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SDS-PAGE technique as biomarker for fish toxicological studies

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ABSTRACT

Although many studies on the hematological and biochemical parameters in fishes have been done, still there are some shortage in the estimation and evaluation of the baseline's values of marine and freshwater fishes. Recently, the use of hematology and biochemistry of fishes in toxicology, aquaculture, environmental pollution, feeding, and antioxidants studies has been increased. In this study we introduced the importance of those parameters and their importance as biomarkers in fish toxicology from previous literature and as new findings. Hemato-biochemical parameters were widely used in fish toxicological studies. Many researches have used the protein electrophoresis as a valid tool to determining intra and inter-specific variation among species. Protein profile was extensively used in determining the health of fish, as indicators of anemia or other diseases provide information about the existence of the disease, and in the diagnosis of disease. So, to carry out the aim of this study, we reported one of the more advanced techniques used SDS-PAGE as molecular biomarker for protein profile analysis in fish with shedding the light on the importance of hematological and biochemical parameters in fish toxicological studies.

1. Introduction

Studies of blood parameters had proven to be a valuable approach for analyzing the health status of fish as these indices provide reliable information on metabolic disorders, deficiencies, and chronic stress status before they are present in a clinical setting [1], and help in understanding the relationship of blood characteristics to the habitat and adaptability of the species to the environment [1,2].

There is growing interest in the study of haemato-biochemical parameters and these parameters are regarded as important for aquaculture purposes [3]. The haemato-biochemical characteristics of some fish species have been investigated with the aim of establishing normal blood values and ranges [4–7]. Also, comparative studies on blood parameters of fish have been carried out to determine the systematic relationship among certain species [8–10]. Svobodova et al. [11] reported that active species display higher values of haematological parameters compared to less active forms. High RBCs values are associated with fast movement, predaceous nature and high activity with streamlined bodies [12]. The ranges of serum biochemistry varied from species to species and can be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age, and sex of the fish [13,14]. Biochemical baselines values established may allow important clinical decisions about fish species [15,16] and commonly used as diagnostic tool in aquatic toxicology and biomonitoring [17]. Plasma protein is the protein component of the blood and it increases with starvation or any other stress [18]. Plasma protein gives an index of the health status of the brood fish [19] and as indicator of nutritional status [20].

Recently, many studies were done using hematological and biochemical in investigation the effects of UVA [21–25], Gamma radiation [26,27], hevey metals [28], 4-nonylphenol [29–32], and pestisides [33]. There is growing interest in the study of the structural features of fish blood cells regarded as important for aquaculture purposes [34] and environmental pollution studies [21]. Biconvex erythrocytes are the predominant cell type found in the blood of the studied species. Using scanning electron microscopy, the erythrocytes of the some species were characterized by a smooth cell surface like those observed such as Dicentrarchus labrax [35], Monopterus cuchia [36] and Sparus aurata [37], and others such as erythrocytes of Salmo gairdneri larvae, which showed an uneven cell surface with small pits and protrusions [38]. Cytochemical staining is particularly useful for the study of cellular lineage [39]. PAS stains glycogen, which is an energetic source for phagocytes [40–44], with the staining intensity increasing during cell maturation due to the accumulation of glycogen [45].

The electrophoresis of proteins is an effective technique for generating systematic data from macromolecules [46] since proteins are

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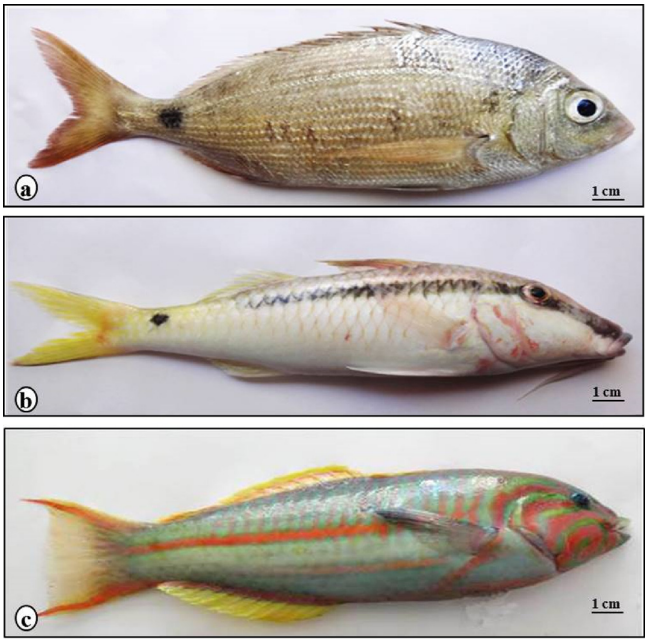

Fig. 1. Photographs of the studied fishes (a) *Diplodus noct* [48], (b) *Parupeneus forsskali*, (c) *Thalassoma klunzingeri*.

species-specific and electrophoretic separations are easily performed [47]. Many researches have used the protein electrophoresis as a valid tool to determining intra and inter-specific variation among species, which may reflect the metabolic level of the organism and its adaptations to environmental fluctuations and the different nutritional status of the fish and feeding habits (McDonald and Milligan [65]; Zowail et al. [66]; Navarro and Gutiérrez [67]). In the present investigation, the liver proteins of *Diplodus noct*, *Parupeneus forsskali* and *Thalassoma klunzingeri* have been analyzed using SDS-PAGE technique and the resemblances and differences between the species was established.

2. Materials and methods

2.1. Fish

Specimens of the Red Sea seabream (*Diplodus noct*) (omnivorous, F: Sparidae) (Fig. 1a; [48]) with a length of (17 ± 0.6 cm, range: 14–21) and a weight of (68 ± 5.05 g, range: 50–95), Red Sea goatfish (*Parupeneus forsskali*) (carnivorous, F: Mullidae) (Fig. 1b) with a length of (18.9 ± 0.3 cm, range: 17–20) and a weight of (65 ± 1.7 g, range: 54–71) and Klunzinger's wrasse (*Thalassoma klunzingeri*) (carnivorous, F: Labridae) (Fig. 1c) with a length of (17.8 ± 0.4 cm, range: 15–20) and a weight of (68.6 ± 3.5 g, range: 51–91) were captured from the Red Sea at Hurghada, Egypt [48]. Netting was the main fishing method used to collect the fish. Fish were examined to be free of external parasites and healthy according to AFS-FHS [64].

2.2. SDS-PAGE technique

Portions of liver (~0.1 g fresh weight) of each fish were suspended in 1.0 ml lysing buffer, heated at 100 °C for 5 min., centrifuged at 10,000 rpm for 30 min, and 50 µL of each extracted protein treatment was used for protein analysis using SDS– PAGE according to [49] in the first dimension. The low molecular weight standards (BioBasic, USA) were run concurrently, and the protein molecular mass was determined using Gel-Pro Analyzer package V3.1 for Windows XP/NT (Media Cybernetica 1993–97). Cluster analysis was done by Past Software version 3.14 based on protein profile. Also, densitometric analysis of protein bands was performed by using Image J program, version 1.48v software (2014).

3. Results

3.1. Protein electrophoresis interpretation

SDS-PAGE protein bands of *Thalassoma klunzingeri*, *Diplodus noct* and *Parupeneus forsskali* are 4, 4 and 6 respectively (Fig. 2). The protein

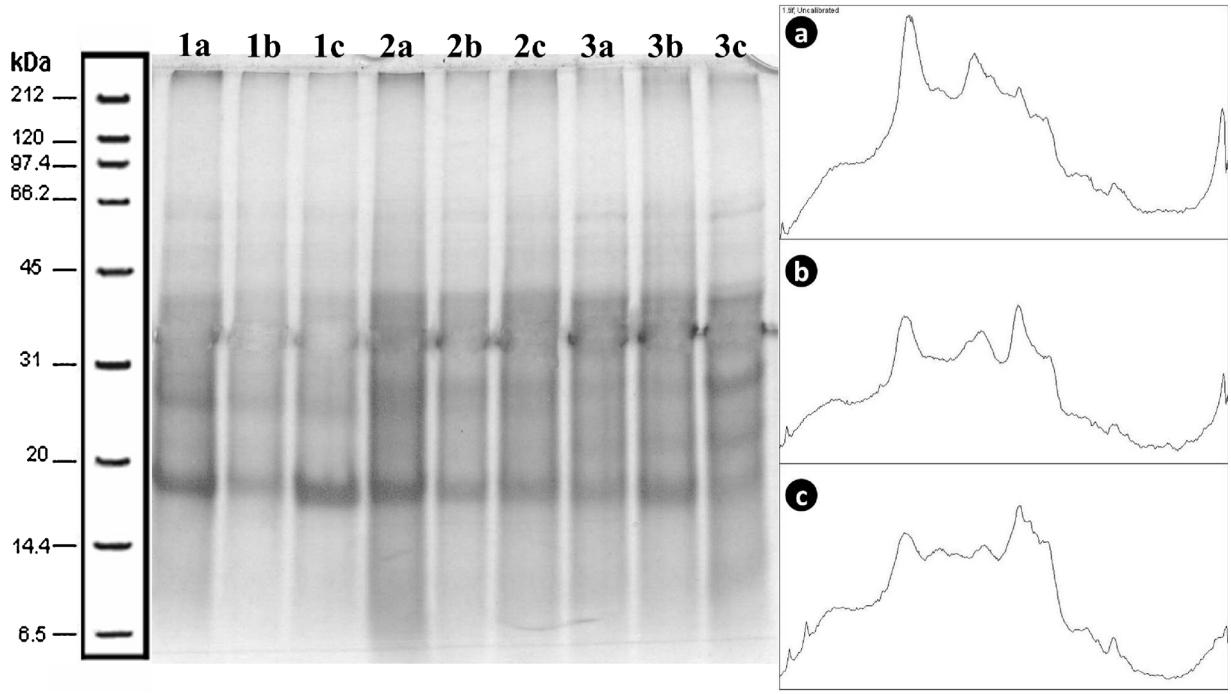

Fig. 2. Electropherogram of Thalassoma *klunzingeri* (lanes 1a, 1b & 1c), *Diplodus noct* (lanes 2a–2c) and *Parupeneus forsskali* (lanes 3a, 3b & 3c) by using liver protein by SDS-PAGE. Densitometric analysis of lane 1a (a), lane 2b (b) and lane 3a (c).

Table 1
Protein fractions (in percent) identified in liver of the Res Sea species. Studied using SDS-PAGE.

Species			
Lanes	*Thalassoma klunzengeri*	*Diplodus noct*	*Parupeneus forsskali*
Protein fractions %			
r1	4.949455136	3.811941	1.744237
r2	0	0	2.374529
r3	12.31764433	0	23.48234
r4	0	39.34948	0
r5	0	31.04771	10.24367
r6	29.70785996	0	0
r7	0	0	50.69507
r8	53.02573615	25.79008	11.462

fractions are identified in terms of their molecular weights. The values of protein fractions (in percent) and molecular weight of each fraction are listed in Tables 1 and 2.

The band obtained from *Thalassoma klunzingeri* is numbered as 3rd protein band with MW of 17.45 kD is not found in the other two species. The band obtained from *Diplodus noct* is numbered as 2nd protein band with MW of 35.62 kD is not found in the other two species. Moreover, the bands obtained from *Parupeneus forsskali* are numbered as 2nd protein band with MW of 40.41 kD and 5th band with MW of 12.31 kD are not found in the other two species (Table 2). According to densitometric analysis (Fig. 2) and cluster analysis (Fig. 3) it is found that *Diplodus noct* and *Parupeneus forsskali* are more similar species than *Thalassoma klunzingeri*. Proteins are the chief source of energy in fishes, since they live in an environment, which is carbohydrate free [50].

4. Discussion

Electrophoresis, have been widely used in the classification of fish, these kinds of studies have brought about a new look to taxonomical evaluation [46]. Discrimination of related taxa can be easily made according to their electrophoretic results of serum proteins [51]. In the present study, electrophoresis of liver protein is used in discrimination of the three species studied. At present, there are number of taxonomical study on fish using SDS-PAGE techniques. In an investigation carried out by [52], the serum proteins of *Capoeta trutta* and *Capoeta capoeta umbla* were analyzed by using SDS-PAGE. These investigators showed that there were 16 bands in *Capoeta trutta* and 11 bands in *Capoeta capoeta umbla*. They concluded that the number of serum protein bands were especially important in taxonomy. Similarly [53], used SDS-PAGE to separate the liver proteins of six species belonging to family Cyprinidae and subfamilies Acheilognathinae, Leuciscinae and

Table 2
Molecular weight (in kda) identification of protein fractions in liver of the Res Sea speciesusing SDS-PAGE.

Species				
Lanes	Marker	*Thalassoma klunzengeri*	*Diplodus noct*	*Parupeneus forsskali*
Protein fractions	Molecular weight (kda)			
r1	212	73.722	74.661	72.794
r2	120			40.418
r3	97.4	36.997		37.468
r4	66.2		35.622	
r5	45		18.798	19.032
r6	31	17.451		
r7	20			12.313
r8	14.4	8.3506	8.4558	8.6704
r9	6.5			

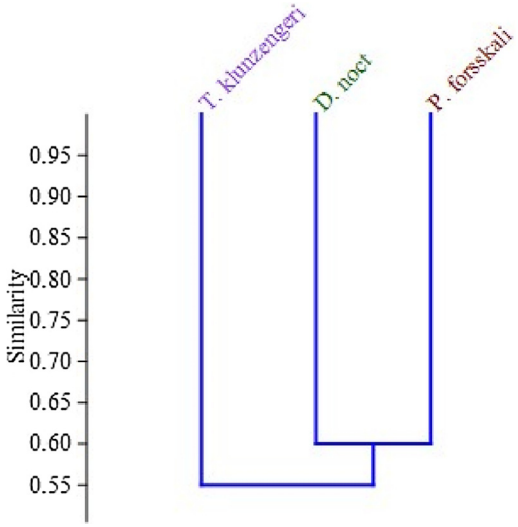

Fig. 3. Cluster analysis for three Red Sea fishes based on profile.

Gobioninae. They pointed out that *Cyprinus carpio* and *Pseudogobius esocinus esocinus* gave the smallest genetic distance.

On the other hand, the sarcoplasmic proteins of *Orthrias insignis euphyraticus* and *Cyprinion macrostomus* were separated by using SDS-PAGE showing that there were differences between the two species in both the number of bands and the molecular weight of the sarcoplasmic proteins [54]. Also, the serum proteins of the female *Cyprinus carpio* and male *Ctenopharyngodon idella* were analysed using SDS-PAGE and it was found that there were differences in the electrophoregrams of each species [55].

Sexual dimorphism in electrophoretic patterns of blood serum proteins of a smelt (*Hypomesus nipponensis*) was investigated by [56]. They found that the blood serum proteins are different in females and males *Hypomesus nipponensis*. The serum protein profiles of parr and of smolt in masu salmon (*Oncorhynchus masou*) have been analysed by two-dimensional SDS-PAGE showing differences in serum protein profiles between the two species [57]. Zowail and Baker [58] studied the serum proteins of five species of freshwater fish (*Sarotheredon galilaeus*, *Tilapia zillii*, *Orecohromis niloticus*, *Clarias lazera*, and *Barbus bynni*) electrophoretically. Eight fractions of serum proteins in *S. galilaeus*, *T. zillii* and *O. niloticus* were reported. In the same research, seven and five fractions of serum proteins were found in *C. lazera* and *B. bynni*, respectively. In the present study, based on cluster analysis *Diplodus noct* and *Parupeneus forsskali* were more similar species than *Thalassoma klunzingeri*. Recent research [59] showed in three fish species (*M. cephalus, S. aurata* and *D. labrax*.) five protein fractions (prealbumin, albumin, α-globulins, β-globulins and γ-globulins) with a different pattern of electrophoretic profile in relation to a different nutritional status. In particular prealbumin is more sensitive to changes in protein-energy status than albumin (Ingenbleek and Young, 2002). Fazio et al. [10], showed the effects of aquatic habitat changes on serum protein electrophoresis of mullet. Other studies [60,61] showed the influence of two different conditions (high stocking density and high salinity levels) on serum protein profile of *Mugil cephalus*.

Electrophoresis cannot demonstrate the identity of two proteins; it can only show differences. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a technique widely used in biochemistry, genetics and molecular biology to separate proteins according to their electrophoretic mobility. In the past, the identification of fish species was carried out mainly by examining the external morphological characteristics. In the present day, electrophoresis of sarcoplasmic proteins, serum proteins, liver proteins and a number of enzymes often has been used by some researchers as an aid in the species identification of fish [53,62,63].

5. Conclusion

Accordingly to the results which mentioned for the first time using SDS-PAGE for some Red Sea fishes, we can conclude that, researchers can use protein profile not only in taxonomy, populations relationship but also to study the toxicological aspects of those species along the Red Sea in some polluted areas.

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