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Assessing ecological impacts of agricultural practices using frogs as bioindicators

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Abstract. *Kurnianto AS, Kusumawati A, Widjayanthi L, Wulanjari D, Ibanah I, Majid MAA, Puspitasari DA, Priambodo B. 2024. Assessing ecological impacts of agricultural practices using frogs as bioindicators. Biodiversitas 25: 3208-3215.* The rapid intensification of agriculture, driven by the increasing global demand for food, heavily relies on chemical inputs like pesticides and synthetic fertilizers. While these practices enhance production, they pose significant challenges to ecological balance and environmental health. Biomonitoring efforts have been developed to detect and evaluate the impact of chemical pollutants on the environment, employing species like the rice field frog (*Fejervarya limnocharis* Gravenhorst 1829) as bioindicators. This study aimed to assess the reliability of rice field frogs' liver gravimetry and morphology as biomonitoring tools using various analytical methods. Samples were collected from rice fields managed both organically and conventionally to analyze liver weight, individual weight, and Snout-Vent Length (SVL). This analysis was conducted to understand the environmental effects on these physiological parameters. Statistical analysis revealed significant differences between the two management practices, indicating environmental conditions notably influence the frogs' physiological parameters, highlighting their potential as bioindicators for chemical pollutants. The findings underscore the need for further research to optimize biomonitoring methods for sustaining environmental health and agricultural practices. This study contributes to the development of more sophisticated and effective biomonitoring methods, supporting sustainable agricultural practices and environmental mitigation strategies.

Keywords: Agricultural sustainability, biomonitoring methods, chemical pollutant, *Fejervarya limnocharis,* liver gravimetry

Abbreviations: C: Conventional management; O: Organic management; RFF: Rice field frog; SVL: Snout-vent Length; VES: Visual encounter survey

INTRODUCTION

As the global population continues to surge, reaching close to 8 billion people in 2022 (World Bank Group 2024), the ever-increasing demand for food has necessitated more intensive agricultural practices. This growth in food demand propels farmers to maximize crop yields, often through the use of chemicals such as pesticides and synthetic fertilizers. These substances, while effective at boosting production, pose severe challenges to environmental health and the delicate balance of ecosystems. The reliance on such chemicals is a critical element in meeting the world's escalating food requirements; however, it simultaneously triggers significant environmental concerns due to pollution (Lu et al. 2015). This pollution can detrimentally affect local ecosystems, human health, and the long-term sustainability of farming practices. The adverse environmental impacts associated with chemical use in agriculture have been well-documented (Pretty and Bharucha 2014; Tittonell 2014; Nicolopoulou-Stamati et al. 2016; Garbach et al. 2017; Mie et al. 2017). Such impacts underscore the urgency for innovative and effective monitoring and mitigation strategies. In response to these challenges, scientists have adopted biomonitoring techniques, utilizing living organisms to assess and monitor the effects of these chemicals on ecosystems. This approach has been applied in various contexts, including the use of rice snails (*Pomacea canaliculata* Lamarck 1822), different types of aquatic insects, and fish to detect the presence of pesticide contamination in aquatic environments (Chiu et al. 2014; Campoy-Diaz et al. 2018; Ernst et al. 2018; Kurnianto et al. 2021).

Biomonitoring offers a dynamic tool for real-time tracking of pollutants and plays a crucial role in efforts aimed at restoring and protecting the environment. Nonetheless, its effectiveness can vary significantly depending on the ecosystem in question and the specific organisms involved. This variability highlights the ongoing need for research to refine and improve biomonitoring methods (Hamza-Chaffai 2014; Schmutz and Sendzimir 2018). Amid the search for more robust environmental monitoring tools, recent research has focused on the potential of using amphibians, specifically frogs, as indicators of environmental health. The Rice Field Frog (*Fejervarya limnocharis* Gravenhorst 1829, RFF), in particular, has attracted attention due to its sensitivity to pollutants, making it a potentially effective bioindicator for agricultural land health. Researchers have been examining various physical and biological parameters of these frogs, especially liver functions, to gauge pollution levels (Baharuddin et al. 2015; Othman et al. 2016; Saputro et al. 2019). The goal of this research is to evaluate and enhance the precision of using the Rice Field Frog in biomonitoring practices.

This study involves the use of statistical methods to verify the reliability of these frogs in signaling the presence of contaminants in agricultural settings. By improving the accuracy with which pollution is detected, the research supports the development of more sustainable farming methods that are less harmful to the environment. The implications of this study are significant not only for enhancing agricultural sustainability but also for advancing broader environmental conservation efforts. Moreover, by establishing more effective tools for environmental monitoring, the research seeks to reconcile the necessity to boost food production with the imperative to safeguard our natural resources. Ensuring a healthy environment for future generations is paramount, and through such innovative research, we can move closer to achieving a balance between these two critical needs. Overall, as the need for sustainable agricultural practices becomes more pressing in the face of global challenges such as population growth and environmental degradation, research like this is crucial. It not only addresses the immediate impacts of pollution on agricultural productivity and ecosystem health but also contributes to the global discourse on sustainable development. Through continuous improvements in biomonitoring techniques and the integration of new bioindicators such as the Rice Field Frog, we can better manage the environmental impacts of agriculture and foster a more sustainable future for all.

MATERIALS AND METHODS

Study area

RFF sampling was conducted in two locations with different management models, namely in organic rice fields in Rowosari Village, Sumberjambe Sub-district, and conventional rice fields in Pontang Village, Ambulu Sub-district, Jember District, East Java, Indonesia. These two locations with different management practices have the potential to impact frogs differently, particularly because conventional management in Pontang will result in higher agrochemical contamination (see Figure 1, and Table 1). The organic rice farm in Rowosari has been certified by the Seloliman Organic Certification Body (LeSos) with the number: 379-LSPr-092-IDN-09-20. It has been managed by farmers since around 2018. The frog search area covers 1000 m^2 based on the Visual Encounter Survey (VES) method. Observations, dissections, and measurements of RFF anatomy and morphology parameters were conducted in the Laboratory of Agrotechnology, Faculty of Agriculture, Universitas Jember, East Java, Indonesia. The research was carried out from January to February 2024.

Procedures

RFF samples were collected through exploratory VES with a distance of 100 meters and a search area width of 2 meters on both the left and right sides of the observer, then repeated until the total search area reached 1000 m^2. The VES process was supported by a 1.2-meter hook and a headlamp. Deadwood, rocks, and debris were lifted to collect RFF. The VES process was conducted from 6-8 pm, regardless of weather conditions. The VES process was carried out for 3 days for each management type Wassens et al. (2017). We collected 20 individuals from each location. The reasons for limiting the samples are twofold: first, to ensure sufficient data for statistical analysis and to maintain equivalent sampling from both locations; second, to minimize the capture of these predator animals due to their important ecological role.

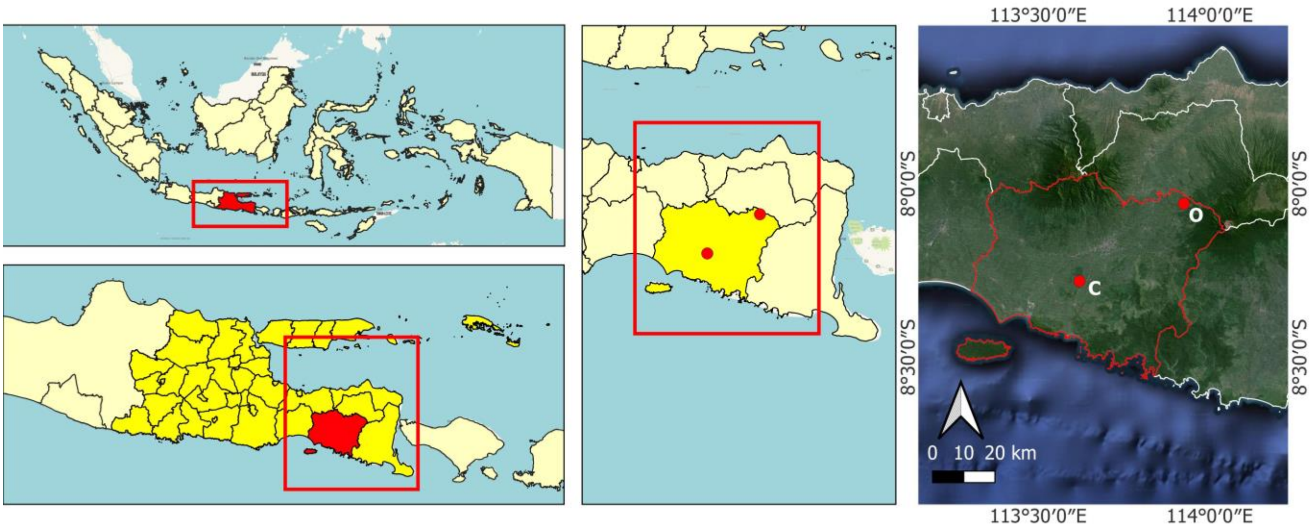

Figure 1. Sampling locations in Jember District, East Java Province, and their positions relative to Indonesia. Notes: O: Organic management; and C: Conventional management

Table 1. Rice field treatments with agrochemical and organic inputs

Management	Fertilizer		Pesticide		Fungicide	
	Brand	Application	Brand/active compound	Application	Brand/active compound	Application
Conventional (C)	Phonska ©	16.66 kg/ha; every 2 weeks (week-5 to week -9)	Fenite© - Emamectin benzoate	200 mL/ season (June- August)	Zole© - difenoconazole	200 mL/ season (June -August)
Organic (O)	-	Organic or manure 222.22 kg/ha; every 2 weeks (week-3 to week-9)	Neem based natural pesticide / azadirachtin compound	1 L/ season (June- August)	-	-

Each RFF sample was documented, collected by hand, placed in a 5 kg breathable plastic bag with local vegetation, and labeled. The collection of plastic sample bags was placed in a large cloth bag for mobilization. All RFF samples were then cleaned and euthanized on the same night by injecting 2 mL of 70% alcohol into the left thigh of each individual and then weighed using an electronic balance to determine individual weight parameters. All procedures involving frog surgeries were conducted under the animal welfare standards outlined in the Animal Welfare Act (https://www.nal.usda.gov/animal-health-and-welfare/animal-welfare-act-quick-reference-guides), using appropriate anesthesia methods to ensure that the animals did not experience pain or stress. Potential risks have been assessed and minimized, and emergency procedures have been established to handle unforeseen events. The samples were then dissected, and their liver was removed. The liver was cleaned of blood, fat, or glands, and then weighed. RFF samples were then measured for Snout-Vent Length (SVL) using a caliper. All measurement results were recorded based on individual labels and locations. Liver samples were then placed in 1.5 mL tubes containing 70% alcohol. The tubes were then tied to the right thigh, and labels were attached to the left thigh of the RFF sample, which was then placed in a 25-liter plastic container containing 10 liters of 70% alcohol, and then tightly closed.

Data analysis

Several parameters were obtained: Liver Weight, Individual Weight, and SVL (Snout-Vent Length). Then, proportional parameters were calculated: Liver/Individual Weight, Liver Weight/SVL, and Individual Weight/SVL. The data were then analyzed statistically using several general and ecological analysis approaches. Statistical tests began with the Shapiro-Wilk normality test, followed by the non-parametric Mann-Whitney U test, and then compared with the non-parametric Kruskal-Wallis test for group differences. To distinguish the largest parameter, descriptive analysis was conducted on the median data. Each parameter was then visualized using various graphs. Violin plots were created to provide an overview of the distribution range comparison among parameters. Principal Component Analysis (PCA) graphs with Kernel Density Estimate (KDE) figures were created to illustrate parameter preferences for the two management types. In our study, the Principal Component Analysis (PCA) included the following variables: Liver Weight, Individual Weight, Snout-Vent Length (SVL), Liver/Individual Weight, Liver Weight/SVL, and Individual Weight/SVL. These variables were selected to capture different aspects of the frogs' morphology and physiology to identify patterns and relationships across the two locations.

Differential analysis was also performed using ecological indices: Bray-Curtis dissimilarity and Jaccard similarity indices, by inputting data from gravimetric and morphometric measurements. These two indices are widely used to show the relatedness between locations with differences in sample characteristics (Podani et al. 2018; Risely et al. 2021). The results of the analysis were then also visualized with Principal Component Analysis (PCA) graphs with Kernel Density Estimate (KDE) figures. All statistical analyses were conducted using Matplotlib with the Seaborn library.

RESULTS AND DISCUSSION

Morphological and morphometric characteristics, as well as the impact of management differences

The summary table of statistics shows the results of statistical tests on data samples of RFF collected from two different types of rice fields: Rowosari, which is an organic field, and Pontang, which is a conventional field. These tests include the Shapiro-Wilk normality test, Mann-Whitney U test, and Kruskal-Wallis test, each assessing the data distribution and differences between the two locations based on various physiological frog parameters (see Table 2). From the Shapiro-Wilk test results, it was shown that the data distribution for Liver Weight, Liver Weight/Individual Weight, and Liver Weight/SVL did not follow a normal distribution (p-value>0.05). Meanwhile, the Individual Weight, SVL, and Individual Weight/SVL parameters showed a normal distribution (p-value<0.05). Furthermore, the Mann-Whitney U and Kruskal-Wallis test results indicated significant differences between the two locations for most parameters. All parameters showed significant differences except for the Individual Weight/SVL parameter (p-value=0.15 and 0.16, respectively).

More clearly, we can observe the differences between the two locations based on violin plot representations. Violin plots are used to visualize the weight, distribution, and range of data. We separate the analysis results into two violin plots to cover two calculation outcomes: direct measurements and ratios. In the first plot (Figure 2), which includes the parameters 'Individual Weight', 'SVL', and 'Individual Weight / SVL', there is a clear difference in the distribution of values between the Rowosari (red) and Pontang (blue) locations. This difference indicates significant morphological variations between the two populations. Especially, 'Individual Weight / SVL' shows quite a large variation, indicating differences in body proportions or health conditions between the two locations. Generally, the proportion values provide a specific overview compared to the comparison between individual weight or SVL measurements independently (see Figures 2 and 3). Next, we can observe the vertical data spread containing weight and liver weight values on the y-axis, which is larger in Pontang compared to Rowosari. This confirms that there is a potential influence between liver weight, which also affects individual weight, but not SVL.

The next graph depicts 'Liver Weight', 'Liver/Individual Weight', and 'Liver Weight/SVL'. Similar to the first graph, there is a striking difference between Rowosari and Pontang, especially in terms of 'Liver/Individual Weight' and 'Liver Weight/SVL'. This can indicate differences in physiology or health conditions related to liver function between these two populations. This visualization provides valuable insights into how environmental conditions and other ecological factors may influence the morphological and physiological characteristics of species in two different locations.

The statistical result indicates that there is no influence between individual weight and SVL. It suggests that the environmental conditions in the organic field of Rowosari and the conventional field of Pontang affect the physiological parameters of RFF differently, impacting liver swelling (Felix-Nascimento et al. 2024), including its ideal proportion to individual weight and SVL. Liver weight in the Pontang location increased by 6% on average compared to liver weight obtained from Rowosari. Conventional agriculture has provided tremendous agrochemical inputs to the ecosystem. This includes chemical fertilizers and pesticides. Conventional agriculture always refers to the excessive and improper use of fertilizers and pesticides (Pimentel and Peshin 2015). Domino effects occur because the residues of substances not absorbed by plants will be absorbed into the soil and flow toward water bodies, becoming sources of pollution (Evans et al. 2019). This has an extraordinary degradative impact on macrofauna such as frogs, which are highly dependent on water for their regeneration phase (Othman et al. 2016; Saputro et al. 2019), where chemical pollutants cause embryonic abnormalities (Strong et al. 2017), defects in the adult phase (Şişman et al. 2021), as well as changes in behavior and community structure in an ecosystem (Sievers et al. 2019).

Table 2. The differences between 6 morphologicals parameters

Parameter	Normality Test (p-value)	Normality Test Inter-pretation	Mann-Whitney U Test (p-value)	Mann-Whitney U Test Inter-pretation	Kruskal-Wallis Test (p-value)	Kruskal-Wallis Test Inter-pretation	Median Rowosari	Median Pontang
Liver weight	0.000005	Not Normal	0.005	Significant	0.29	Significant	0.04	0.07
Individual weight	0.07	Normal	0.04	Significant	0.02	Significant	3.59	3.83
SVL	0.40	Normal	0.0007	Significant	0.0007	Significant	3.64	3.98
Liver /individual weight	0.01	Not Normal	0.03	Significant	0.03	Significant	0.01	0.02
Liver weight / SVL	0.001	Not Normal	0.02	Significant	0.02	Significant	0.01	0.02
Individual weight / SVL	0.50	Normal	0.15	Not Significant	0.16	Not Significant	0.94	0.97

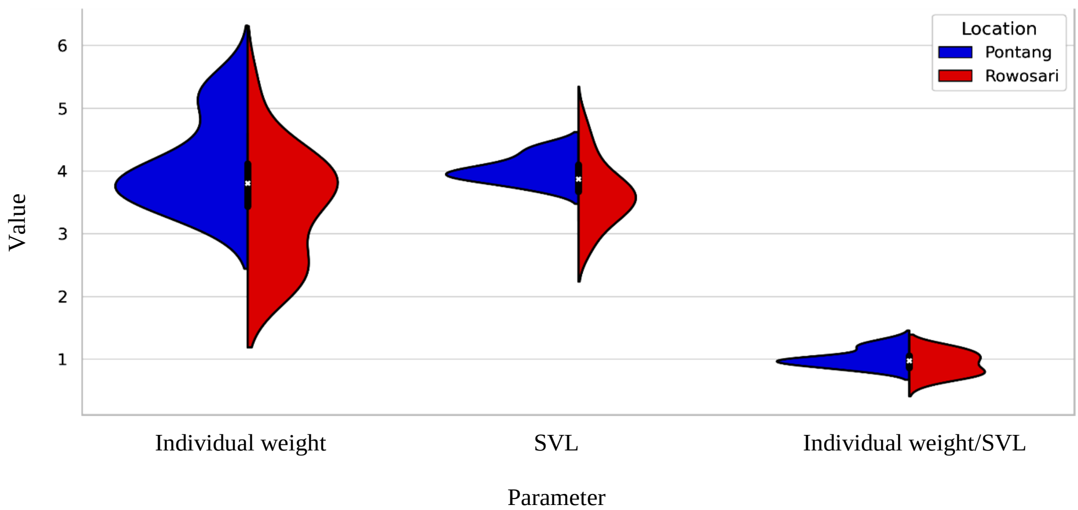

Figure 2. Violin plots for individual weight, SVL, and the ratio of individual weight/SVL

Among the compounds used in conventional rice fields, Fenite© (emamectin benzoate) is likely the most harmful to *Fejervarya* sp. Emamectin benzoate is an insecticide known for its potential toxicity to aquatic organisms, including amphibians. Its usage has also been studied for its impact on other biota, including the natural cycle of the American lobster, *Homarus americanus* H.Milne Edwards 1837 (Waddy et al. 2010).

The liver is an organ capable of neutralizing toxins and pollutants in the body. The more absorption of pollutants, the higher the liver's performance, resulting in swelling due to faster regeneration compared to other organs (Gaber et al. 2015). This can illustrate that RFF is able to accumulate pollutants and express them morphologically and gravimetrically (Saputro et al. 2019). The fact of this research shows that chemical pollution provides a range of anomalies in the liver. Emamectin, as the impact of the active ingredient that has been published, is stated not lethal to water vertebrates or other biota (Bronzwaer et al. 2019; Xiao et al. 2021). However, pesticide producers, as well as other agrochemicals, have not provided a deep insight into the regeneration process and other impacts on gravimetric and physiological parameters. This is mostly studied through separate experimental studies, which demonstrate the dangers of agrochemical accumulation on biota (Temiz 2020; Khan et al. 2021).

The summary table of statistics shows significant differences in most physiological parameters of RFF between organic and conventional rice fields. Liver Weight, Liver Weight/Individual Weight, and Liver Weight/SVL did not follow a normal distribution, while Individual Weight, SVL, and Individual Weight/SVL did. Violin plots illustrate these differences, with larger liver weight in Pontang indicating the impact of agrochemical pollutants. Emamectin benzoate from conventional fields is particularly harmful to frogs, affecting liver swelling and other physiological aspects, emphasizing the need for better agrochemical management to protect aquatic organisms.

Preference towards location

The influence of variables on separation is shown with the Biplot (Figure 4). Liver weight and individual weight are parameters that contribute to the main components in both locations. In the graph, we see that Pontang samples are mostly concentrated due to liver weight, both measured individually and compared with other parameters. There is valuable insight into how samples from the organic fields of Rowosari differ from the conventional fields of Pontang from several perspectives of the measured biological characteristics. Furthermore, we can understand the liver weight and individual weight that most contribute parameters to these differences. This is important to evaluate that the biological characteristics of RFF can serve as a representation of contamination, where liver swelling occurs, as well as an increase in total individual weight, compared to frogs obtained from Rowosari.

RFF demonstrates its quality as a bioindicator. These frogs are capable of accumulating and expressing the impacts of contamination inputs, as seen in this research. The advantage of a bioindicator is to provide a broad range of absorption and contamination readings. Additionally, it provides a comprehensive picture of all life phases, as it lives in a contaminated environment. The use of adult RFF frogs provides advantages, such as the ease of liver extraction and individual weighing, as well as more visible accumulation of contaminants in the morphology of adult frogs. RFF can indicate the presence of environmental contaminants such as pesticides, heavy metals, and industrial pollutants. These frogs also reflect the accumulation of pollutants like nitrates and phosphates from agricultural runoff, offering insight into the overall health of the ecosystem (Slaby et al. 2019). However, as seen in this research, only a representation of the presence or absence of contamination at a location is provided. This is certainly beneficial for activities requiring sensitive resource monitoring systems, such as the use of water sources for drinking water or the consumption of raw materials (Karbasdehi et al. 2016; Blaise et al. 2017).

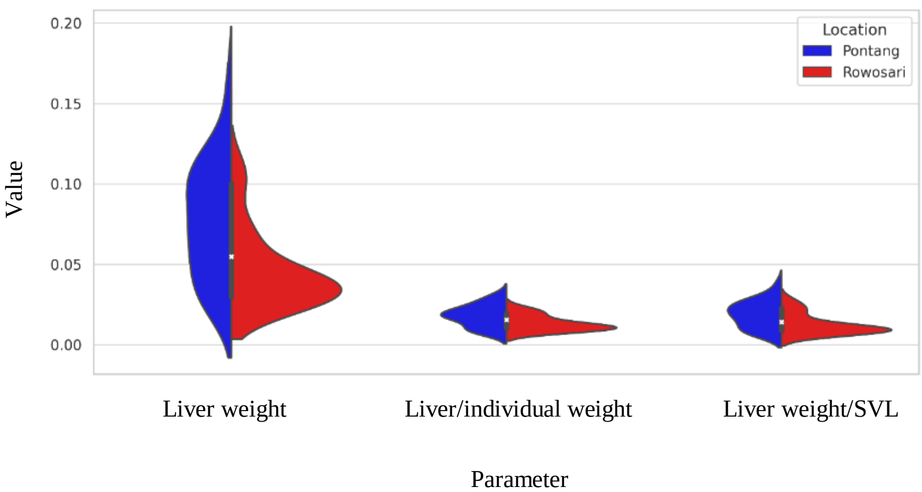

Figure 3. Violin plots for liver weight, ratio of liver/individual weight, and liver weight/SVL

The study found that liver weight is a key factor in differentiating samples, with heavier liver samples predominantly found in Pontang, likely due to frogs absorbing contaminants, resulting in increased liver activity. This underscores the effectiveness of RFF frogs as bioindicators. The use of adult RFF frogs is advantageous for easy liver extraction and clear visibility of contaminant accumulation.

Differences based on ecological standards

The Bray-Curtis Index (IBC) indicates that both locations have a value of 0.5061. This value represents the average dissimilarity among samples in the dataset. In the context of Bray-Curtis, values closer to 0 indicate greater differences between samples, while values closer to 1 indicate greater similarity. The PCA graph based on Bray-Curtis dissimilarity depicts the morphological variation between samples from the two locations, Rowosari and Pontang, in a two-dimensional space (Figure 5). From this visualization, it is apparent that samples from Rowosari (red) and Pontang (blue) have different distributions, indicating significant morphological variations between the two locations.

The KDE areas show higher sample densities in PCA space, with some overlap between the two locations, indicating certain morphological similarities despite dominant differences. These results may indicate the influence of the environment or other external factors on the morphological characteristics of species in both locations. Environmental factors such as soil type, water quality, and availability of food resources can lead to variations in morphology. Additionally, differences in climate and habitat structure might also play a significant role in shaping these characteristics. Furthermore, the difference in management practices, with Pontang using conventional methods and Rowosari employing organic farming, likely contributes to the observed morphological differences by affecting. The results depicted by IBC are quite interesting, where ecologically, the measurements of morphological and gravimetric aspects in frogs show neutral differences. This suggests that the criteria for these differences are not well captured by an index (Ricotta and Podani 2017). Additionally, more complex sampling points are needed to demonstrate fundamental differences between the two locations (Hardersen and La Porta 2023).

The Jaccard similarity index for the dataset is approximately 0.0515. This value indicates that, on average, the similarity between samples in the dataset is at a relatively low level. In the context of morphology, this suggests that there are significant differences between samples from the Rowosari and Pontang locations. These differences are reflected in the PCA visualization based on Jaccard similarity, where the distribution of samples from both locations shows clear separation patterns, indicating significant morphological variation among species in the two areas (see Figure 6). The dataset index can confirm the results of the IBC calculations, which indicate the difficulty of representing the differences between the two locations due to the variation between the two locations in the combination of morphology and gravimetric parameters.

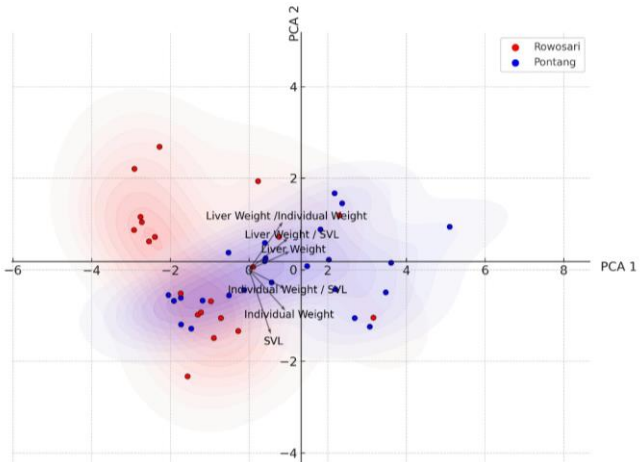

Figure 4. The PCA graph of 2 locations. Note: Red: Pontang; Blue: Rowosari

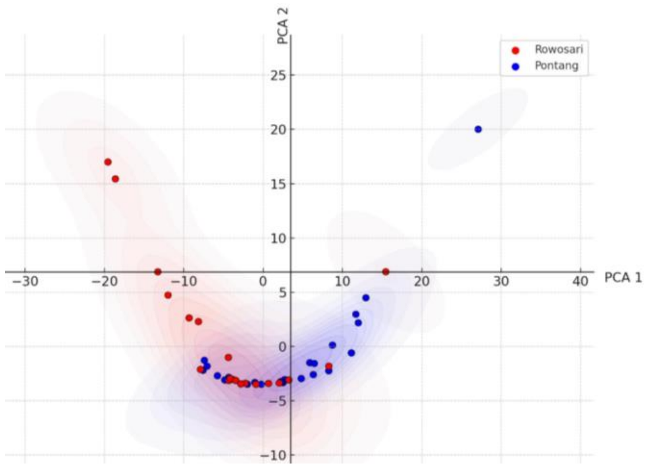

Figure 5. The PCA graph IBC between of 2 locations. Note: Red: Pontang; Blue: Rowosari

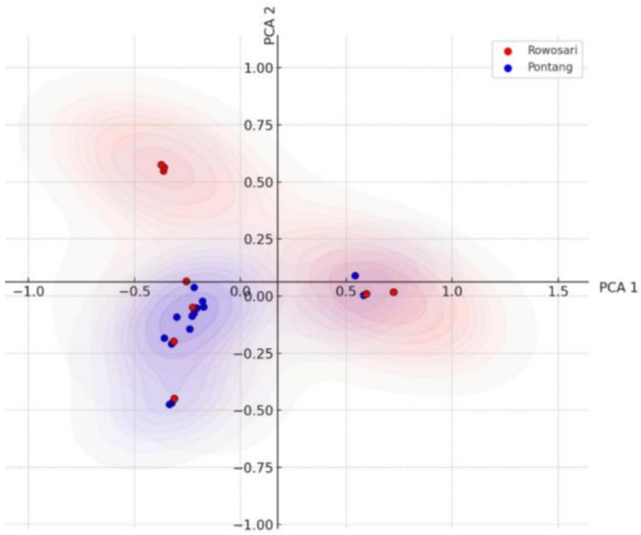

Figure 6. The PCA graph Jaccard between of 2 locations. Note: Red: Pontang; Blue: Rowosari

The study found that liver weight is a key factor in differentiating samples, with heavier liver samples predominantly found in Pontang, likely due to frogs absorbing contaminants and their livers working harder. This is due to frogs absorbing contaminants, causing their livers to work harder, clearly indicating that higher contamination levels are prevalent in Pontang. Furthermore, this study compared the use of ecological indices such as Bray-Curtis and Jaccard indices to evaluate the similarity and differences in morphology among samples from different locations. The Bray-Curtis index measures dissimilarity between samples based on species composition, while the Jaccard index focuses on the similarity of species presence. The Jaccard index enriches the interpretation of data by providing an ecological perspective on the variability of observed bioindicators, affirming that farming practices impact the health and biological diversity of freshwater ecosystems, whereas the Bray-Curtis Index does not depict differences or similarities from the comparison of parameters used.

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