sustainability

Article

Proximate Composition, Predictive Analysis and Allometric Relationships, of the Edible Water Frog (*Pelophylax epeiroticus*) in Lake Pamvotida (Northwest Greece)

Marianthi Hatziioannou, Efkarpia Kougiagka, Ioannis Karapanagiotidis and Dimitris Klaoudatos *

Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Fytokou Street, 384 45 Volos, Greece; mxatzi@uth.gr (M.H.); ekougiagka@uth.gr (E.K.); ikarapan@uth.gr (I.K.)
* Correspondence: dklaoud@uth.gr

Abstract: The edible water frog *Pelophylax epeiroticus*, distributed mainly in Northwest Greece and utilized commercially as food, was investigated in lake Pamvotida (Ioannina). The objective was to assess aspects of population structure (sex ratio, morphometric characteristics, allometric relationships) and proximate composition of the Epirus water frog (*Pelophylax epeiroticus*). Commercial samples (31 females and 54 males) were obtained and sex ratio, morphometric characteristics, allometric relationships and proximate composition were assessed. A significantly lower abundance of females was indicated (31 females and 54 males). Body length range was higher in females (females 3.4 mm, males 2.6 mm), whereas total weight range was higher in males (females 45.08 gr, males 48.35 gr). Differences in allometric relationships were indicated between sexes. The high protein (15.93 ± 3.32) and low lipid (0.25 ± 0.13) contents indicated that *P. epeiroticus* is an excellent food source of high nutritional value. A tree classification algorithm indicated that the principal contributing component for sex classification was dry matter, followed by a proportion of edible flesh and protein content. A predicted future increase in demand for wild-caught individuals requires the use of a suitable management plan, coupled with the development of farming practices aiming to assure the sustainable exploitation of this important resource and alleviate the pressure on its populations.

Keywords: *Pelophylax epeiroticus*; water frog; lake Pamvotida; allometric relationships; proximate composition; predictive analysis

check for updates

Citation: Hatziioannou, M.; Kougiagka, E.; Karapanagiotidis, I.; Klaoudatos, D. Proximate Composition, Predictive Analysis and Allometric Relationships, of the Edible Water Frog (*Pelophylax epeiroticus*) in Lake Pamvotida (Northwest Greece). *Sustainability* **2022**, *14*, 3150. https://doi.org/10.3390/su14063150

Academic Editor: Tim Gray

Received: 12 February 2022
Accepted: 6 March 2022
Published: 8 March 2022

1. Introduction

Pelophylax epeiroticus is an edible water frog species native to the Ionian zone [1], distributed mainly in the western part of Greece along the Ionian coast, including the island of Kerkyra [2]. The species is also present in Albania; however, the main part of its range is within Greece [3]. The western Palaearctic water frog of the genus *Pelophylax* includes several distinct species and has been investigated in several phylogenetic, morphological, and ecological studies [4–10]. The populations of *P. epeiroticus* (Epirus Water Frog) are suspected to be decreasing due to ongoing decline in the extent and quality of its habitat, thus improved management measures are required for the protected areas and Natura 2000 sites where the species appears [3]. Subpopulations are fragmented due to extensive areas of unsuitable habitat within its range. The species occurs in many protected areas within its range and is listed in Appendix III of the Berne Convention. The main threats for the species are general loss of wetland habitat, water pollution, climate change, dam construction and hydro-power developments [3]. *P. epeiroticus* is utilized commercially as food; however, consumption is not currently considered a major issue for its sustainability since it is consumed in low numbers. Furthermore, there is no evidence that the species is collected excessively in Albania, despite the 2008 assessment that reported an established fishery harvesting measurable quantities [3], and Greece [11].

The amphibian fauna of Greece includes 22 species out of a total of 64 European species, a significant number given the small size of Greece [2]. Perhaps the most important threat that amphibians face in Greece is the degradation and loss-destruction of their habitats and breeding sites, which occurs either because of natural change (increase in temperature, decrease in precipitation, drought) or as a result of anthropogenic activities (inland water agricultural and industrial pollution, livestock activities, development of residential and tourist infrastructure, water overdraft, recreational activities) [2,3,12]. Aquatic frogs are an important part of biota in the natural wetlands, playing an essential role in the food chain, functioning both as predators (adults feed mainly on invertebrates: insects and arthropods) and prey (they can serve as food for both aquatic and terrestrial predators). Adult anurans have highly permeable skin [13], making them sensitive to various environmental stressors (including toxins of anthropogenic origin). The latest research has indicated that human industrial activity (e.g., mining, agriculture) is amongst the main reasons for the decline of amphibian anuran populations [13–20].

Large-bodied frogs are under additional pressure by the national and international demand for their meat [21–24]. In some cultures—notably Asian, Greek and Roman—frog meat has been considered a delicacy for centuries [25]. Consumption and trade of frogs are of great interest in Europe and the USA, with the largest importers of frog legs being France, USA, Belgium and Luxembourg, whereas the largest exporters are Indonesia, China, Belgium and Luxembourg [26–29]. European frog populations, particularly of the European green frog complex (*Pelophylax* spp.) were heavily exploited, especially in France (with 40–70 tonnes of frogs captured per year), followed by Belgium and the Netherlands [30].

The present study is the first that describes the proximate composition of wild-caught *P. epeiroticus*. Frog leg meat is considered a traditional delicacy in Western Greece and of great gastronomic interest worldwide, offering an excellent source of protein [31,32]. Frog meat has the potential to attract the attention of food technologists and dieticians as a new healthy food source based on its excellent nutritional composition. European countries tend to prefer species of the genus *Pelophylax* that are small in weight (i.e., 50–60 g) [33]. Proximate frog leg composition of wild and cultured species (*P. ridibundus, P. esculentus, Lithobates catesbeianus* and *Holobatrachus rugulosus*) is characterized by low lipid content [34–37]. According to [22], in 2018 the global production of frogs was 107.3 thousand tonnes. Frog farming is not sufficiently developed in Greece [38], resulting in the increased fishing pressure on wild populations, threatening their sustainability.

The aim of this study was to contribute to the literature of the Epirus water frog *Pelophylax epeiroticus* in Lake Pamvotida (Ioannina) on aspects of population structure through assessing sex ratio, morphometric characteristics and allometric relationships. Furthermore, we aimed to highlight its nutritional value by comparing its proximate composition with other popular freshwater commercial species. Predictive analysis on proximate composition was further used to identify principal contributing components that could classify the opposite sexes of the species.

2. Materials and Methods

In total, 85 water frogs *P. epeiroticus* were sampled in 2016 from lake Pamvotida (Figure 1) monthly between March and May (24, 30, 31 individuals in March, April, and May accordingly). Adult water frogs were captured by net or hand and were directly transported to the Laboratory of Aquaculture (using plastic 50 lt containers containing a small volume of freshwater and plant material from the lake) in the Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, where they were kept for one day in freshwater aquariums (40 × 20 × 20 cm).

Figure 1. Location and view of Lake Pamvotida (sampling location is indicated by an arrow).

2.1. Study Area

Pamvotida is the largest lake of Epirus, located in the northwestern part of Greece in the Ioannina regional unit, with mount Mitsikeli located at its northwestern edge. Lake Ioannina (also known as lake Pamvotida) is a shallow lake with an average depth of 4.5 m and a polymictic stratification regime, situated at 470 m elevation, occupying an area of 22 km^2 [39]. The local climate consists of cold wet winters with daily averages <0 °C and hot dry summers with temperatures in excess of 30 °C. The location of Ioannina within the Pindus mountains and the proximity of the Ionian Sea result in high levels of precipitation, with annual means of 1100–1200 mm recorded in the proximity of the lake. Annual mean evaporation and precipitation are approximately equal [40].

The lake was formed by water concentration and is fed by the mountain Mitsikeli springs, with no physical surface outlet. The outflow of the water is carried through the Lapsista's ditch and is diverted to the Kalamas river. It is a sensitive ecosystem, which belongs to the European Network of Protected Areas, NATURA 2000 because of the important habitats and the rare species of flora and fauna [41]. Lakes in the Mediterranean climate zone experience high variation in rainfall and are vulnerable to changes in climate, land cover and anthropogenically induced effects on water level and salinity [42].

A major city (Ioannina, pop. 100,000) lies along its western shoreline. A decrease in the external load of nutrients and pollutants occurred in 1990 with the removal of the Ioannina sewage outfall from the lake along with industrial discharges on the southern shoreline. The lake has undergone cultural eutrophication over the past 40 years and is currently eutrophic. Fish stocking has occurred annually in the lake since 1986 to promote commercial fishery [40]. Sampling was conducted in the proximity of the village Amphithea (latitude 39.6832448, longitude 20.8697307) in the north part of the lake next to the shore.

2.2. Morphometry

Morphometric characteristics were recorded for all 85 individuals caught (31 females and 54 males): total body weight (TW) with a digital scale (accurate to 0.1 g) and body length (BL), femur length (FL), tibia length (TL), maximum head length (MHL) from the tip of the snout to the posterior margin of the tympanum, maximum head width (MHW) the largest pre-tympanum head width [43] and snout eye distance (SED) from the tip of the head to the posterior corner of the eye, with a digital caliper (accurate to 0.01 mm) (Figure 2).

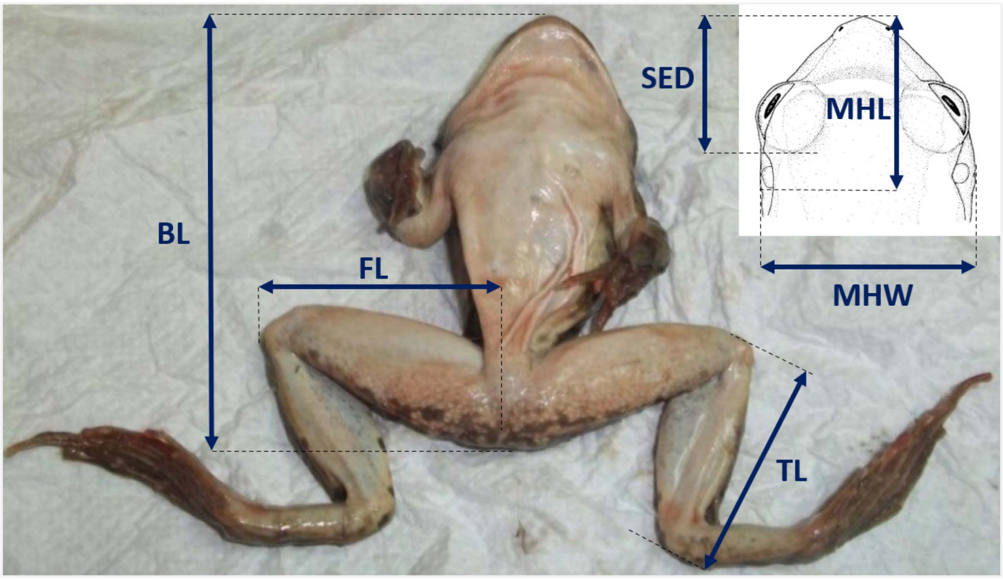

Figure 2. Morphological traits measured (MHL = maximum head length, MHW = maximum head width, BL = body length, FL = femur length, TL = tibia length, SED = snout eye distance).

2.3. Proximate Composition

A total of 27 individuals (31 females and 54 males) were analyzed for the estimation of edible parts and proximate composition. All animals were euthanized by dipping in 0.5% MS-222 solution. The removal of the skin and clipping of the feet were carried out immediately after. The hind legs from each frog were removed by full extension and severed from the body by an incision close to the waist. The total weight of hinds was assessed immediately after anatomy. Using body weight (BW) and net weight of frog leg hinds (LW), the edible part (EP) (%) was assessed according to the equation:

$$EP = (LW/BW) \times 100 \quad (1)$$

Moisture, protein (as total nitrogen content) and lipid (as total lipid content) and ash content of frog leg meat were determined in line with the procedures of the Association of Official Analytical Chemists in triplicate [44]. Samples were placed for 24 h at 105 °C to determine moisture content. Crude protein and crude fat were assessed by Kjeldahl (N × 6.25; Behr Labor-Technik, Dusseldorf, Germany) and exhaustive Soxhlet extraction with petroleum ether (40–60 °C) using a Soxtherm Multistat/SX PC (Sox-416 Macro, Gerhardt, Germany) respectively, whereas a water-free sample was combusted in a muffle furnace (Nabertherm L9/12/C6, Lilienthal, Germany) by heating at 600 °C for 3 h and the ash content was measured gravimetrically [44]. The proximate composition was expressed as a percentage of dry matter and as a percentage of wet weight.

2.4. Statistical Analysis

Data for statistical analysis were evaluated for normal distribution by employing the Shapiro–Wilk test for normality and homogeneity of variance by employing Levene's test.

Student's t-test was employed for comparisons of body length and total weight among sexes since data were approximately normally distributed and homogenous. Welch's analysis of variance (ANOVA) was employed for monthly comparisons when data were normally distributed but violated the assumption of homogeneity of variance [45]. Mood's median test was used for comparisons where data failed both assumptions of normality and homogeneity [46]. Chi-Square goodness-of-fit and Chi-Square test for association were employed for the comparison of categorical values. Statistical analysis was performed with Jamovi Software (2.2.5) [47]. Nonlinear regression was performed with Minitab software (Minitab, PA, USA) between body length, total weight, femur length, tibia length, maximum head length, maximum head width and snout eye distance. Proximate composition of *P. epeiroticus* was compared with frog and fish proximate composition values from the literature, using one sample *t*-test. A tree algorithm with forward pruning was used to compute the contribution of proximate composition to the phyletic identification. Before classification, data preprocessing techniques were applied to improve the efficiency of the algorithm and correctly classify the data [48]. A prediction was inferred by measuring the increase in the prediction error of the model after permuting the feature's values, using Orange software (3.30.2) [49].

3. Results

3.1. Sex Ratio

The Chi-Square goodness-of-fit test (Table 1) indicated a significant difference between observed (31) and expected (42.5) male and female (observed 54 and expected 42.5) abundance, with a significantly lower abundance of females and higher in males than expected.

Table 1. Chi-Square goodness-of-fit test for sex ratio.

Sex		Number of Individuals	Proportion	χ^2 Goodness of Fit		
Female	Observed	31.0	0.36	χ^2	df	*p*
	Expected	42.5	0.50	6.22	1	<0.05
Male	Observed	54.0	0.64			
	Expected	42.5	0.50			

The Chi-Square test for association indicated no significant difference in the sex ratio between different sampling periods ($p > 0.05$).

3.2. Population Structure

No significant difference was indicated in body length and total weight between sexes (*t*-test, $p > 0.05$). No significant difference was indicated in body length between sampling periods (Mood's Median test, $p > 0.05$); however, a significant difference was indicated in total weight between sampling periods with a significantly higher total weight for individuals sampled in April (Mood's Median test, $p < 0.05$).

Range of body length among sexes (Figure 3) was higher for females (3.4 mm) compared to males (2.6 mm), whereas the opposite trend was indicated for total weight where females exhibited lower range (45.08 gr) compared to males (48.35 gr).

Monthly distribution of body length and total weight indicated a greater range in body length during all sampling periods for females, and similarly, the range of total weight was greater in females during most sampling periods except March, where the opposite trend was indicated (Figure 4).

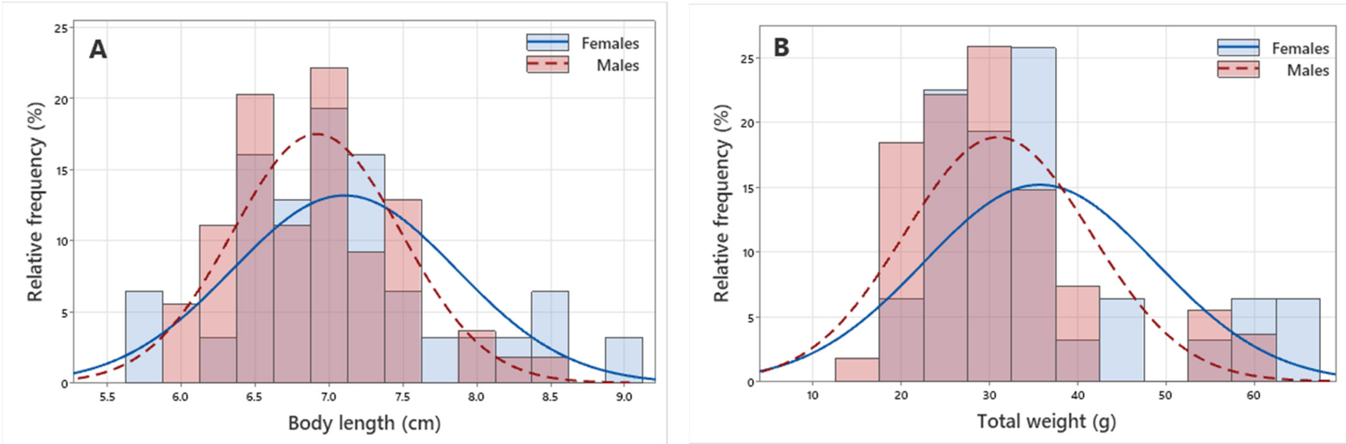

Figure 3. Body length (**A**) and total weight (**B**) frequency distribution of *P. epeiroticus* male and female populations with overlaid fitted normal distribution.

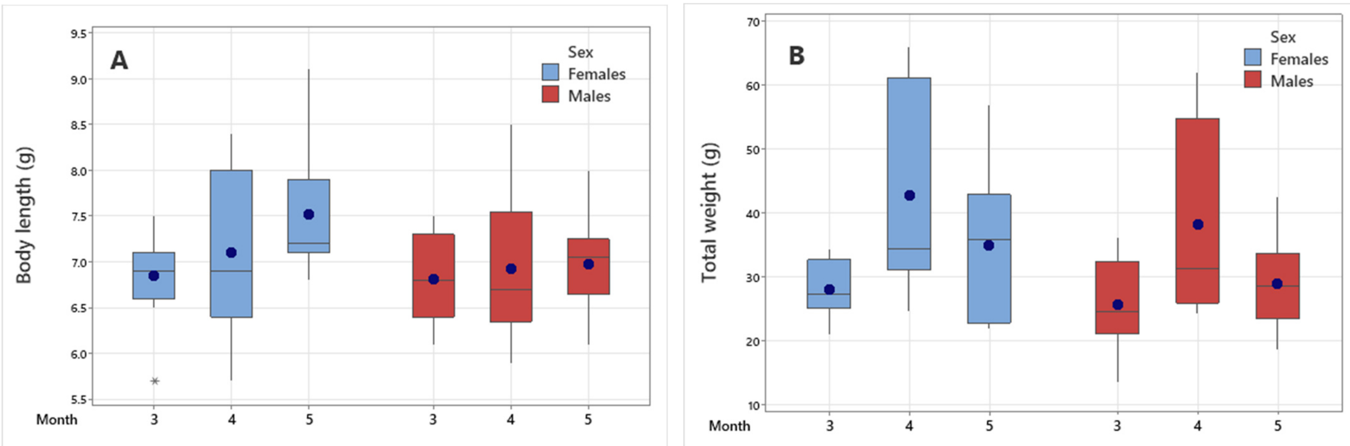

Figure 4. Monthly body length (**A**) and total weight (**B**) distribution of male and female *P. epeiroticus* populations. Values are means (dots), medians (lines), standard deviation (interquartile range box), minimal and maximal value (whiskers) and outliers (asterisks *).

3.3. Allometry

A significant allometric relationship was indicated between body length, femur length, tibia length and snout eye distance. A significant difference in the allometric relationships among sexes was indicated between body length and femur length and between body length and snout eye distance (Table 2). No significant allometric relationship was indicated between total weight and body length as well as between maximum head length and maximum head width.

3.4. Proximate Composition

The proximate composition of frog and freshwater fish species (cultured and wild) and their comparison with estimated values for *P. epeiroticus* in lake Pamvotida is illustrated in Tables 3 and 4.

Table 2. Allometric equations between body length (BL), total weight (TW), femur length (FL), tibia length (TL), maximum head length (MHL), maximum head width (MHW) and snout eye distance (SED) of *P. epeiroticus*. populations.

Morphometric Relationships						Equation Comparison	
Sampling Site	**Equation**	**N**	**R^2**	***t*-Test**	**Allometry**	**Slopes**	**Intercepts**
Total population	$TW = 0.0793345 \times BL^{3.08249}$	85	84.4%	ns	Positive	ns	ns
Females	$TW = 0.147744 \times BL^{2.78335}$	31	85.7%	ns	Negative		
Males	$TW = 0.0446303 \times BL^{3.36766}$	54	85.6%	ns	Positive		
Total population	$BL = 2.62897 \times FL^{0.723272}$	85	93.3%	***	Negative	***	***
Females	$BL = 2.2427 \times FL^{0.839318}$	31	96.3%	ns	Negative		
Males	$BL = 2.9084 \times FL^{0.647612}$	54	95.6%	***	Negative		
Total population	$BL = 2.90683 \times TL^{0.696432}$	85	92.8%	***	Negative	ns	ns
Females	$BL = 3.13275 \times TL^{0.632722}$	31	94.7%	***	Negative		
Males	$BL = 2.70909 \times TL^{0.758638}$	54	96.5%	***	Negative		
Total population	$BL = 6.14082 \times SED^{0.70285}$	85	87.8%	**	Negative	***	ns
Females	$BL = 5.81649 \times SED^{0.95948}$	31	92.9%	ns	Negative		
Males	$BL = 6.25962 \times SED^{0.592945}$	54	92.5%	***	Negative		
Total population	$MHL = 1.24333 \times MHW^{0.958627}$	85	94.8%	ns	Negative	ns	ns
Females	$MHL = 1.41502 \times MHW^{0.829075}$	31	96.8%	ns	Negative		
Total population	$MHL = 1.24333 \times MHW^{0.958627}$	85	94.8%	ns	Negative		

N: number of individuals, R^2: coefficient of determination, *t*-test: statistical significance of the allometric relationship, allometry: allometric relationship between the two variables, slopes (b): statistical comparison between the slopes of the equations, intercepts (a): statistical comparison between the intercepts of the equations, significance level (ns: non-significant, **: $p < 0.01$, ***: $p < 0.001$).

Table 3. Proximate composition (percentage of wet weight) of wild and cultured frog species, and statistical comparison using one sample *t*-test, with values estimated for *P. epeiroticus* in lake Pamvotida. Data are mean values.

Origin	Species	Moisture	Ash	Protein	Fat	Origin	Reference
Cultured	*Pelophylax ridibundus*	74.79 ± 0.25 ***	1.00 ± 0.15 ns	22.95 ± 0.16 ***	0.93 ± 0.20 ***	Turkey	[34]
	Pelophylax ridibundus	79.15 ± 0.77 ***	1.03 ± 0.01 ns	19.69 ± 0.17 ***	0.71 ± 0.03 **	Turkey	[33]
	Lithobates catesbeianus	84.81 ± 0.12 ***	0.94 ± 0.27 ns	15.24 ± 0.67 ns	0.06 ± 0.0 *	Brazil	[35]
	Holobatrachus rugulosus	79.47 ± 0.12 **	0.77 ± 0.05 **	21.89 ± 0.16 ***	0.8 ± 0.07 ***	Thailand	[36]
Wild	*Pelophylax epeiroticus*	81.5 ± 3.63	1.10 ± 0.3	15.93 ± 3.32	0.25 ± 0.13	Greece	Present study
	Pelophylax ridibundus	79.37 ± 0.50 **	1.37 ± 0.23 *	18.52 ± 0.70 ***	0.74 ± 0.15 **	Turkey	[34]
	Pelophylax ridibundus	81.67 ± 0.11 ns	0.82 ± 0.05 **	16.58 ± 0.14 ns	0.89 ± 0.07 ***	Turkey	[50]
	Pelophylax ridibundus	81.2 ± 2.12 ns	1.26 ± 0.06 ns	18.88 ± 0.48 ***	0.82 ± 0.03 ***	Turkey	[33]
	Pelophylax esculentus	79.72 ± 0.94 *	0.56 ± 0.02 ***	19.23 ± 0.15 ***	0.68 ± 0.03 **	Turkey	[37]
	Pelophylax esculentus	79.47 ± 0.59 **	1.83 ± 0.16 ***	22.21 ± 1.19 ***	1.05 ± 0.34 ***	Turkey	[28]
	Pelophylax esculentus	78.83 ± 0.48 ***	0.85 ± 0.02 **	18.83 ± 0.48 ***	1.04 ± 0.00 ***	Turkey	[51]
	Lithobates catesbeianus	80.77 ± 0.74 ns	0.63 ± 0.04 ***	17.01 ± 0.12 ns	0.61 ± 0.07 **	Mexico	[52]
	Lithobates catesbeianus	80.02 ± 0.13 *	0.58 ± 0.06 ***	19.31 ± 0.06 ***	0.71 ± 0.05 **	Thailand	[36]

Significance level (ns: non-significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Table 4. Proximate composition (percentage of wet weight) of wild freshwater fish species and statistical comparison using one sample *t*-test, with values estimated for *P. epeiroticus* in lake Pamvotida. Data are mean values.

Species	Moisture	Ash	Protein	Fat	Origin	Reference
Pelophylax epeiroticus	81.5 ± 3.63	1.10 ± 0.3	15.93 ± 3.32	0.25 ± 0.13	Greece	Present study
Salmo trutta fario	78.3 ***	1.16 ± 0.03 ns	17.36 ± 0.37 *	2.71 ± 0.21 ***	Turkey	[53]
Salvelinus fontinalis	76.54 ***	1.89 ± 0.04 ***	16.31 ± 0.09 ns	2.33 ± 0.02 ***	Turkey	[54]
Oreochromis niloticus	81.2 ± 1.0 ns	0.5 ± 0.2 ***	16.4 ± 0.60 ns	2.00 ± 0.60 ***	Poland	[55]
Cyprinus carpio	77.7 ± 2.6 ***	0.6 ± 0.3 ***	16.7 ± 0.80 ns	5.1 ± 3.00 ***	Poland	[55]
Cyprinus carpio	73.73 ± 0.24 ***	0.88 ± 0.05 *	16.69 ± 0.40 ns	7.13 ± 0.10 ***	Serbia	[56]
Oncorhynchus mykiss	73.00 ± 1.5 ***	0.80 ± 0.2 **	18.90 ± 0.80 ***	7.4 ± 1.60 ***	Poland	[55]
Liza abu	80.2 ns	1.10 ns	19.60 ***	1.40 ***	Iraq	[57]
Aspius aspius	78.51 ± 0.2 ***	1.16 ± 0.05 ns	18.07 ± 0.09 ***	2.78 ± 0.11 ***	Serbia	[56]
Abramis brama	78.66 ± 0.19 ***	0.80 ± 0.04 **	17.59 ± 0.22 *	3.24 ± 0.15 ***	Serbia	[56]
Barbus barbus	72.39 ± 0.29 ***	1.33 ± 0.03 *	18.61 ± 0.37 ***	7.78 ± 0.15 ***	Serbia	[56]
Acipenser ruthenus	75.38 ± 0.35 ***	0.93 ± 0.09 ns	17.54 ± 0.23 *	5.39 ± 0.14 ***	Serbia	[56]
Esox lucius	79.00 ± 0.14 **	0.64 ± 0.03 ***	18.43 ± 0.21 ***	1.61 ± 0.01 ***	Serbia	[56]

Significance level (ns: non-significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

Moisture, crude protein, crude fat and ash content of *P. epeiroticus* were found to be 81.5 ± 3.63, 15.93 ± 3.32, 0.25 ± 0.13 and 1.10 ± 0.30, respectively. Cultured frog species recorded a protein content ranging from 15.24 to 22.95% (Table 3). Proximate composition exhibited a high degree of similarity to wild-caught *P. ridibundus* from Turkey and *Lithobates catesbeianus* from Mexico.

Compared to freshwater fish, protein content of the Epirus water frog (15.93 ± 3.32) was similar to *Salvelinus fontinalis* (16.31 ± 0.09), *Oreochromis niloticus* (16.4 ± 0.6) and *Cyprinus caprio* (16.7 ± 0.8). Moisture content (81.50 ± 3.63) exhibited high similarity to *Oreochromis niloticus* (81.20 ± 1.0) and *Liza abu* (80.20). However, fat content (0.25 ± 0.13) was significantly lower compared to all freshwater fish species.

Predictive Analysis

Predictive analysis was applied to identify patterns and generate predictions for sex identification based on proximate composition. Through visual programming, five supervised machine learning algorithms were utilized (CN2 induction, random forest, logistic regression, neural network and decision tree algorithms) and the one that applied best to our dataset was identified (decision tree algorithm) by scoring the performance of each model. The workflow applied for the identification of the appropriate model classifier identification is shown in Figure 5.

The tree classification algorithm used to compute the contribution of proximate composition to the phyletic identification of *P. epeiroticus* identified that the principal contributing component was the proportion of dry matter (Figures 6 and 7) followed by the proportion of edible flesh and protein.

The output of the decision tree algorithm is shown in Figure 6.

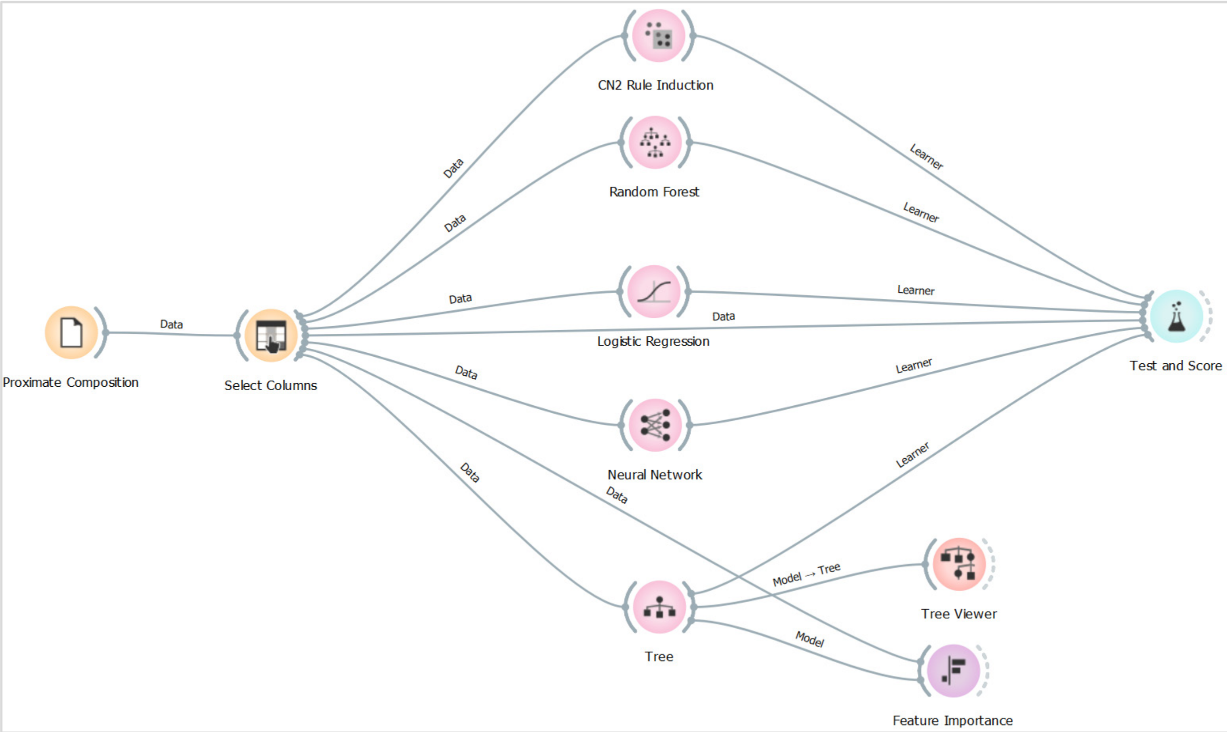

Figure 5. Workflow of the most appropriate model classifier identification.

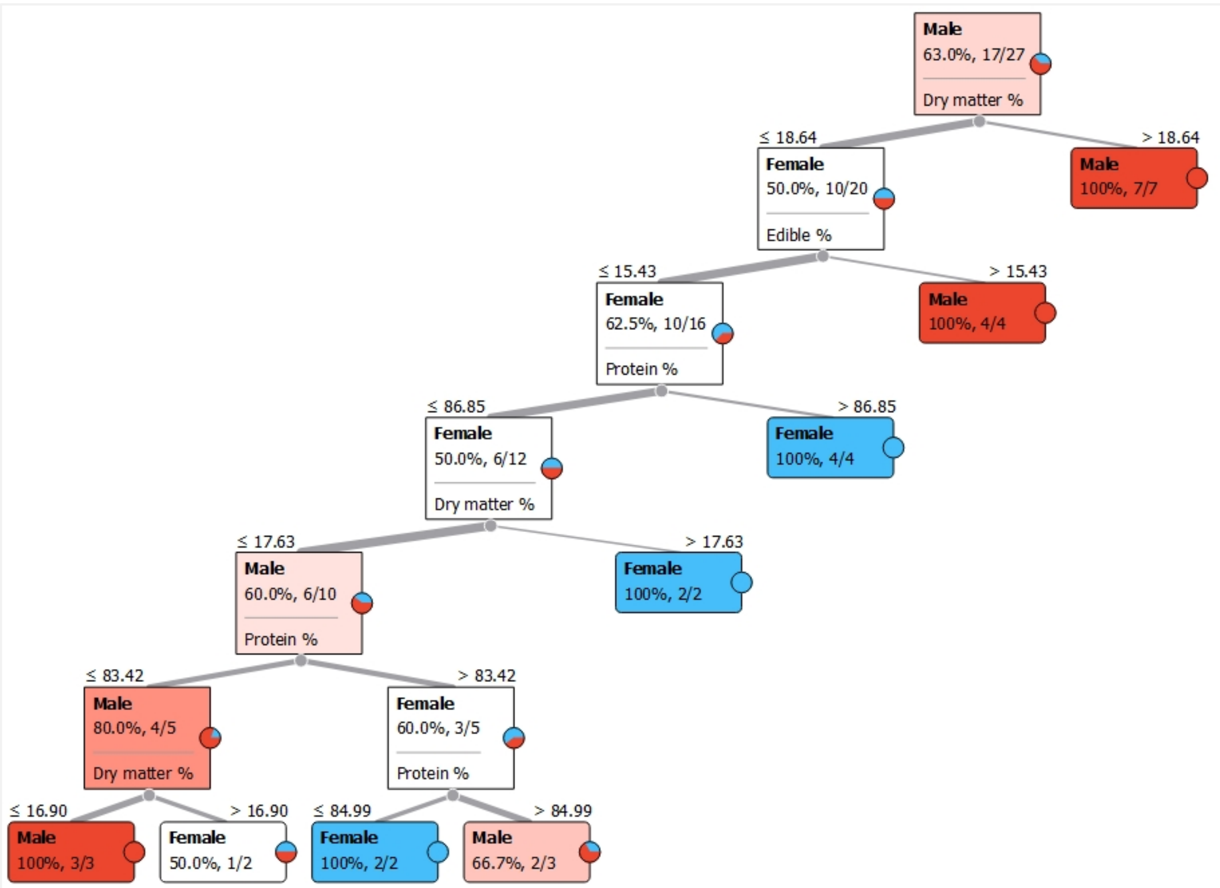

Figure 6. Predictive analysis classification of *P. epeiroticus* according to its proximate composition.

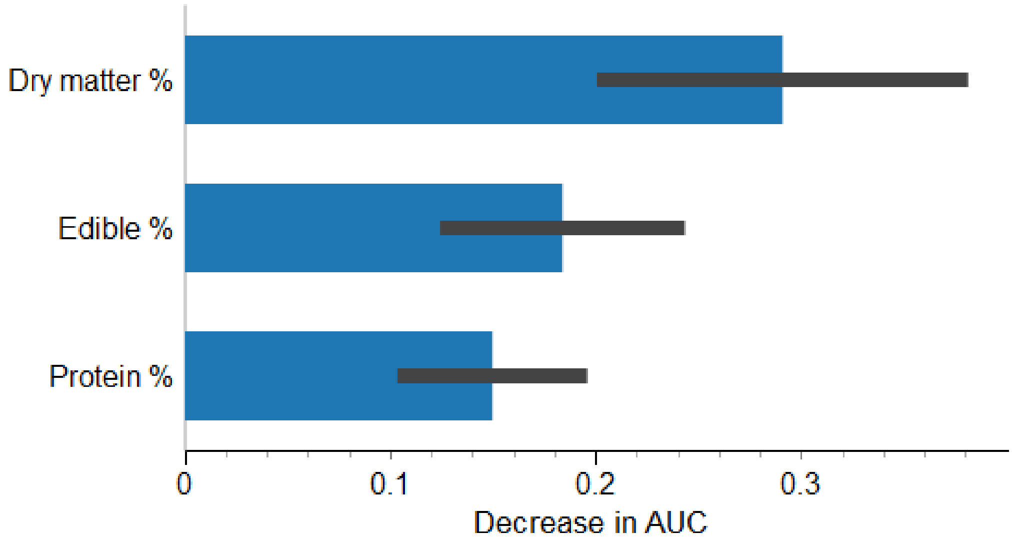

Figure 7. Identification of the most important proximate composition component in the phyletic identification of *P. epeiroticus* according to the tree classification algorithm.

4. Discussion

Significantly lower abundance of females than expected (Table 1) could be potentially attributed to differential mortality between sexes, previously reported in frogs, where males exhibit more conspicuous behavior than females and can be easily detected by predators [12]. According to [58], differential mortality between sexes has been previously reported in frogs, where males exhibit more conspicuous behavior than females and can be easily detected by predators.

Females exhibited higher weight (35.69 ± 13.13) compared to males (31.01 ± 10.57) and larger body length (females: 7.11 ± 0.76, males: 6.92 ± 0.57); however, this is not statistically significant (Figure 3). According to [33], female frogs weigh more than their male counterparts. Alternations of morphological traits can be explained by the biological cycle of the frog and variation of abiotic and biotic parameters. Significant morphological variation reported across *Pelophylax* species, and particularly between mainland *P. epeiroticus, P. ridibundus* and *P. kurtmuelleri* species, does not always allow a reliable morphological discrimination of the different species in their overlapping regions [8]. According to [59], there is a strong sexual size dimorphism in *P. epeiroticus,* with females growing larger than males, related to differences in the age at which maturity is reached. Furthermore, a significant difference in body length between *P. epeiroticus* sexes was reported [12].

A significant difference was indicated in total weight between sampling periods with a significantly higher total weight for individuals sampled in April (Figure 4) possibly attributed to hibernation of the species. It has been reported [60] that minimum fat body weight was observed in February in *R. epeiroticus* due to the fact that lipid was used for ovarian follicle growth through hibernation.

The divergence of shape and size, due to the development of an organism is known as allometry [61]. Allometry is common in nature and is exhibited during the developmental stage of an organism between individuals of a population at the same developmental stage and between different species [62,63]. Allometric relationships identified several morphological differences among *P. epeiroticus* sexes (Table 2). The relationship of total weight versus body length exhibited the opposite allometric growth, with negative allometry indicated for females and positive for males; however, this is not statistically significant. Significant differences among sexes were indicated between body length, femur length and between body length and snout eye distance (Table 2). Environmental conditions coupled with ecological interactions and habitat utilization are known to affect development [64,65]. Both size and shape affect the biological processes of an organism [66]. According to [67], anurans are known to vary little in body size compared to other vertebrates, resulting in high lability of body dimensions as a result of abiotic factors [68].

Overall, *P. epeiroticus* proximate composition exhibited a high degree of similarity with cultured and wild frog species *Lithobates catesbeianus* from Brazil and Mexico, respectively [34,47]. *P. epeiroticus* moisture and protein content were similar to the frog species *P. ridibundus* and *L. catesbeianus* species caught in the wild [33,50,52].

Significant differences were exhibited with wild-caught frog species *Pelophylax esculentus* and *Lithobates catesbeianus* [28,37,51] (from Turkey and Thailand respectively) and cultured *Holobatrachus rugulosus* [36]. Protein content was higher in females (16.89 ± 4.88) in comparison to males (15.36 ± 1.91); however, this is not statistically significant.

Notably, fat content exhibited significantly lower value compared both to cultured and wild frog species [28,33,34,50,51], possibly attributed to the timing the individuals were caught, in the onset of spring right after winter when body fat tissue had not returned to normal values after a fasting period. No significant difference was indicated for *P. epeiroticus* fat content between sexes, with males exhibiting higher fat content (0.28 ± 0.12) compared to females (0.11 ± 0.07). A number of studies showed that marked seasonal changes in fat body and liver occur in amphibians living in seasonal environments [69,70]. In a study by [71], on *P. epeiroticus*, the emergence from wintering and the subsequent breeding season (February to May) was accompanied by a sharp decrease in fat body weight.

In amphibians, climate change mainly affects habitat suitability, the spread of infectious diseases, population size, phenology of reproduction, growth, and morphology [72–75]. In temperate areas, the increase in air and water temperature during spring appears important for initiating certain behaviors, including emergence from hibernation and initiation of reproductive activities [76]. Studies on Phelophylax in the Balkans [75] and north Europe [74] highlighted the important role of temperature increase on development, growth and body size.

The considerable similarity was indicated in the protein content between *P. epeiroticus* and several fresh water fish species, notably *Salvelinus fontinalis*, *Oreochromis niloticus*, and *Cyprinus carpio* [54–56], whereas lipid content was significantly lower, indicating *P. epeiroticus* excellent nutritional value. Proximate composition of *P. epeiroticus* was used in predictive analysis aiming to identify the principal contributing proximate composition components that classify opposite sexes (Figures 3–5). Predictive models are used to construct a model by using the results of the known data and predict the results of unknown data sets by using the constructed model [77].

The tree classification algorithm indicated that the principal contributing component for sex classification was dry matter, followed by a proportion of edible flesh and protein content. Sexual dimorphism was indicated in the limb muscles of *Pelophylax nigromaculata* [78]. Sexual dimorphism in size and morphology is widespread in the animal kingdom [79,80]. Three major hypotheses proposed to explain the evolution of dimorphism in secondary sexual characters include: mechanisms of sexual selection, intersexual food competition and reproductive role division [79,81]. Sexual size dimorphism is widespread among anurans [82,83], linked to intersexual differences in growth rate, time of sexual maturation, age structure and mortality schedules [84,85].

Epirus water frog is included in the list of European edible green frog complex (*Pelophylax* spp.) and is a sought-after food item, captured and consumed extensively in Western Greece. Frog leg meat is considered by many to be a delicacy and except for alive frogs and frozen frog legs, the food industry uses preservation techniques and cooking methods in order to prepare new food products [23,35] and increase shelf life [28]. *P. epeiroticus* could be marketed and further promoted as an excellent food choice with a rich nutritional profile. However, an increase in demand for frog leg meat should be combined with a suitable management plan regulating fishing pressure and fishing practices of wild captures, thus assuring a sustainable exploitation of the species. Steps should be taken to facilitate the development of farming of indigenous frog species in Greece in a further measure to alleviate the pressure on the wild populations.

5. Conclusions

This is the first study that describes the proximate composition of wild-caught *P. epeiroticus* distributed mainly in Northwest Greece in lake Pamvotida (Ioannina) and utilized commercially as food. Proximate composition with high protein and low lipid content demonstrated the high nutritional value of the species in comparison with other popular freshwater commercial species. Aspects of the population structure of the species indicated that the sex ratio was in favor of males. Females exhibited a higher body length range, whereas males exhibited a higher total weight range. A tree classification algorithm identified dry matter, followed by a proportion of edible flesh and protein content as the principal contributing components for sex classification. The combination of habitat degradation, coupled with a predicted increase in demand, will require the implementation of a suitable management plan to ensure the sustainability of the edible water frog populations in the western part of Greece along the Ionian coast.

Author Contributions: Conceptualization, M.H. and D.K.; data curation, M.H.; formal analysis, I.K. and D.K.; funding acquisition, M.H.; investigation, E.K.; methodology, M.H., I.K. and D.K.; software, D.K.; validation, M.H., E.K. and I.K.; visualization, D.K.; writing—original draft, M.H., E.K. and D.K.; writing—review and editing, M.H., E.K., I.K. and D.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: All experimental procedures were conducted according to the guidelines of the EU Directive 2010/63/EU regarding the protection of animals used for scientific purposes. The experiments were performed at the registered experimental facility (EL-43BIO/exp-01) of the Laboratory of Aquaculture, Department of Ichthyology and Aquatic Environment, University of Thessaly by FELASA accredited scientists (functions A–D).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the article itself.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sofianidou, T.S.; Schneider, H. Distribution range of the Epeirus frog Rana epeirotica (Amphibia: Anura) and the composition of the water frog populations in western Greece. *Zool. Anz.* **1989**, *223*, 13–25.
2. Valakos, E.D. *The Amphibians and Reptiles of Greece*; Edition Chimaira: Frankfurt, Germany, 2008; ISBN 3899734610.
3. IUCN SSC Amphibian Specialist Group. *Pelophylax Epeiroticus. The IUCN Red List of Threatened Species*; IUCN SSC Amphibian Specialist Group: Cambridge, UK, 2020.
4. Kyriakopoulou-Sklavounou, P.; Sjögren, P.; Jansson, H. Discordant genetic and morphological differentiation between three Rana vidibunda populations from northern Greece. *Hereditas* **1991**, *115*, 51–61. [CrossRef]
5. Herczeg, D.; Vörös, J.; Christiansen, D.G.; Benovics, M.; Mikulíček, P. Taxonomic composition and ploidy level among European water frogs (Anura: Ranidae: Pelophylax) in eastern Hungary. *J. Zool. Syst. Evol. Res.* **2017**, *55*, 129–137. [CrossRef]
6. Pafilis, P.; Kapsalas, G.; Lymberakis, P.; Protopappas, D.; Sotiropoulos, K. Diet composition of the karpathos marsh frog (Pelophylax cerigensis): What does the most endangered frog in europe eat? *Anim. Biodivers. Conserv.* **2019**, *42*, 1–8. [CrossRef]
7. Hermaniuk, A.; Czajkowska, M.; Borkowska, A.; Taylor, J.R.E. Body size variation in hybrids among populations of European water frogs (Pelophylax esculentus complex) with different breeding systems. *Amphib. Reptil.* **2020**, *41*, 361–371. [CrossRef]
8. Sagonas, K.; Karameta, E.; Kotsakiozi, P.; Poulakakis, N. Cross-species testing of nuclear markers in Pelophylax water frogs in Greece and examination of their power to detect genetic admixture. *Amphib. Reptil.* **2020**, *41*, 253–259. [CrossRef]
9. Zhelev, Z.M.; Arnaudova, D.N.; Popgeorgiev, G.S.; Tsonev, S.V. In situ assessment of health status and heavy metal bioaccumulation of adult Pelophylax ridibundus (Anura: Ranidae) individuals inhabiting polluted area in southern Bulgaria. *Ecol. Indic.* **2020**, *115*, 106413. [CrossRef]
10. Katsiyiannis, P.; Tzoras, E. First record of Pelophylax kurtmuelleri preying on Hyla arborea in Greece. *Parnass. Arch.* **2020**, *8*, 17–18.
11. Karatsivou, E. *Sustainability Model of Frog Farming in Greece*; Unveristy of Thessaly: Volos, Greece, 2014.
12. Kyriakopoulou-Sklavounou, P.; Stylianou, P.; Tsiora, A. A skeletochronological study of age, growth and longevity in a population of the frog Rana ridibunda from southern Europe. *Zoology* **2008**, *111*, 30–36. [CrossRef]
13. Duellman, W.E.; Trueb, L. *Biology of Amphibians*; JHU Press: Baltimore, MD, USA, 1994; ISBN 080184780X.

14. Marques, S.M.; Chaves, S.; Gonçalves, F.; Pereira, R. Evaluation of growth, biochemical and bioaccumulation parameters in Pelophylax perezi tadpoles, following an in-situ acute exposure to three different effluent ponds from a uranium mine. *Sci. Total Environ.* **2013**, *445*, 321–328. [CrossRef]
15. Franco-de-Sá, J.F.O.; Val, A.L. Copper toxicity for Scinax ruber and Rhinella granulosa (Amphibia: Anura) of the Amazon: Potential of Biotic Ligand Model to predict toxicity in urban streams. *Acta Amaz.* **2014**, *44*, 491–498. [CrossRef]
16. Taiwo, I.E.; Henry, A.N.; Imbufe, A.P.; Adetoro, O.O. Heavy metal bioaccumulation and biomarkers of oxidative stress in the wild African tiger frog, Hoplobatrachus occipitalis. *Afr. J. Environ. Sci. Technol.* **2014**, *8*, 6–15.
17. Mahmood, I.; Imadi, S.R.; Shazadi, K.; Gul, A.; Hakeem, K.R. Effects of pesticides on environment. In *Plant, Soil and Microbes*; Springer: Berlin/Heidelberg, Germany, 2016; pp. 253–269.
18. Jayawardena, U.A.; Angunawela, P.; Wickramasinghe, D.D.; Ratnasooriya, W.D.; Udagama, P.V. Heavy metal–induced toxicity in the Indian green frog: Biochemical and histopathological alterations. *Environ. Toxicol. Chem.* **2017**, *36*, 2855–2867. [CrossRef] [PubMed]
19. Corduk, N.; Hacioglu-Dogru, N.; Gul, C.; Tosunoglu, M. Monitoring of micronuclei and nuclear abnormalities in Pelophylax ridibundus erythrocytes from the Biga Stream (Canakkale, Turkey). *Fresenius Environ. Bull.* **2018**, *27*, 147–153.
20. Pollo, F.; Bionda, C.; Otero, M.; Grenat, P.; Babini, S.; Flores, P.; Grisolia, M.; Salas, N.; Martino, A. Morphological abnormalities in natural populations of the common South American toad Rhinella arenarum inhabiting fluoride-rich environments. *Ecotoxicol. Environ. Saf.* **2019**, *177*, 32–38. [CrossRef]
21. Altherr, S.; Goyenechea, A.; Schubert, D.J. *The International Trade in Frogs'legs and Its Ecological Impact*; Pro Wildlife Munich (DE): München, Germany, 2011.
22. Agriculture Organization of the United Nations. *The State of World Fisheries and Aquaculture*; Food & Agriculture Org.: Rome, Italy, 2000; Volume 3, ISBN 9251044929.
23. Blé, Y.C.; Yobouet, B.A.; Dadié, A. Consumption, proximate and mineral composition of edible frog Hoplobatrachus occipitalis from midwest areas of Côte d'Ivoire. *AJSR* **2016**, *5*, 16–20.
24. Neveu, A. La raniculture est-elle une alternative à la récolte? Etat actuel en France. *Prod. Anim.* **2004**, *17*, 167–175. [CrossRef]
25. Teixeira, R.D.; Mello, S.C.R.P.; dos Santos, C.A.M.L. *The World Market for Frog Legs*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2001.
26. Tokur, B.; Gürbüz, R.D.; Özyurt, G. Nutritional composition of frog (Rana esculanta) waste meal. *Bioresour. Technol.* **2008**, *99*, 1332–1338. [CrossRef]
27. Warkentin, I.G.; Bickford, D.; Sodhi, N.S.; Bradshaw, C.J.A. Eating frogs to extinction. *Conserv. Biol.* **2009**, *23*, 1056–1059. [CrossRef]
28. Baygar, T.; Ozgur, N. Sensory and chemical changes in smoked frog (Rana esculanta) leg during cold storage (4 °C ± 1). *J. Anim. Vet. Adv.* **2010**, *9*, 588–593. [CrossRef]
29. Onadeko, A.B.; Egonmwan, R.I.; Saliu, J.K. Edible amphibian species: Local knowledge of their consumption in Southwest Nigeria and their nutritional value. *West Afr. J. Appl. Ecol.* **2012**, *19*, 67–76.
30. Mohneke, M.; Rödel, M.-O. Declining amphibian populations and possible ecological consequences—A review. *Salamandra* **2009**, *45*, 203–210.
31. Omoniyi, L.O.; Ajibola, M.E.; Bifarin, J.O. Demand Analysis for Frog Meat in Ondo State. *Glob. J. Sci. Front. Res. Agric. Biol.* **2012**, *12*, 9–12.
32. Schaefer, G.L.; Cosh, M.H.; Jackson, T.J. The USDA natural resources conservation service soil climate analysis network (SCAN). *J. Atmos. Ocean. Technol.* **2007**, *24*, 2073–2077. [CrossRef]
33. Alkaya, A.; Sereflisan, H.; Dikel, S.; Sereflisan, M. Comparison of Pond-Raised and Wild Female Marsh Frog (Pelophylax ridibundus) with Respect to Proximate Composition and Amino Acids Profiles. *Fresenius Environ. Bull.* **2018**, *27*, 6330–6336.
34. Cagiltay, F.; Erkan, N.; Selcuk, A.; Ozden, O.; Devrim Tosun, D.; Ulusoy, S.; Atanasoff, A. Chemical composition of wild and cultured marsh frog (Rana ridibunda). *Bulg. J. Agric. Sci.* **2014**, *20*, 1250–1254.
35. Afonso, A.M.; Fonseca, A.B.M.; Conte-Junior, C.A.; Mársico, E.T.; de Freitas, M.Q.; Mano, S.B. Cauda de rã: Uma fonte proteica para alimentar o futuro. *Bol. Do Inst. Pesca* **2017**, *43*, 112–123. [CrossRef]
36. Charasrosjanakul, A.; Laohasongkram, K.; Chaiwanichsiri, S. Effects of Garlic and Rosemary Essential Oil and Packaging Conditions on Physical and Chemical Properties of Frog Leg Meat during Refrigerated Storage. In Proceedings of the 5th International Conference on Agriculture, Environment and Biological Sciences (ICAEBS-16), Pattaya, Thailand, 28–29 April 2016.
37. Özogul, F.; Özogul, Y.; Olgunoglu, A.I.; Boga, E.K. Comparison of fatty acid, mineral and proximate composition of body and legs of edible frog (*Rana esculenta*). *Int. J. Food Sci. Nutr.* **2008**, *59*, 558–565. [CrossRef]
38. Hatziioannou, M.; Karatsivou, E. Bioeconomic Analysis of a Pilot Commercial Production for Frogs (Lithobates Catesbeianus), In Greece. *Oceanogr. Fish. Open Access J.* **2020**, *12*, 137–140. [CrossRef]
39. Wilson, G.P.; Frogley, M.R.; Roucoux, K.H.; Jones, T.D.; Leng, M.J.; Lawson, I.T.; Hughes, P.D. Limnetic and terrestrial responses to climate change during the onset of the penultimate glacial stage in NW Greece. *Glob. Planet. Chang.* **2013**, *107*, 213–225. [CrossRef]
40. Romero, J.R.; Kagalou, I.; Imberger, J.; Hela, D.; Kotti, M.; Bartzokas, A.; Albanis, T.; Evmirides, N.; Karkabounas, S.; Papagiannis, J. Seasonal water quality of shallow and eutrophic Lake Pamvotis, Greece: Implications for restoration. *Hydrobiologia* **2002**, *474*, 91–105. [CrossRef]
41. Perdikaris, C.; Georgiadis, C. Co-occurrence of narrow-clawed crayfish (Astacus leptodactylus sensu lato) and noble crayfish (*Astacus astacus* L.) in the southwestern Balkans: The case of Lake Pamvotida (NW Greece). *North-West. J. Zool.* **2017**, *13*, 18–26.

42. Stefanidis, K.; Kostara, A.; Papastergiadou, E. Implications of human activities, land use changes and climate variability in Mediterranean lakes of Greece. *Water* **2016**, *8*, 483. [CrossRef]
43. Borzée, A.; Park, S.; Kim, A.; Kim, H.-T.; Jang, Y. Morphometrics of two sympatric species of tree frogs in Korea: A morphological key for the critically endangered Hyla suweonensis in relation to H. japonica. *Anim. Cells Syst.* **2013**, *17*, 348–356. [CrossRef]
44. AOAC. Official Methods of Analysis. In *Association of Official Analytical Chemistrs*, 19th ed.; AOAC: Washington, DC, USA, 2012.
45. Krishnamoorthy, K.; Lu, F.; Mathew, T. A parametric bootstrap approach for ANOVA with unequal variances: Fixed and random models. *Comput. Stat. Data Anal.* **2007**, *51*, 5731–5742. [CrossRef]
46. Hampton, R.E.; Havel, J.E. *Introductory Biological Statistics*; Waveland Press: Long Grove, IL, USA, 2006; ISBN 1577663802.
47. Şahin, M.D.; Aybek, E.C. Jamovi: An easy to use statistical software for the social scientists. *Int. J. Assess. Tools Educ.* **2019**, *6*, 670–692. [CrossRef]
48. Yadav, S.K.; Pal, S. Data mining: A prediction for performance improvement of engineering students using classification. *arXiv* **2012**, arXiv:1203.3832.
49. Demšar, J.; Curk, T.; Erjavec, A.; Gorup, Č.; Hočevar, T.; Milutinovič, M.; Možina, M.; Polajnar, M.; Toplak, M.; Starič, A. Orange: Data mining toolbox in Python. *J. Mach. Learn. Res.* **2013**, *14*, 2349–2353.
50. Cagiltay, F.; Erkan, N.; Tosun, D.; Selcuk, A. Chemical composition of the frog legs (Rana ridibunda). *Fleischwirlschaft Int.* **2011**, *26*, 78–81.
51. Çaklı, Ş.; Kışla, D.; Cadun, A.; Dinçer, T.; Cağlak, E. Determination of Shelf Life in Fried and Boiled Frog Meat Stored in Refrigerator in 3.2 ± 1.08 °C. *Ege J. Fish. Aquat. Sci.* **2009**, *26*, 115–119.
52. Haddad, L.R.; Tejada-Ortigoza, V.; Martín-del-Campo, S.T.; Balderas-León, I.; Morales-de la Peña, M.; Garcia-Amezquita, L.E.; Welti-Chanes, J. Evaluation of nutritional composition and technological functionality of whole American Bullfrog (Lithobates catesbeianus), its skin, and its legs as potential food ingredients. *Food Chem.* **2022**, *372*, 131232. [CrossRef] [PubMed]
53. Kaya, Y.; Erdem, M.E.; Turan, H. Monthly differentiation in meat yield, Chemical and amino acid composition of wild and cultured brown trout (Salmo trutta forma fario Linneaus, 1758). *Turk. J. Fish. Aquat. Sci.* **2014**, *14*, 479–486. [CrossRef]
54. Şahin, Ş.A.; Başçinar, N.; Kocabaş, M.; Tufan, B.; Köse, S.; Okumuş, I. Evaluation of meat yield, proximate composition and fatty acid profile of cultured brook trout (Salvelinus fontinalis Mitchill, 1814) and black sea trout (Salmo trutta labrax Pallas, 1811) in comparison with their hybrid. *Turk. J. Fish. Aquat. Sci.* **2011**, *11*, 261–271. [CrossRef]
55. Usydus, Z.; Szlinder-Richert, J.; Adamczyk, M.; Szatkowska, U. Marine and farmed fish in the Polish market: Comparison of the nutritional value. *Food Chem.* **2011**, *126*, 78–84. [CrossRef]
56. Ljubojevic, D.; Trbovic, D.; Lujic, J.; Bjelic-Cabrilo, O.; Kostic, D.; Novakov, N.; Cirkovic, M. Fatty acid composition of fishes from Inland Waters. *Bulg. J. Agric. Sci.* **2013**, *19*, 62–71.
57. Al-Anbari, I.H.A.; Abbas, A.A.; Al-Ani, M.T.H. Effect of adding table salt and citric acid on some properties of Iraqi fish (Liza abo mugilidae) after cookingin a microwave oven. *Plant Arch.* **2019**, *19*, 3043–3049.
58. Shirose, L.J.; Brooks, R.J.; Barta, J.R.; Desser, S.S. Intersexual differences in growth, mortality, and size at maturity in bullfrogs in central Ontario. *Can. J. Zool.* **1993**, *71*, 2363–2369. [CrossRef]
59. Tsiora, A.; Kyriakopoulou-Sklavounou, P. A skeletochronological study of age and growth in relation to adult size in the water frog Rana epeirotica. *Zoology* **2002**, *105*, 55–60. [CrossRef]
60. Tsiora, A.; Kyriakopoulou-Sklavounou, P. Female reproductive cycle of the water frog Rana epeirotica in northwestern Greece. *Amphib. Reptil.* **2002**, *23*, 269–280. [CrossRef]
61. Gould, S.J. Allometry and size in ontogeny and phylogeny. *Biol. Rev.* **1966**, *41*, 587–638. [CrossRef]
62. Klingenberg, C.P. Size, shape, and form: Concepts of allometry in geometric morphometrics. *Dev. Genes Evol.* **2016**, *226*, 113–137. [CrossRef] [PubMed]
63. Klingenberg, C.P. Heterochrony and allometry: The analysis of evolutionary change in ontogeny. *Biol. Rev.* **1998**, *73*, 79–123. [CrossRef] [PubMed]
64. Losos, J.B. Ecological character displacement and the study of adaptation. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5693–5695. [CrossRef] [PubMed]
65. Moen, D.S.; Irschick, D.J.; Wiens, J.J. Evolutionary conservatism and convergence both lead to striking similarity in ecology, morphology and performance across continents in frogs. *Proc. R. Soc. B Biol. Sci.* **2013**, *280*, 20132156. [CrossRef]
66. Schmidt-Nielsen, K.; Knut, S.-N. *Scaling: Why Is Animal Size So Important?* Cambridge University Press: Cambridge, UK, 1984; ISBN 0521319870.
67. Landis, M.J.; Schraiber, J.G. Pulsed evolution shaped modern vertebrate body sizes. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 13224–13229. [CrossRef]
68. Levy, D.L.; Heald, R. Biological scaling problems and solutions in amphibians. *Cold Spring Harb. Perspect. Biol.* **2016**, *8*, a019166. [CrossRef]
69. Morton, M.L. Seasonal changes in total body lipid and liver weight in the Yosemite toad. *Copeia* **1981**, *1981*, 234–238. [CrossRef]
70. Schlaghecke, R.; Blüm, V. Seasonal variations in fat body metabolism of the green frog *Rana esculenta* (L.). *Experientia* **1978**, *34*, 1019–1020. [CrossRef]
71. Tsiora, A.; Kyriakopoulou-Sklavounou, P. Male reproductive cycle of the water frog Rana epeirotica in northwestern Greece. *Amphib. Reptil.* **2001**, *22*, 291–302.
72. Carey, C.; Alexander, M.A. Climate change and amphibian declines: Is there a link? *Divers. Distrib.* **2003**, *9*, 111–121. [CrossRef]

73. Alan Pounds, J.; Bustamante, M.R.; Coloma, L.A.; Consuegra, J.A.; Fogden, M.P.L.; Foster, P.N.; La Marca, E.; Masters, K.L.; Merino-Viteri, A.; Puschendorf, R. Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature* **2006**, *439*, 161–167. [CrossRef] [PubMed]
74. Tryjanowski, P.; Sparks, T.; Rybacki, M.; Berger, L. Is body size of the water frog *Rana esculenta* complex responding to climate change? *Naturwissenschaften* **2006**, *93*, 110–113. [CrossRef] [PubMed]
75. Mosavi, J.; Vaissi, S.; Dastansara, N.; Sharifi, M. Effects of temperature on growth, development and survival in larvae of Pelophylax ridibundus (Pallas, 1771) (Amphibia: Anura): Linking global warming to amphibian development. *Acta Zool. Bulg.* **2017**, *69*, 541–546.
76. Reading, C.J. The effect of winter temperatures on the timing of breeding activity in the common toad Bufo bufo. *Oecologia* **1998**, *117*, 469–475. [CrossRef] [PubMed]
77. Al-Radaideh, Q.A.; Al Nagi, E. Using data mining techniques to build a classification model for predicting employees performance. *Int. J. Adv. Comput. Sci. Appl.* **2012**, 144–151.
78. Mao, M.; Mi, Z.P.; Yang, Z.S.; Zhou, C.Q. Sexual dimorphism in the limb muscles of the dark-spotted frog, Pelophylax nigromaculata. *Herpetol. J.* **2014**, *24*, 147–153.
79. Andersson, M.B. *Sexual Selection*; Princeton University Press: Princeton, NJ, USA, 1994.
80. Liao, W.B. Evolution of sexual size dimorphism in a frog obeys the inverse of Rensch's rule. *Evol. Biol.* **2013**, *40*, 293–299. [CrossRef]
81. Hedrick, A.V.; Temeles, E.J. The evolution of sexual dimorphism in animals: Hypotheses and tests. *Trends Ecol. Evol.* **1989**, *4*, 136–138. [CrossRef]
82. Shine, R. Sexual selection and sexual dimorphism in the Amphibia. *Copeia* **1979**, 297–306. [CrossRef]
83. Li, S.T.; Wu, X.; Li, D.Y.; Lou, S.L.; Mi, Z.P.; Liao, W.B. Body size variation of odorous frogs (*Odorrana grahami*) across altitudinal gradients. *Herpetol. J.* **2013**, *23*, 187–192.
84. Halliday, T. Intersexual selection and alternative mating behaviour. *Amphib. Biol.* **1995**, *2*, 419–468.
85. Liao, W.B.; Zeng, Y.; Yang, J.D. Sexual size dimorphism in anurans: Roles of mating system and habitat types. *Front. Zool.* **2013**, *10*, 65. [CrossRef] [PubMed]