P = 0.021453$).

From the above information, the performance of the selected area for the hue values was evaluated by ROC analysis (Figure S10). The AUCs for the pixel [2:46] and the whole [0:180] as hue values were calculated to be 0.656 (Figure S10A) and 0.529 (Figure S10C), respectively, whereas that for pixel [106:176] was calculated to be 0.386, which was a low value (Figure S10B). These results indicate that the qualitative differences in the fish surfaces observed in this study could be quantitatively evaluated using HSV analysis.

DISCUSSION

The current environmental problems involve a complex combination of factors, and it is necessary to use nitrogen and phosphorus efficiently and reduce the use of artificial antibiotics. Under such circumstances, technological developments that could be solutions need to be disseminated quickly. The results of this research provide important information for the operation of high-value-added recycling technology that leads to reduced levels of artificial antibiotics via the development of technology that can efficiently evaluate the added value using images of the surface of the fish body. Based on these observations, the potential mechanisms underlying the effects of thermophile-fermented compost were predicted as a summarized concept (Figure 7). Specifically, the following two points were clarified: (1) the admin-

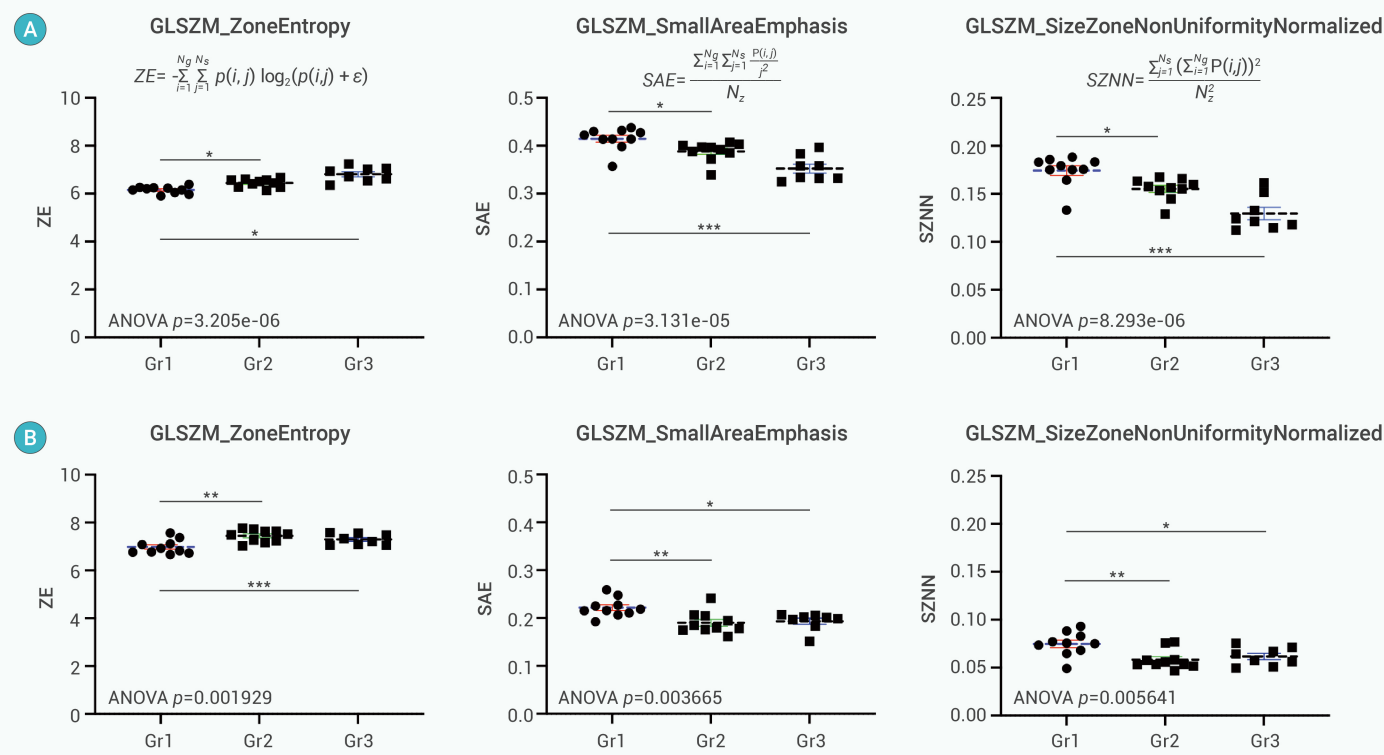


Figure 5. Statistical comparison of texture features in normal and Gaussian photographs (A) Main features of the original photos. (B) Main features of the Gaussian-treated photos. In (A), the formula for the texture metrics (<https://pyradiomics.readthedocs.io/en/latest/features.html#module-radiomics.glszm>) is shown below each metric. The abbreviations were as follows: GLSZM, a grey level size zone matrix; ZE, GLSZM_ZoneEntropy; SAE, I GLSZM_SmallAreaEmphasis; SZNN, GLSZM_SizeZoneNonUniformityNormalized. The asterisks indicate the degree of significance for Turkey's HSD (honestly significant difference) as multiple tests: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

istration of thermophilic compost reduces infections in fish, and (2) such effects can be detected as quantitative differences not only by in-body evaluation but also by noninvasive evaluation using field photographs, which are rough original or unclear Gaussian ones. On the basis of these multiple perspectives, this study is the first to assess effectively sustainable fisheries technology, especially the antipathogenic effects of thermophile-fermented compost, using field photo diagnostic imaging.

In recent years, noninvasive fish evaluation has been promoted in various forms,⁵⁴⁻⁵⁷ but the results of this research focus on dimensional evaluation, texture analysis, and HSV analysis. The scope of application is that it is applied to the evaluation of circular technologies.

The thermophile *Bacillaceae*-fermented compost used in this study appears to play an antipathogenic probiotic role in fish. In addition to the properties of general compost that promote the efficient use of nitrogen and phosphorus, thermophile-fermented compost should possess a novel probiotic functionality as an alternative to antibiotics. In previous studies, compost itself was shown to reduce the stillbirth rate of pigs.¹⁸ It has also been reported to increase productivity and/or muscle quality in flatfish,¹⁵ carp,¹⁶ chickens, and pigs.^{17,18} While the mechanism by which compost acts is not clear, but experiments on rodents as model animals have demonstrated its positive effect on faecal IgA secretion in the immune system,^{23,24} although the complement activity has not been examined. Rodent experiments have shown that the oral administration of compost affects the gut microbiota and is involved in the gene expression of granzyme B in the gut.²³ Interestingly, granzyme B is known to be involved in complement activity.⁵⁸ Since the improvement in the survival rate of Gr2 and Gr3 did not have a dose-response relationship, the effect was assumed to be due to the improvement in the biological defence function associated with exposure to compost above a certain level. Moreover, the function of this compost was strongly influenced by the compost-derived *Caldibacillus hisashii*.^{24,59,60} It is necessary to study this fermentation-mediating thermophile that affects the physiological responses of fishes.

In general, it is known that the surface of a fish body is affected by health conditions and the environment.²⁶⁻³² However, no method has been estab-

lished to quantitatively evaluate these conditions. Therefore, another goal of this study was noninvasive evaluation. In general, it is difficult to evaluate the body of a fish in the field unless it is extremely sick, and it is not possible to evaluate it quantitatively and quickly. However, the results of this study showed that a quantitative assessment can be easily performed using a single photograph via the developed commands.

Depending on the ratio calculation, various possibilities exist for the measurement. Since this result is consistent with the results of manual measurement using ImageJ (Table S1), we have succeeded in calculating accurate data semiautomatically via commands developed in this study. Furthermore, texture analysis can evaluate the degree of smoothness of images; therefore, it is less susceptible to the effects of light. In the field, this analytical method was considered useful because it was generally difficult to adjust the lighting in the photographs. This analysis method is particularly advanced in the medical field,^{37,39,40} and there are no examples of its use in primary industries other than our research cases. In addition, calculation commands are not always easy to operate. Therefore, one of the important points of this research is that the results can be applied to fish to increase convenience in real-world operation. Consequently, the tendency of entropy to increase under the experimental conditions of this study was confirmed. This trend was also confirmed in the edited image of the Gaussian model, the quality in which the photograph was bad.

Next, an HSV analysis was performed to rule out the effects of light. Under these experimental conditions, the effect of light was partly observed as V (value, brightness) (Figure S9). The results revealed that there was a significant difference in V (value, brightness), except for one part, but the effect was low, and a characteristically significant difference was observed in H (hue, colour tone) under these experimental conditions (Figures 6B-C). These results indicated that the effect of compost administration can be statistically evaluated as the difference observed on the surface of the fish body. As mentioned above,²⁶⁻³² the body surface can change depending on health conditions, pathogenic infection, and other environmental conditions. Thus, it was suggested that disease resistance against the pathogen *Edwardsiella* was predicted by a multifaceted evaluation of the fish surface. Consequently,

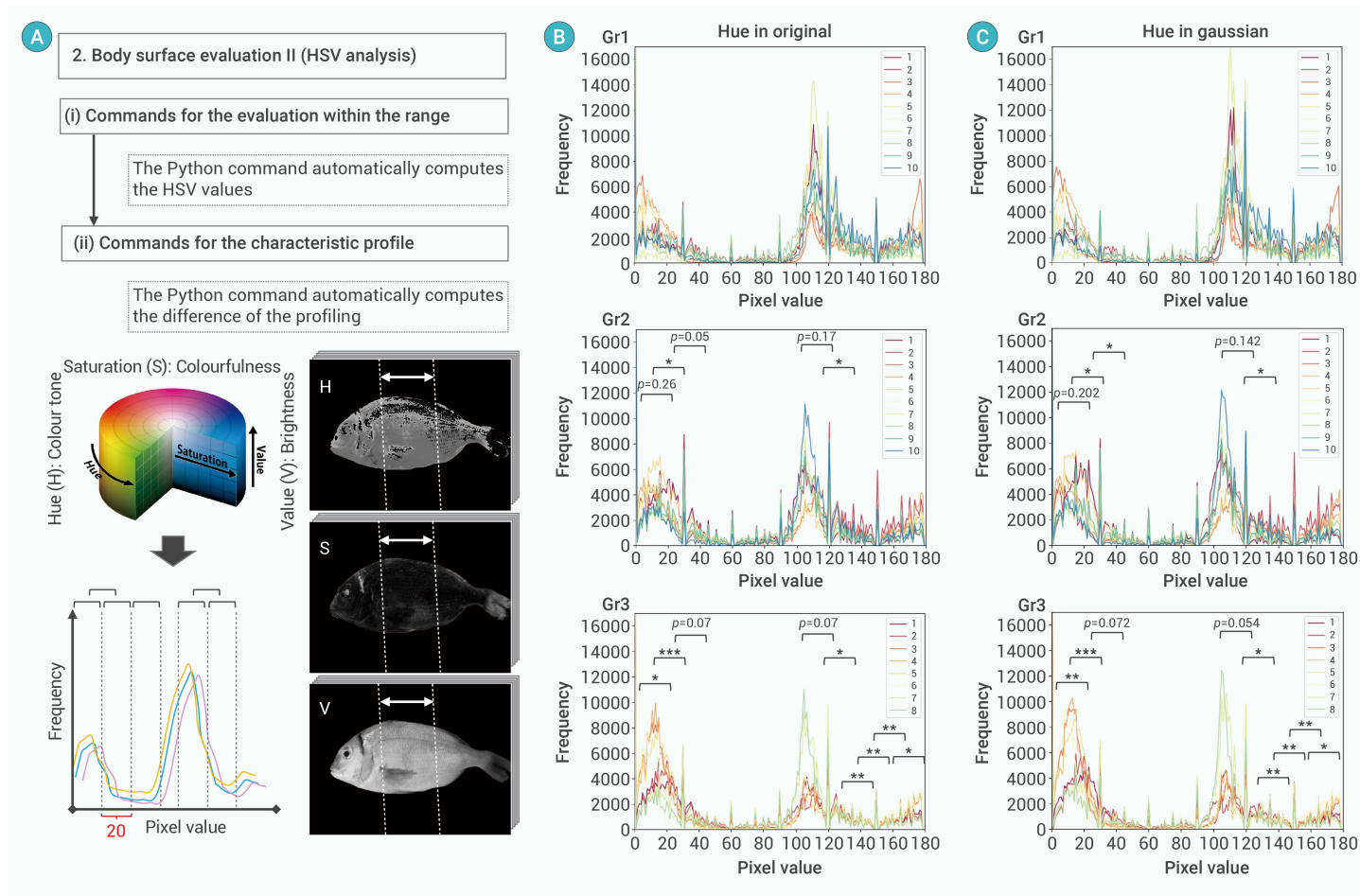


Figure 6. HSV analysis of body surfaces (A) Workflow of the analytical steps for HSV analysis used in this study. The process of splitting the photographs is performed in the same way as for texture analysis. A developed command then calculates the HSV values for the photo (marked as H, S, and V) in step (i). Next, the HSV profiles are statistically computed in the step (ii). A part of the profile is calculated for each of the 20 pixels. The abbreviations are as follows: H, hue as colour tone; S, saturation as colourfulness (colour intensity); and V, value as brightness. The hue values from (B) the original photographs and (C) the Gaussian-treated photographs are shown for each group. The vertical Y axis represents the frequency. The horizontal x-axis shows the range of 180 pixels, which is based on the OpenCV specification. The asterisks indicate the degree of significance: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

changes that have been evaluated qualitatively thus far may be used as indicators in noninvasive analysis methods.

In the future, it is necessary to evaluate the relationships between various conditions, such as different fish species, growth stages, and environmental factors, but it is expected that these relationships can be used as indices to quantitatively evaluate visual differences under relevant experimental conditions. The analysis of these studies is based on a limited number of samples, and universality and adjustment factors need further discussion. If it is possible to add an internal standard in image analysis, a more rigorous evaluation will be possible. However, since features are calculated by multiple machine learning algorithms via evaluation by the Gaussian model, which deteriorates the quality of the image and results in multiple indicators, it means that more important indicators have been successfully selected under limited conditions. For example, three types of ML algorithms (random forest, XGBoost, LightGBM) have effective training frequencies (num_boost_round: 1000, early_stopping_round: 400, and learning rate: 0.01). In the future, there may be room for more efficient methods to use these algorithms, but it is assumed that their value as a model is high. Furthermore, the results of this study indicate that compost, which can positively affect the circulation of nitrogen, phosphorus, and antibiotics, can be evaluated noninvasively, easily and quantitatively through changes in the surface of the fish body. In addition, aquaculture facilities are located in areas that are generally densely populated with several facilities, which could lead to the spread of infectious diseases. This area results in the excessive use of antibiotics, which has a negative impact on biodiversity. Therefore, the development of the model in this study is also an attempt to reduce the environmental load of antibiotics and simultaneously contribute to the productivity and quality of fish by efficiently assessing

the degree of smoothness and gloss of the body surface.

Thus, the approach used in this study provides a new perspective on ecosystem sustainability and a natural symbiotic society. Further research to evaluate the images efficiently could contribute not only to environmental studies, biology, and medicine, but also to several other fields.

CONCLUSION

In conclusion, our developed noninvasive diagnostic imaging method using field photographs could estimate anti-pathogenic property of thermophile-fermented compost as a feed additive for fish. The noninvasive diagnostic imaging method should offer novel insights into the efficient evaluation of fish health. In addition, this method will be quickly adopted help build a sustainable society.

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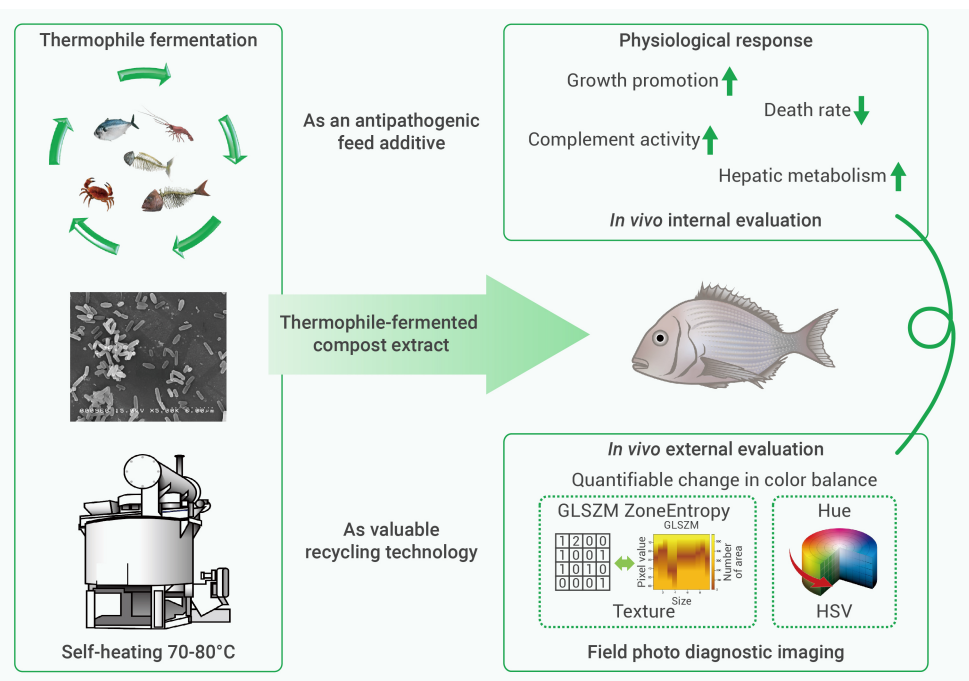


Figure 7. Summary of this study Compost fermented nonedible marine resources with the thermophile *Bacillaceae* act as a disease-resistant feed additives and are highly valuable for use in recycling technology. By internal evaluation, growth promotion, mortality reduction, and the potential activities of serum complement and hepatic metabolism can be enhanced. Simultaneously, different degrees of smoothness and gloss of the fish body surface can be observed by external evaluation: diagnostic imaging using field photographs reveals a significant increase in GLSZM ZoneEntropy as a metric for texture analysis and the hue value as a metric for HSV analysis.

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FUNDING AND ACKNOWLEDGMENTS

We are grateful to Seichi Wada (Chuubushiryō Co. Ltd.), Toshihito Shinmyo (Keiyo Gas Energy Solution Co., Ltd.), Jirou Matsumoto, and Kazuo Ogawa (Sermas Co., Ltd.) for help with breeding facilities, sampling, and/or preparation of feed and to Teruno Nakaguma (Japan Eco-science Co., Ltd.) and Chitose Ishii (Sermas Co., Ltd.) for discussion of the imaging results. Special thanks to Professor Hideaki Haneishi (Chiba University) for providing technical advice on the HSV image analysis. In addition, special thanks to Ms. Izumi Jouzuka and Ms. Ami Urasaki for helping with the figure design. This study was partly supported by the grants from the Research and Development Support Program for Regional Revitalization of the Food Industry Sector of the Ministry of Agriculture, Forestry, and Fisheries of Japan (No. 22331219048) and the Ministry of Economy, Trade, and Industry of Japan (No.21231219119). The funders had no role in the

study design, data collection, analysis, decision to publish, or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

Hirokuni Miyamoto and Hisashi Miyamoto conceived and designed the experiments; Shunsuke Ito performed the fish experiments; Hisashi Miyamoto and Motoaki Udagawa prepared the compost extract; Hirokuni Miyamoto, Shunsuke Ito, Takayuki Nagatsuka, and Takashi Satoh performed the biological assay and the data arrangement; Hirokuni Miyamoto, Kenta Suzuki, Shingo Tamachi, and Shion Yamada performed the creation of commands for image analysis; Hirokuni Miyamoto, Kenta Suzuki, Shingo Tamachi, Shion Yamada, and Jun Kikuchi analyzed the raw data.; Hirokuni Miyamoto, Hiroshi Ohno, and Jun Kikuchi supervised the study; Hirokuni Miyamoto, Kenta Suzuki, and Jun Kikuchi wrote the manuscript. All authors contributed to the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

ETHICAL STATEMENT AND PATIENT CONSENT

All experimental breeding procedures involving fish were conducted according to the animal care guidelines of Chubushiryō Co., Ltd. as feed manufacturer.

DATA AND CODE AVAILABILITY

The commands and scripts for image analysis used in this study are provided across five GitHub repositories. These repositories cover the full pipeline from raw field photographs of fish specimens to quantitative feature extraction and analysis of body shape, texture, and colour characteristics: (i) program_tai3-main (https://github.com/hmiyamoto2000/program_tai3-main/tree/v0.1.1), for the automatic measurement of fish body length from field photographs. The workflow includes image preprocessing (drawing reference lines, orientation correction), length measurement (measurement_program.py), and output of measurement results in Excel format. Example images and preprocessed data are also included.; (ii) program_tai1_com (.../program_tai1_com), for combined texture and HSV colour space analysis of fish body images. It supports horizontal adjustment (line_split_ver.py), extraction of texture features (feature_value_image_whole.py), computation of HSV metrics (program2h_*, program2s_*, program2v_*), and visualization. This repository can be used for comprehensive diagnostic imaging on the basis of both structural and colorimetric features.; (iii) program_tai1_Texture_main (.../program_tai1_Texture_main/tree/v2.0.1), for texture analysis using grey level co-occurrence matrix (GLCM)-based feature extraction. It includes machine learning-based analysis (machine_learning.py) and visualization tools (graph.py). Example configuration files and test images are provided for reproducibility.; (iv) program_tai1_HSV_main(.../program_tai1_HSV_main/tree/v1.0.5), for HSV colour space analysis of segmented fish body images. The workflow includes computation of hue, saturation, and value metrics, statistical analysis (program2h_statistics.py, program2s_statistics.py, program2v_statistics.py), and profile comparisons using post hoc tests. This analysis is typically performed after preprocessing and segmentation using program_tai1_Texture_main.; (v) cv2_jpg_preprocessing (https://github.com/kecosz/cv2_jpg_preprocessing), for JPEG image preprocessing, particularly Gaussian filtering for noise reduction and image smoothing. The core script is implemented as a Google Colab notebook (jpg_cv2_gaussianfilter.ipynb), with example JPEG images included for testing. This tool can be used as an optional preprocessing step before feature extraction. All versions of the code used in this study have been archived on Zenodo under the following DOIs: 10.5281/zenodo.15080985; 10.5281/zenodo.15081665; 10.5281/zenodo.15828298; 10.5281/zenodo.15081661; 10.5281/zenodo.15081995. Detailed installation instructions, software dependencies, and step-by-step guidelines for executing each part of the analysis pipeline are provided in README.md or Setup Instruction.txt files in each repository. In addition, the photos used for the GitHub commands, a raw data file, and an optional command file were stored on the figshare site and registered (10.6084/m9.figshare.28646039).

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-life.2025.100155>

LEAD CONTACT WEBSITE

<https://www.yokohama.riken.jp/ies/index.html>
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