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Associations of Isotopes ^{13}C, ^{15}N, ^{34}S on the Mercury Levels of Different Marine Finfish and Invertebrate Species

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ABSTRACT

It is well known that the stable isotopes of carbon (δ13C), sulfur (δ34S), and nitrogen (δ15N) have several effects on biochemical parameters of different marine finfish and invertebrate species. The current article examines the roles δ13C, δ34S and δ15N on the mercury levels (Hg) of different marine finfish and invertebrate species based on a real data set of 16 marine finfish species as well as invertebrates of 56 different sample units. It is derived here in that the mean Hg level is negatively associated with δ13C (p=0.0059), positively associated with δ15N (p=0.0019), and it is positively associated with their joint interaction effect δ13C* δ15N (p=0.0021). In addition, mean Hg is negatively partially associated with δ34S (p=0.1493). Mean Hg level is negatively associated with species Type 2(=Free Swimming) (p=0.0023) and species Type 3(Invertebrate species) (p=0.0556). The variance of Hg level is positively associated with δ13C (p=0.0419) and the species Type 3 (p=0.0063). Stable δ13C and δ34S can reduce the Hg level, while δ15N and δ13C*, δ15N can increase Hg level. Free swimming fish species and Invertebrate species have lower Hg levels than bottom dowelling finfish species.

Keywords Carbon isotope; Invertebrates; Joint mean variance modeling; Marine finfish species; Mercury level; Nitrogen isotope; Supper isotope; Unequal variance.

INTRODUCTION

Heavy metal mercury (Hg) is a global health concern (Mergler *et al*., 2007, WHO 2010). More than 6000 tons of Hg is released into the environment annually, and its concentration is continuously increasing in many regions over the world (WHO 2010, Zhu *et al*. 2012). Most amount of Hg is released from the coal-fired power plants, and there are several such point sources in many countries such as China,

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India, (ZHu *et al*., 2012, Compbell *et al*. 2005), Hg is disseminated throughout the globe via natural and anthropogenic processes and the mercury toxicity has resulted in concerns for our food chain (Zhu *et al.* 2012, Bisi *et a*l. 2012).

Human beings are primarily exposed to Hg (as methylmercury) by fish consumption (Mergler et al. 2007, Campbell *et al*., 2006, Bisi *et al.* (2012).The principal source of accumulation of Hg in humans is through the consumption of marine fish and other marine animals contaminated with methylmercury. It is known that the accumulation of Hg in marine animals increases with increased trophic levels predators accumulate higher levels of Hg (Mergler *et al*. 2007, Chatterjee *et al*. 2014). Hg is released from most industries as an inorganic compound and it is generally deposited into aquatic ecosystems and then the microorganisms can methylate this form of Hg into methylmercury. In the form of methylated chemical, Hg can effectively pass through the biological membranes and deposit in organisms and biomagnify via aquatic food chains, and deposit in the tissues of fish consumers (Cambell *et al.* 2005, Chatterjee *et al.* 2014, Ray *et al*. 2019)).

The use of stable isotopes δ13C, δ15N, δ34S has been intensified very recently (Habson and Wassenaar 1999). These isotopes are adopted to illustrate the energy sources and trophic relationships in food chains of marine and freshwater, terrestrial ecosystems (Peterson and Fry 1987).The δ13C commonly recognizes carbon transference pathways, starting from the primary producers, while the δ15N recognizes the trophic position of the organisms in food chains (Vander zanden *et al.* 1997, Jennings *et al.* 1997).The δ15N quantity in tissues of consumers is generally enriched by 3% with respect to their prey relationship. However, δ13C is very slightly increased by 1% with the increase in trophic levels (Pereira *et al.* 2007).

The relationships of Hg concentration along with stable isotope ratios such as δ13C, δ15N, δ34S are derived in some research articles based on Pearson's correlation coefficient test, multiple linear regression, logistic regression, meta analysis, which are not appropriate statistical method for deriving associations of heteroscedastic data (Sackett *et al.* 2917, Endo *et al.* 2015). Note that physiological data sets are always heteroscedastic. Best of our knowledge, there is no article which has derived these associations considering the original nature of the data set. The present article makes an attempt to derive the relationships of Hg concentration along with stable isotope ratios such as δ13C, δ15N, δ34S considering the original nature of the data set. The article is ordered as follows. The next section presents the material and methods. The remaining sections are statistical analysis, results & discussion, and conclusions.

MATERIALS AND METHODS

Materials

The article considers a data set of 16 marine finfish and invertebrate species of 56 sample units. The data set contains stable isotope ratios (δ13C, δ15N and δ34S) and Hg concentrations for various fish and invertebrates that are common prey of Thick-billed murres from Coats Island, Canada, which is given in the link--https://data.mendeley.com/datasets/4rngbjg4r8/3 The data set contains two separate categories of animals such as marine finfish and marine invertebrates. The marine finfish can be categorized into two major groups such as Type 1 and Type 2, according to their habitats and choice of food. Type 1 fish-group includes the fishes of marine bottom dwellers and predominantly benthivorous (benthivore) and the rest fishes (free swimming) are considered as Type 2 fish-group. The marine invertebrates can be grouped as Type 3, which includes Squids and Flying snail (Mollusks), Amphipod Gammarid and Euphausiids (Crustaceans) and Jellyfish (Small) and Jellyfish (Large) (Cnidarians). These groups are formed using the trophic levels and habitats as differential characters. Therefore, the data set contains four continuous variables such as δ13C, δ15N, δ34S, Hg and one attribute character marine finfish and invertebrate species, which includes three types such as Type 1 (marine bottom dwellers finfish=1), Type 2 (free swimming finfish=2), Type 3 (invertebrates=3).

Statistical Methods

The aimed study response mercury level is positive, continuous and heteroscedastic which belongs to exponential family distributions. The associations of δ13C, δ34S, δ15N and fish and invertebrate species with mercury levels can be obtained by deriving appropriate statistical modeling. Based on our knowledge, the response mercury level is very little examined based on its original nature such as positive, heteroscedastic and non-normal, which can be modeled by using variance stabilizing transformation when it is stabilized with that transformation. Otherwise, it can be suitably modeled by joint generalized linear (JGL) gamma and Log-normal models (Lee *et al*. 2017, Das 2014.The response mercury level is searched herein adopting JGLMs with both the Log-normal and gamma distributions, and it is found that both the models give similar results. These two models are given explicitly in the book by Lee *et al.* (2017), Das (2014) for ready reference, these are very briefly presented as follows.

JGLMs with Log-normal distribution: For a positive random response variable, herein Hg=y_i's with unequal variance σ_i^2 (dispersion parameters) and average (or mean) E (y_i) = μ_i (mean parameters) and Var (y_i) = σ_i^2 μ_i^2=σ_i^2 V(μ_i) say, the log transformation z_i=log (y_i) is frequently adopted to stabilize the variance Var (z_i) ≈ σ_i^2, but it is not stabilized in most of the cases (Myers *et al*. 2002).. Then, JGLMs for both the mean and dispersion are to be derived. JGLMs of the mean and dispersion under log-normal distribution for z_i=log (y_i) are as follows:

$$E(z_i) = \mu_{z\,i} = x_i^t\,\beta \text{ and } Var\,(z_i) = \sigma^2_{zi}, \text{ with } \log\,(\sigma^2_{zi}) = g_i^t\,\gamma,$$

where x_i^t and g_i^t are the explanatory variables vectors connected respectively,with the mean regression coefficients β and γ (variance model parameters).

JGLMs with gamma distribution: In practice, the GLM family distributions is recognized by V(μ_i), which is called as the variance function, and it is gamma if $V(\mu)=\mu^2$, or Normal if $V(\mu)=1$, or Poisson if $V(\mu)=\mu$,. JGLMs of the mean and dispersion with amma distribution are as follows:

$$\eta_i = g\,(\mu_i) = x_i^t\,\beta \text{ and } \epsilon_i = h\,(\sigma_i^2) = w_i^t\,\gamma,$$

where g (.) and h (.) are GLM link functions related respectively, with the mean and dispersion linear predictors, while x_i^t, w_i^t are the explanatory factors vectors related respectively, with the mean and dispersion parameters. In practice, the maximum likelihood (ML) and the restricted ML (REML) method are used respectively, for estimating the mean and dispersion parameters (Lee *et al*. 2017)

Statistical Analysis

Hg level is considered as the aimed response random variable and the remaining 3 variables (δ13C, δ34S, δ15N) and 1 factor (species) are considered as the explanatory variables. It is detected that Hg level is heteroscedastic, which is modeled using JGL under both the log-normal and gamma distributions. Final fitted models are chosen based on the lowest Akaike information criterion (AIC) value (in each class), which diminishes both the predicted additive errors and squared error loss [20, p. 203-204]. JGL Ms gamma fit (AIC=--220.442) and log-normal fit (AIC=--220.6) give almost similar analysis results under the AIC criterion, as the AIC difference between the two models is less than one, which is insignificant (Lee *et al.* 2017). Partially significant effect δ34S is considered in the mean model for better fitting (Das and Lee 2009, Hasfic *et al.* 2009).Final Hg level analysis summary resultsare reported in Table 1.

Data generated probabilistic modelsare accepted based on model checking diagnostic procedures. Note that all the valid interpretations are drawn from the accepted model, which should be appropriate for the unknown true model. Figure 1 shows the model checking diagnostic plots for the fitted Hg level gamma model (Table 1). In Fig. 1(a), the Hg level gamma fitted (Table 1) absolute residuals are plotted against the fitted values that is nearly a flat straight line, except the left tail, concluding that variance is constant with the running means. At the left boundary, there is a larger absolute residual, so the left tail is little increasing. Figure 1(b) reveals the mean Hg level gamma fitted normal probability plot (Table 1), which does not indicate any sign of fit discrepancy. Thus, both the Figure 1(a) & Figure 1(b) imply that the gamma fitted Hg level model fits the data (Table 1).

Table 1. JGLMs gamma and log-normal fit of Hg levels with three isotopes.

Model	Covariate	Gamma fit				Log-normal fit			
		Estimate	se	t(49)	p-value	Estimate	se	t(49)	p-value
Mean	Constant	-24.384	8.026	-3.038	0.0038	-26.155	8.246	-3.172	0.0026
	δ13C	-1.104	0.383	-2.882	0.0059	-1.173	0.393	-2.989	0.0044
	δ15N	1.886	0.573	3.291	0.0019	1.952	0.589	3.317	0.0017
	δ13C* δ15N	0.090	0.028	3.243	0.0021	0.093	0.029	3.276	0.0019
	δ34S	-0.058	0.039	-1.465	0.1493	-0.041	0.039	-1.042	0.3025
	Type 2	-0.288	0.089	-3.222	0.0023	-0.301	0.087	-3.470	0.0011
	Type 3	-0.788	0.402	-1.961	0.0556	-1.156	0.438	-2.639	0.0111
Dispersion	Constant	6.884	4.298	1.602	0.1156	8.556	3.805	2.248	0.0291
	δ13C	0.464	0.222	2.089	0.0419	0.548	0.196	2.790	0.0075
	Type 2	0.026	0.497	0.053	0.9579	0.090	0.494	0.182	0.8563
	Type 3	2.385	0.835	2.856	0.0063	2.826	0.802	3.525	0.0009
AIC		-220.442				-220.6			

RESULTS AND DISCUSSION

The Hg level summarized analysis results are reported in Table 1. It is derived herein that the mean Hg level is negatively associated with δ13C (p=0.0059), positively associated with δ15N (p=0.0019) and it is positively associated with their joint interaction effect δ13C*δ15N (p=0.0021). In addition, mean Hg is negatively partially associated with δ34S (P=0.1493).). Mean Hg level is negatively associated with species Type 2(= Free Swimming) (P=0.0023) and species Type 3(Invertebrate species) (P=0.0556). The variance of Hg level is positively associated with δ13C (p=0.0419) and the species Type 3 (p=0.0063).

Gamma fitted Hg level mean $\hat{\mu}$ model (Table 1) is $\hat{\mu}=\exp(--24.384 -- 1.104\,\delta 13C + 1.886\delta 15N + 0.090\,\delta 13C^*\,\delta 15N -- 0.058\,\delta 34S - 0.288\,\text{Type 2} - 0.788\,\text{Type 3})$,
and from Table 1, the gamma fitted Hg level dispersion (σ^2) model is

$\sigma^2 = \exp(6.884 + 0.464\,\delta 13C + 0.026\,\text{Type 2} + 2.385\,\text{Type 3})$.
From Table 1, it is observed that the mean Hg level is negatively associated with δ13C (p=0.0059), concluding that Hg level is reduced for the marine finfish and invertebrate species with the increase of δ13C.

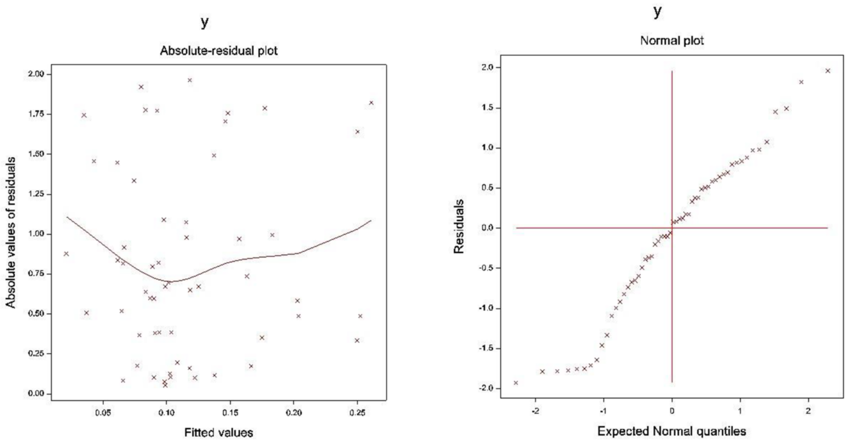

Figure 1(a) **Figure 1(b)**

Figs. 1. For the JGLMs gamma fitted Hg model (Table 1), the (a) absolute residuals plot with respect to fitted values and (b) normal probability plot for mean model.

Also the mean Hg level is positively associated with δ15N (p=0.0019), implying that Hg level is increased for the marine finfish and invertebrate species with the increase of δ15N. In addition, the mean Hg level is positively associated with δ13C*δ15N (p=0.0021), interpreting that that Hg level is increased for the marine finfish and invertebrate species with the increase of the interaction effect δ13C*δ15N. If both the isotopes (δ13C &δ15N) are in lower amounts, Hg level is reduced. On the other hand, mean Hg level is negatively partially associated with δ34S (p=0.1493), implying that Hg level is reduced for marine finfish and invertebrate species with the increase of δ34S. Note that there is no interaction effect of δ34S with δ13C and δ15N. Mean Hg level is negatively associated with species Type 2(= Free Swimming) (p=0.0023) and species Type 3(Invertebrate species) (=0.0556), concluding that Hg level is lower for the marine free swimming finfish and invertebrate species than the bottom dowelling finfish species.

From variance model (Table 1), it is observed that the variance of Hg level is positively associated with δ13C (p=0.0419), concluding that Hg level is highly scattered for the marine finfish and invertebrate species with higher amount of δ13C. In the mean model, it is found that a larger amount of δ13C and δ15N increases Hg level. Also, the dispersion model shows similar results. Also, the variance of Hg level is positively associated with the species Type 3 (p=0.0063), implying that Hg level is highly scattered for the marine invertebrate species.

Hg level concentration in stable isotopes of δ13C and δ15N has been analyzed in the above (Table 1) and it is seen from the data that the δ15N has shown significant impact on methyl mercury level (>11.63 in finfish and >9.18 in invertebrates), in comparison with δ13C isotopes and Hg level assemblage. The Hg level of bionts has shown higher values (from the considered sample data) when jointly measured both the δ13C and δ15N isotopes, which is simply due to the bioaccumulation of mercury through the process of biomagnification. The biotic methylmercury concentration is higher with trophic levels, so it is highly correlated with benthivore fishes, in comparison with planktivore fishes like Capelin (in case of smaller Capelin, but it may be higher in larger Capelin who devours krills). Note that Capelin shows lower Hg levels due to their food habits. Similarly the Hg level is also low in marine invertebrates due to their position in trophic level. However, the Hg level in arctic fish and invertebrates is a cause of great concern for the ecosystem functioning through the food chain which involves humans.All these biological phenomena support the current analysis findings.

Most of the earlier studies regarding the associations of the isotopes with the Hg level for the marine finfish and invertebrate species are based on Pearson's correlation coefficient, meta-analysis, logistic and multiple regression analysis, which are not appropriate statistical data analysis methods for physiological heteroscedastic data and they can miss many significant variables, or factors.The current article has focused many novel associations of isotopes with Hg level for the marine finfish and invertebrate species, which are very little studied in earlier articles. The current determinants of the Hg dispersion model are completely new in the marine finfish Hg accumulation studies. So, the current outcomes are little comparable with earlier articles.

CONCLUSION

The current article has derived the associations of the isotopes δ13C, δ15N, δ34S with the Hg level for the marine finfish and invertebrate species based on JGLMs, which has identified many associations that support many biological phenomena. The best model fittings have been accepted hereinbased on lowest AIC value,graphical diagnostic plots, small standard errors of the estimates and comparison of log-normal and gamma distributions. The article has derived that the stable δ13C and δ34S isotopes can reduce the Hg level, while δ15N and δ13C*δ15N can increase Hg level. Free swimming fish species and invertebrate species have lower Hg levels than bottom dowelling finfish species. Research workers in the areas of marine aquaculture as well as marine product consumers and industries related with marine products, environmentalists, ecologists will be benefited from the current article.

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