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DNA barcode assisted labels - a novel step in sustainable seafood labeling with reference to crab

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ABSTRACT

Mislabeled seafood is a widely spread problem on extreme geographical scale. Efforts have been made by various countries for seafood labeling so as to support sustainable fisheries and reduce seafood fraud. During this endeavor accurate species nomenclature had been ignored and seafood had been labeled with common terminologies like fish, crab and shrimps, despite the availability of more number of species with varied taste less than one particular seafood group. In this situation, chances of mislabeling and seafood fraud are more. In order to overcome this problem, DNA barcode assisted seafood labels can be used. The process is simple, low cost and lead to seafood labels that are species specific with accuracy supported at molecular scale. The application of such labels would surely reduce the chances of mislabeling and seafood fraud. The universal initiatives with collaborations of policy makers from different countries are required to standardize this process.

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INTRODUCTION

Labelling is an essential tool, which ought to be seen as a necessary piece of correspondence between traders to buyers. This is a critical mechanism for promotion of food security as it not just gives the complete subtle elements of the item additionally empowers data about the producer. It is one of the essential means by which shoppers separate between particular foods and their makes. Utilization of labelling had an old history of the thirteenth century when it was utilized for controlling weight and nature of bread and beer for which the King of England had passed the first law concerning proper labeling requirements (Ross, 1956). In 1758, the Georgia Legislature passed the Act for Regulating the Assize of Bread which administers the bakers to label the bread with a source of origin (Georgia, 1758). The first direct statutory regulation of food labeling was created by the United States and known as the pure food and drug act of 1906. Then onwards, there were lot of amendments in the act in 1938, 1966 and 1996 for the wellbeing, security and welfare of the buyer. Regardless of the fact that the labelling regulations were into force and also followed by the supplier, there were risks that the items could be mislabeled. Mislabeled items can extensively be termed as deliberately concealing the identity or uniqueness of the thing on a mark, sign, or menu either at the generation level or its prepared structure. Mislabeled commercial resources are a broadly spread issue on wide-ranging geographical scale. The aquatic resources are one

of the central amongst the food resources as they exist in practically every nation of the globe. These resources are more inclined for mislabeling because of the more number of species accessible under every class. Amongst aquatic resources, marine resources are important for every nation as they fetch foreign revenue through export. These resources comprise various groups like fishes, crustaceans, mollusks, bivalves, sea weeds etc. and possess different species under these groups. All the species under each group do not contribute for food resources of human. In this way, the marine resources which are enabling to suffice hunger, supply of protein and key nourishment are recognized as seafood. The demand for seafood has shown the rapid increase with the increasing population, which in turn resulted in extremely lucrative returns for the fishing industry and overridden sustainable practices (Owens, 2008). Thus to fulfill the need of the population, the acts of overfishing had started, which resulted in the depletion of the seafood stocks from the capture fisheries resources.

Sea food is most preferable food item amongst non-vegetarian food doters due to its taste and nutritional value. There are various sea food items, which not only includes fishes but also includes crustaceans and mollusks with numerous species that are edible and served in food plate with different price tags that vary according to the size and taste of respective sea food. Amongst fishes, Pomfret, Seer fish, Salmon fish, Sea bass fish, Catla, Rohu, Mrigal etc.

are popular names, which consumer knows and either purchase in retail fish market or order in restaurant to satisfy taste buds. Like different fishes, various shrimps, crabs, lobsters are famous crustaceans and squid, octopus; clams are well-known mollusks, which are preferred by consumers. Considering more number of seafood species, chances of mislabeling and species substitution are more.

Seafood comprises numerous species, but only few of them are in huge demand due to tasty meat. Similarly, these species are fetching more price and merely affordable to the higher and upper middle class families in the society. This does not mean that the low cost seafood deserves nothing, instead such seafood suffice the need of middle or lower middle class families due to low price of purchase. Such a major difference in prices of seafood is found to be the prime reason for the mislabeling, as low cost could be substituted with the high cost food. To avoid such mislabeling, the Federal Government of United States has worked through the years, to provide consistent and scientifically sound recommendations to industry and consumers about acceptable market names for seafood sold in interstate commerce. Accordingly, first list of fishes was published by FDA in 1988 in cooperation with the National Marine Fisheries Service to provide a source of names that would facilitate consistency and order in the U.S. market place and reduce confusion among consumers. The seafood list provides information to assist manufacturers in properly labeling seafood and to reflect the acceptable market names of new species introduced into the U.S. marketplace (<http://en.wikipedia.org>). However, this fish list could not solve the problem of mislabeling as it has different species under one genus named with single tag despite the differences in the fish species under the particular genus. The common labeling like crab, shrimp, pomfret, tuna etc. used in export as well as food chains is a threat to consumer interest and pocket owing to the availability of more than one species under each of these categories. For example, there are more than one shrimp available as seafood and each shrimp has its own common name, taste and the price. But all shrimps are labelled as 'shrimp' rather than its common name at the point of sale. Thus such type of common labelling gives way to substitute low cost species with the high cost ones. Such activity increases the chance of price fraud as inferior meat may serve on par with high priced meat and the consumer has forced to pay extra money from his pocket for low value meat. Some fish and crabs are poisonous and may also pose serious threat to consumer health (Leo and Peter, 1988). Seafood mislabeling can lead towards serious health concerns due to mask on undeclared allergens and contaminants (Warner *et al.*, 2013). One of the instances in this regard was mislabeling of the toxic puffer fish which was sold as headless monkfish (Cohen *et al.*, 2009). The prime investigation of seafood fraud was conducted by Oceana, which is the international organization focused solely on ocean conservation, collecting more than 1,200 seafood samples from 674 retail outlets in 21 states of United States. Their study revealed that the mislabeling was highest (74 percent) in the sushi venues, followed by restaurants (38 percent) and grocery stores (18 percent). They also discerned seafood substitutions including species carrying health advisories (e.g. king mackerel sold as

grouper; escolar sold as white tuna), low cost farmed fish sold as wild (e.g. tilapia sold as red snapper), and over-fished, imperiled or vulnerable species sold as more sustainable catch (e.g. Atlantic halibut sold as Pacific halibut) (Warner *et al.*, 2013). Thus their study gave the factual condition of seafood mislabeling prevailing in the United States (U.S.). This problem is not only restricted to U.S. but might be range over the vast geographical regions of the world. The extent of the mislabeling in any nation is so adverse that it may badly affect the export revenue of that country. It has also impact at the domestic level, as fisheries resources provide food security for the poor; livelihoods; rural and urban nutrition; and have cultural values in some societies (Gardiner and Viswanathan, 2004). Apart from this, there are other threats associated with the seafood mislabeling which are directly or indirectly imperative for every human being. These hazards are threat to ecology, biodiversity and fisheries resource management of every nation. Mislabeling of catch data at the deck level results in wrong estimates of stock size and affects the overall fisheries resource database and related management. Thus wrong estimates of the resources would complicate the policy makers during strategic planning of sustainable fisheries management. The act of mislabeling accelerates the capture of low cost or low consumed seafood species for substitution with the high cost seafood. This in turn leads to overfishing, which causes collapse of these stocks. Due to the extinct of one single resource, the whole food chain would collapse that further leads towards the threat to ecology or ecosystem. In the wake of these sustainability challenges, various nations have initiated the local food movement and sustainable seafood initiatives (Gilliland, 2013). Efforts have been made by different nations for labelling fish in order to back sustainable fisheries and lessen misrepresentation. To defeat this sort of future risk, a few firms in nations like Europe (Natureland), America (Ecofish) and UK (Marine Stewardship Council) have begun maintainable labelling of marine resources. However, the manifestation of mislabeling is not under control due to the multipart seafood sources on world-wide level. This indeed advanced by lack of systematic government assessment backed with seafood regulations. Hence, without verification of the seafood supply chain it becomes difficult to ascertain the level of fraud occurrence either at the place of capturing, during processing, at the wholesale level, at the retail counter or somewhere else along the way (Warner *et al.*, 2013). Even if the seafood inspection is ruled out, the assessment of the processed forms is difficult owing to loss of the morphological characters and appearance. This includes all the processed forms such as restaurant seafood, fish fillets, and processed fish products (Smith *et al.*, 2008). In order to overcome such problems a concrete method of inspection is required, which could discern the seafood at live as well as its processed forms. Still the identification of live specimens is not an easy task and indeed a taxonomist work, as numbers of seafood species are more, and many species under single genus bears a like morphometric characters, leading to confusion. The DNA barcoding is the method which has potential to validate the identity of specimen in live or processed forms. In the present study, crabs were targeted for validating their identity using modern method of DNA barcoding.

Sea food mislabeling and substitution is not newfangled act as various researchers have already discerned this. Mislabeling is not only threat to the consumer but also to the biodiversity as it facilitates substitution of inferior or undersized seafood item. Inferior seafood may be a different low coast species or would be an undersized species of same category. If smaller versions are sold with the same price tag, it could be one of the seafood frauds, as small size varieties have less tasty meat than their fully grown varieties (Pers. comm.). Thus, selling of undersized fish indirectly promotes undersized fishing leading to threat to sustainable fisheries and biodiversity. According to the United Nations Food and Agriculture Organization statistics, 52% of the world's fish stocks are fully exploited, 24% are over exploited, depleted, or recovering from depletion, 21% are moderately exploited, and only 3% are underexploited (Owens, 2008). The researchers have studied the effect of over fishing, which suggested a complete worldwide disintegration of international marine fisheries by 2050 (Worm *et al.*, 2006). To overcome this kind of future peril, some firms in countries like Europe (Natureland), America (Ecofish) and UK (Marine Stewardship Council) have started sustainable labeling of seafood. The 'Ecolabelling' schemes are formulated by these organizations to certify and encourage labels for products from controlled marine capture fisheries so as to spotlight on problems related to the sustainable use of fisheries resources. These labels provide information on the source of seafood right back to the boat or the pond it was caught by. These labels are certainly effective in providing the source of seafood and promote sustainable fisheries but lacks taxonomic details of the species. When the seafood menu served in restaurant was analyzed, it was found that general terminologies were used instead of specific species of seafood. For example menu includes names as Fish curry, Prawn curry, Crab curry etc. Sometimes some restaurants add more details like pomfret fish curry, Jumbo shrimp curry, King crab curry in their menu card, which is also insufficient and prop up seafood fraud. Owing to huge numbers of species under each seafood group, proper labels assisted with species identity details are paramount requirement in restaurants to reduce seafood fraud (Armani *et al.*, 2012; Vartak *et al.*, 2015). Currently, the Maryland Food, Drug, and Cosmetic Act as well as guidelines set in place by the U.S. Food and Drug Administration prohibit mislabeling of seafood products (<http://cnsne.w s/1cz W7Gb>). However, it is very important to note that the labeling seafood without strong taxonomic and molecular database support could not solve the actual purpose of labeling. The exact nomenclature for the particular seafood up to species level is extremely important and shall be made mandatory without any pretexts. Then only the labeling programmes would come up with the success and lessen seafood fraud. Miller *et al.*, (2012) has raised the need of global investigations to substantiate the facts about species supersession and other forms of seafood fraud on a broader geographic scale. Hence, in this paper, we have come up with the pragmatic solution for this kind of global problem, which has support of modern molecular method i.e. DNA barcoding. In the present work, we have taken crabs to explain the process of modern labeling but almost all important seafood species could be labeled with this process as DNA barcodes are

available for almost all major seafood species. Even if they are not available, they could be developed with the help of DNA barcoding technique. The process of seafood labeling proposed is very simple, economical and easy to adopt. In the present scenario, this kind of process would be very innovative approach for the restaurant owners and seafood consumers of India. But this should take the start and the present work is aimed to initiate this start. Those restaurants that adopt and follow this process would surely get the high consumer preference as consumers get valid information and proof about what they eat. In India seafood labeling regulations are required to be formulated so as to support the consumer and food safety. The present method of labeling suggested may be helpful in the process of formation of seafood labeling regulation in India.

DNA barcode assisted seafood labels- with an example of crabs

Crabs are one of the famous seafood items throughout the world. They are generally served with the shell on in the restaurants. This affects food safety as it leads to mislabeling and species substitution in the seafood industry. In order to address this problem authentication of seafood and related products has become a market priority (Pardo *et al.*, 2009). Amongst the numerous molecular methods, DNA barcoding is very recent and effective technique. This method was satisfactorily utilized for identification and authentication of crustaceans. However, till date there is no such tool for rapid detection of samples using DNA barcoding. In this scenario, we have developed a process which could be used for labeling the seafood product by DNA barcode assisted tags. This would be helpful for consumers as they would feel safe during purchase and consumption of authenticated labeled products. Once DNA barcode labels become mandatory, the supplier would be conscious and truthful while selling of the product. Due to this labeling, seafood trader would be unable to sell the product with a different name other than mentioned in the barcode label. Hence the proposed DNA barcode with electronic barcode for crabs would have applicability for all food item at any point of sale like restaurants, exports etc.

The process of DNA barcode assisted seafood barcode labels

DNA barcodes were developed for 12 edible crabs using DNA barcoding protocol that involves DNA extraction from crab tissue, PCR and sequencing. Further, these sequences were edited using bioinformatics tools to get the DNA barcodes. These barcodes were submitted to NCBI to get the accession numbers for each DNA barcode. Then these accession numbers for twelve identified crab species from the national Centre for Biotechnology Information (NCBI) GenBank and scientific name of the species has been used as input data for barcode creation software to create the automated barcode label. These barcodes are readable by an electronic barcode scanner, which analyzes the barcode's image data with sensor and provides information of species. These barcode labels are printable and help in labeling sea food items.

Application and use of the DNA barcode assisted electronic barcodes seafood labels in restaurants

Presently, there are no instant methods for species identification of tissues with DNA barcoding tool. Hence, a

process of seafood labeling with DNA barcodes assisted electronic barcodes has been developed. These are made by the combination of DNA barcode accession numbers and scientific name of the species (Table 1).

Table 1 - List of species with their DNA barcode assisted electronic barcode labels.

Scientific Name of Species	Electronic Barcodes
<i>Atergatis integerrimus</i>	 KC928392-Atergatis integerrimus
<i>Barytelphusa cunicularis</i>	 KC928397-Barytelphusa cunicularis
<i>Charybdis feriata</i>	 KC760152-Charybdis feriata
<i>Charybdis helleri</i>	 KF574086-Charybdis helleri
<i>Charybdis lucifera</i>	 KC760154-Charybdis lucifera
<i>Portunus pelagicus</i>	 KF443208-Portunus pelagicus
<i>Portunus reticulatus</i>	 KF717538-Portunus reticulatus
<i>Portunus sanguinolentus</i>	 KC760159-Portunus sanguinolentus
<i>Scylla serrata</i>	 KC760161-Scylla serrata
<i>Scylla tranquebarica</i>	 KC760166-Scylla tranquebarica
<i>Varuna litterata</i>	 KF443206-Varuna litterata

Likewise QR codes could be generated which would have all the necessary information of the species and supported by DNA barcode. Once these barcodes are scanned by a barcode scanner the output will be the amalgamation of species and the NCBI accession number which will be displayed in the final bill. Similarly QR codes could be scanned by the QR scanner which is easily available in the smart phones. This benefits customers in two ways, one that he will have an authentic bill with species being accommodated in his plate and two he can utilize this as proof for claiming if he doubts that the

seafood accommodated is different from what sanctioned. A consumer could further utilize DNA barcoding method from sanctioned laboratory to support the species being accommodated to him. If such systematic labeling with accession numbers would come into regulation seafood supplier could not possess the courage to mislabel the product due to fear of becoming exposed for this sham. The conception of utilizing these labels is put forth so as to make DNA barcoding technique consumer cordial and applicable in seafood labeling ventures.

CONCLUSION

There is a need for the development of universal seafood labeling policy on larger geographical scale. The process as such looks simple but need lot of equivalence, research on seafood market and public preference before bringing into force. The labeling can be used by anyone in the sea food industry. But it will give impact if government accepts it and make it mandatory. But even the one who will use this process in the sea food industry on their own will get recognition for the genuineness. This also requires technical and administrative collaborations of technicians and policy makers on global scale. But once this is done, this policy would be beneficial for consumer protection and food safety which shall make DNA barcode assisted seafood labeling as a mandatory procedure at the point of sale of seafood in restaurants, retails markets as well as seafood export.

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