Effect of water quality on hematological and biochemical parameters of *Gobius niger* caught in Faro lake (Sicily)

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In many countries, environmental risk assessment is still based solely on chemical analyses that do not suffice to evaluate the complex toxic effects on the environment. This latter derives from the combined effects of chemicals, their decomposition products, and the physical environment can only be evaluated by biological test using biomarkers (McCarthy and Shugart, 1990). Biomarker analysis of field-collected organisms can provide information on the status of the environment, avoiding the need and uncertainty inherent to the extrapolation of laboratory results (Menezes et al., 2006). Biomarkers are defined as a change in a biological response, ranging from molecular to cellular and from physiological responses to behavioural changes which can be related to the toxic exposure or to the toxic effects of environmental chemicals (Depledge et al., 1995). The use of selected biomarkers has become attractive and useful for monitoring environmental quality and the health of fish inhabiting polluted ecosystems (Fernandes et al., 2008). Fish are largely used for the assessment of aquatic environment quality and are accepted as bio-indicators of environmental pollution (Borkovic et al., 2008). Fish, in fact, live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components. It should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Adeyemo, 2007). Obviously animals did not show a consistent response to all stressors, and the physiological

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changes vary with the species and the stressor (López-Olvera et al., 2006), so stress indicators must be established for each species and circumstance. The haematological profile represents a good indicator of physiological dysfunctions (Elahee and Bhagwant, 2007) and it provides information not only about the health status of fish and the physical and chemical parameters of water in which they live, but even to evaluate the relationship among these factors and correlate them with the status of health of organism respect to environmental conditions (Elahee and Bhagwant, 2007; Debala Devi and Usha Anandhi, 2010; Maceda-Veiga et al., 2010; Ayoola et al., 2011).

Species used in biomonitoring programmes should be sensitive and the measured biomarkers should give a consistent response. Moreover, to assess the local detrimental effects of pollutants reliably on marine biota, a non-migratory species is recommended. *G. niger*, generally called black goby, is a euryhaline teleost fish that can live in brackish waters and is common in estuaries and lagoons. This led us to consider the black goby as a good model organism because it is a territorial species living and feeding at the bottom on sediment. This species has an important ecological value (Filiz and Toğulga, 2009) and an important role in the food chain in Faro Lake. It is also found in polluted and unstable environments, in which their skeletal abnormalities (Cunha and Antunes, 1999) and impaired hematological parameters (Katalay and Parlak, 2004) are considered as indicators of pollution. Our previous work reported the hematological changes in this species caught in different site confirming the use of black goby as model organism to study the pollution conditions in the Mediterranean areas (Fazio et al., 2012a). In particular, it is known that the male of this species is more susceptible respect to female to environmental and water quality changes. The male builds the nest under shells or stones and they guard eggs: these behaviors force him to stay in close contact with the bottom and to be exposed most from changes in the sediment.

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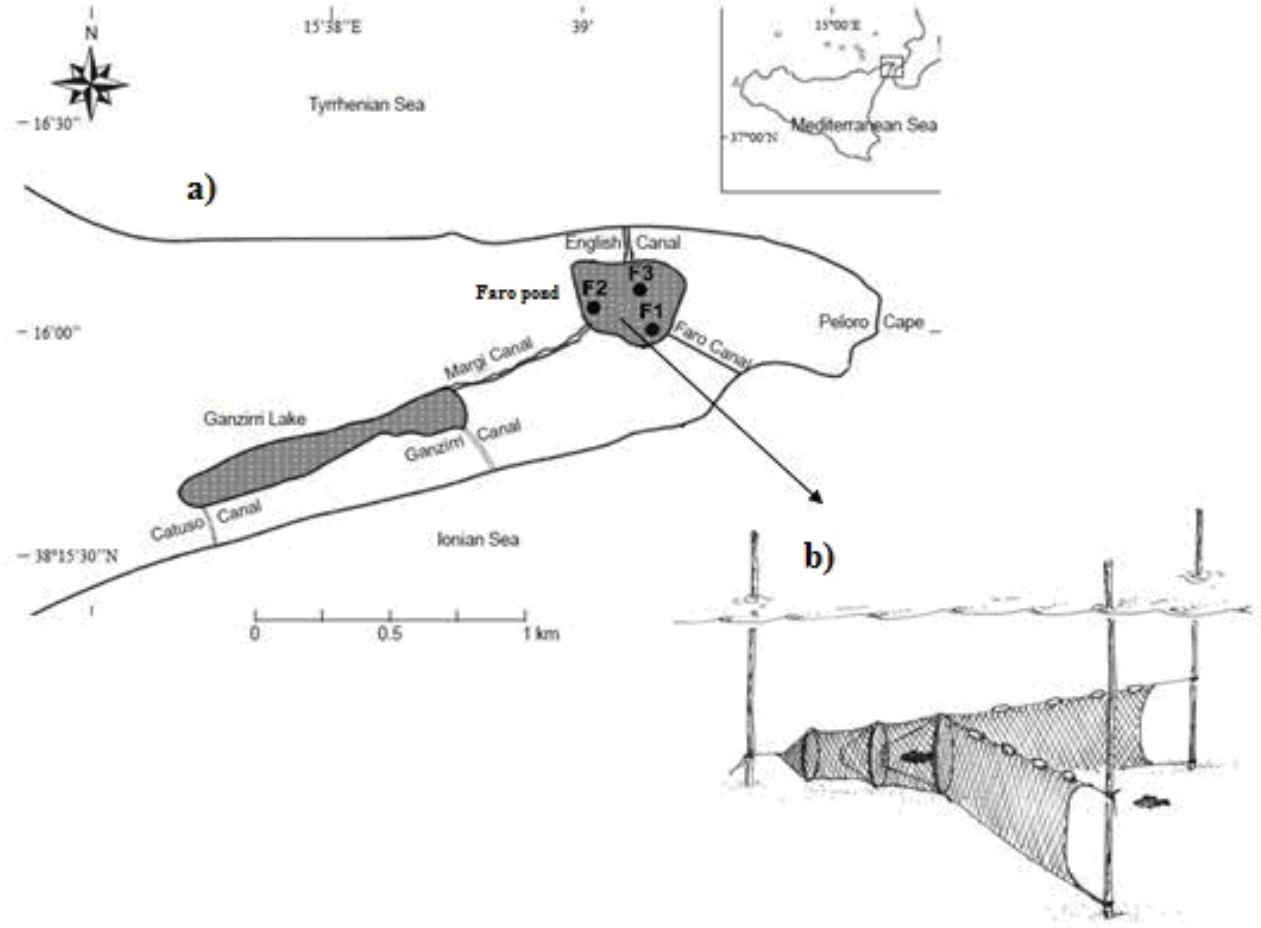

Figure 1: Map of the study site (a) and fishing method (b).

In the present study, 20 specimens of *G. niger*, caught in three different sites (6 fish from F1, 6 from F2 and 8 from F3) of Faro lake (Fig. 1), were used with the aim to assess the influence of water quality on some blood parameters. The three stations were selected randomly and the distances among them were about 3 m. Faro is a small meromictic marine pond (26 ha), locate close to Capo Peloro (Eastern Sicily; 38°15'57" N; 15° 37' 50" E). It is a circular basin with a 500 m diameter, and is deeper in its central part (30 m), whereas its mean depth ranges from 0.5–5 m (Manganaro et al., 2009). The fish were caught with bottom-set nets placed on bottom in May 2010. All animals captured were considered as healthy on the basis of their external appearance, the absence and of obvious sign of disease and considered male and sexually mature on the basis of their length and weight (Bouchereau and Guelorget, 1998).

At the same time of fish collection water samples were collected in F1, F2 and F3 at 50-80 cm below the water surface with plastic containers of 2 L , previously treated with a solution of 0.1 N HCl. Temperature, salinity and dissolved oxygen were measured *in situ* using a multiparametric probe YSI 85 System and pH by a HANNA HI 83140 pH- meters. To assess the other parameters, water samples were kept in an icebox and carried

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to the laboratory in glass water sampling bottles. Water samples were screened through a 200 μm mesh net in order to remove large zooplankton and debris. Sub-samples (500–2000 ml) were filtered onto pre-washed, pre-combusted (450 °C, 4 h) and pre-weighed Whatman GF/F filters (0.45 μm nominal pore size).

Total suspended matter (TSM) determination was carried out gravimetrically after desiccation (105°C, 3 h), using a Mettler M3 balance (accuracy ±1 μg). Nutrient concentrations were determined from prefiltered (through a 0.45 lm Millipore filter) and frozen subsamples with a VARIAN Cary50 spectrophotometer using methods described by Innamorati et al. (1990) for nitrate ($N\text{-}NO_3$), nitrite (NO_2–N) and ammonia nitrogen (NH_4–N).

After capture ten *G. niger* were assigned to experimental group (3 from F1, 3 from F2 and 4 from F3). They were immediately subjected to blood collection, weighting (69.00 ± 3.00 g) to the nearest 0.1g (Kern 440-49N) and measuring (16.50±2.00 cm) with Scubla mm 600 and then they were reintroduced in their habitat. The other ten fish were assigned to control group (3 from F1, 3 from F2 and 4 from F3) and were transported to the laboratory in a well-aerated tank containing 40 L of lake-water. Once they arrived in the laboratory (after ten minutes) the fish were transferred in 180L tank with flowing artificial seawater with the same composition of the natural one. The tank has been set up one month before the experimental start; it had a double bottom, a filter system equipped with an electric pump through which the tank could be well ventilated and cleaned continuously.

In tank, the fish were acclimated for a period of 15 days under natural spring photoperiod (sunrise at 06:00 h, sunset at 19:00 h) and were fed daily with shell-less mussels (*Mytilus galloprovincialis*) crushed in order to avoid the possible effect of starvation on any of the haematological and haemato-chemical parameters, but the animals were fasted for 24 h prior to blood collection. After the acclimate period, fish were subjected to blood collection, weighting (70.23 ± 2.40 g) and measuring (17.27 ± 2.40) and water characteristics of tank were determined with the same methods using to assess water parameters of Faro lake.

On all animals, blood samples were collected by caudal vein using a sterile plastic syringe (2.5 mL) and transferred to special tubes (Miniplast 0.6 ml, LP Italiana Spa, Milano) containing EDTA (1.26 mg/0.6 mL) as an anticoagulant agent. In order to avoid changes in the variables induced by the manipulation during sampling, fish were anesthetized with 2-phenoxyethanol that have no effect on hematological profile (Velisek et al., 2007). Samplings were made at the same hour of the day for both groups in order to minimize circadian variations (Benneman, 1977). For the assessment of glucose and lactate on whole blood a portable blood glucose analyzer (ACCU-Chek Active, Roche Diagnostics GmbH, Mannheim, Germany) and a portable blood lactate analyzer (Accusport, Boehringer Mannheim, Germany) were used. An automated haematology analyzer (HeCo

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Vet C, SEAC, Florence, Italy) with special lysing reagent for fish (SEAC, Code 71010460), already used to investigate haematological profile in *G. niger* and in *S. aurata* and in (Fazio et al., 2012b, 2012c), was used to assess the hematological profile that involves the determination of the Red Blood Count (RBC), Haematocrit (Hct), Haemoglobin concentration (Hgb), White Blood Cell Count (WBC), Thrombocytes Count (TC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC).

An unpaired t-test was used to determine significant differences in blood glucose, blood lactate and hematological parameters between experimental group and control group. P value < 0.05 was considered statistically significant. Data were analyzed using statistical software Prism v. 4.00 (Graphpad Software Ldt., USA, 2003). Physical and chemical water parameters measured in Faro lake and in tank are represented in Table 1.

Table 1. Chemical and physical parameters of water investigated in Faro lake and tank

Parameters	Units	Groups	Value	SEM	Δ	Percent change
T	°C	*Control*	24.80		0.50	2.0%
		Experiment	25.30	0.10		
pH		*Control*	8.00		0.10	1.0%
		Experiment	8.10	0.00		
Sal	‰	*Control*	32.80		3.20	8.9%
		Experiment	36.00	0.15		
O_2	ml/L	*Control*	6.10		0.20	3.4%
		Experiment	5.90	0.06		
O_2 sat%	%	*Control*	124.60		0.90	0.7%
		Experiment	123.70	1.02		
TSM	mg/L	*Control*	3.10		3.32	51.7%
		Experiment	6.42	1.21		
N-NH_4	mg/L	*Control*	0.006		0.025	80.0%
		Experiment	0.031	0.012		
N-NO_2	mg/L	*Control*	0.004		0.002	33.3%
		Experiment	0.006	0.004		
N-NO_3	mg/L	*Control*	0.003		0.104	97.0%
		Experiment	0.107	0.090		

The presence of any substance in the water produces changes in their quality, which are not always favourable to development and survival of aquatic organisms. When the water quality is affected by toxicant, any physiological changes will be reflected in the values of one or more of the biochemical and haematological parameters (Adham, 2002; Ishikawa et al., 2007).

Each physical parameter is intimately linked with each others, so changes in one parameter affected other factors and then healthy of aquatic organism. Water temperature is

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the most important environmental variable, it together with salinity, affects solubility of gases in water and so oxygen disponibility. The increase of water temperature and salinity are strongly linked with the decrease of dissolved O_2 and then with oxygen saturation. For chemical parameters, our results showed that TSM, N-NH_4, NO_2–N and N-NO_3 have enormous differences in percentage between 33.3 and 97.0% (Table 1). The highest levels of some chemical parameters in Faro Lake is probably due to the phytoplankton blooming, photosynthetic activities of aquatic plants and microscopic algae. Increased in TMS are directly linked with large quantity of algae (Osmann et al., 2010) that is in turn linked with high concentration of NO_2 and NO_3. Nitrite and nitrate is intermediate compound of the nitrification process and elevated levels during unbalanced nitrification can seriously damage fish health and may lead to mass mortalities (Svobodova et al., 2005). Another parameter strongly linked with parameters already listed and with pH is total ammonia-nitrogen. In water, ammonia exists in two forms, unionized ammonia (NH_3) and ammonium (NH_4^+). In Lake NH_3 concentration fluctuates daily because of the effect of photosynthesis and respiration on pH. On the basis of our results on chemical physical parameters, we can assume that the changes found in our biomarkers were strongly due to chemical parameters.

In *G. niger*, glucose levels were significantly lower ($P<0.0003$; $t_{(10)}= 5.481$) in control group than in experimental group, with a difference of 111.87 mg/L. This increase due to high percent change in chemical parameters between two sites of monitoring is probably linked in an attempt to mobilize energy resources to cope with stress and maintain homeostasis (Ackerman et al., 2006). Under stress conditions, hypothalamo-pituitaty interregnal axis elevated blood cortisol which in turn leads to glycogenolysis, lypolysis and gluconeogenesis to provide energy.

Also blood lactate exhibited decrease ($P<0.0001$; $t_{(9)}= 12.84$) in control group compared to that of experimental (difference of 2.68 mmol/L). Vedel et al. (1998) showed that fish exposed to nitrite accumulated it in plasma to approximately twice the ambient concentration, and that it is associated with a small significant increase in blood lactate. In recent study, Sinha et al. (2012) showed that ammonia exposure induced plasma lactate accumulation in *Carassius auratus.*Significantly higher levels of MCH and MCHC were recorded in control group compared with the experimental group ($P<0.003$; $t_{(18)}= 3.31$) with an increase of 7.55 Pg and 4.04 g/L respectively. Various authors (Jee et al., 2004; Saravanan et al., 2011) showed that in fish exposed to different agents, the MCHC and MCH values decreased respect to control group. MCHC is a measure of the concentration of hemoglobin in a given

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volume of packed red blood cells and it is calculated by dividing the hemoglobin by the hematocrit. In our study two groups showed similar value of Hct while Hb decreased in the experimental group, even if not significantly. So low concentration obtained of MCHC in experimental group is due to decrease in Hb that could be due to its decrease synthesis or its oxidation in methemoglobin. Some researches showed that the major outcome of nitrite poisoning is the oxidation of haemoglobin to methemoglobin in erythrocytes (González et al., 2000; Svobodova et al., 2005; Kroupovaa et al., 2008). Consequently blood oxygen transport is compromised, since methemoglobin does not bind oxygen. The decrease in MCHC in experimental group could be due to swelling of RBC also (Milligan and Wood, 1982). Variations of this haematological parameter was found in previous studies on *G. niger* confirming that blood profile is strongly related to environmental changes (Fazio et al., 2011; Fazio et al., in press). Significant decrease in MCH found in experimental group respect to control group could be due to high percentage of immature RBC in the circulation, as seen by Saravanan et al. (2011) in *Cyprinus carpio* exposed to chemicals. Decreases in circulating erythrocytes in *Oreochromis niloticus* exposed to ammonia were reported by Ahamed et al. (1992) and Ishikawa et al. (2007) that found the decrease Hct also. The results of the present study indicate that the highest level of some chemical parameters in Faro lake influence the haematological profile, glucose and lactate levels of *G. niger*. The alterations of these parameters may provide an early warning signals for the determination of toxic effects of chemicals on healthy of fish and to ascertain water quality of Faro Lake. There is a real need to study the interrelationships between the pollution of surface waters by a wide range of chemicals and diseases in natural fish populations, and the processes involved. This represents an important but at present under-developed field of scientific research.

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Table 2. Mean values (± SEM) of hematological profile, glucose and lactate of *G. niger* caught in Faro lake and acclimate in tank.

Parameters	Experimental group x ± SEM	Control group x ± SEM	Experimental group CV %	Control group CV %	Δ	P
RBC ($x10^6/\mu L$)	1.02 ± 0.07	1.11 ± 0.09	21.21%	25.85%	0.09	0.4673
Hct (%)	17.12 ± 1.17	17.13 ± 0.83	21.68%	15.37%	0.01	0.9929
Hgb (g/dL)	4.46 ± 0.29	5.16 ± 0.20	20.45%	12.50%	0.70	0.0620
WBC ($x10^3/\mu L$)	8.92 ± 0.46	8.04 ± 0.25	16.36%	10.06%	0.88	0.1176
TC($x10^3/\mu L$)	33.50 ± 3.11	37.18 ± 2.86	29.32%	24.34%	3.68	0.3957
MCV (fL)	166.40 ± 1.67	169.30 ± 2.23	3.16%	4.17%	2.90	0.3134
MCH (pg)	43.70 ± 1.98	51.32 ± 1.14	14.26%	7.03%	7.62	0.0039
MCHC (g/dL)	26.31 ± 1.12	30.35 ± 0.47	13.51%	4.93%	4.04	0.0039
Glucose (mg/dL)	166.70 ± 19.57	54.83 ± 5.79	37.13%	33.41%	111.87	0.0003
Lactate (mmol/L)	3.20 ± 0.21	0.54 ± 0.04	20.17%	21.10%	2.66	<0.0001

References

Ackerman, P. A., Wicks, B. J., Iwama, G. K. and Randall, D. J., 2006. Low level of environmental ammonia increase susceptibility to disease in Chinook Salmon Smolts. *Physiological and Biochemical Zoology*, 79 (4), 695-707.

Adham G.K., 2002. Subletal effect of aquatic pollution in Lake Maryût on the Afrcan shaerptooth catfish, *Clarias gariepinus*(Burchell,1822). *Journal of Applied Ichthyology*, 18, 87-94.

Adeyemo, O. K., 2007. Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. *Turkish Journal of Fisheries and Aquatic Sciences* 7, 1636-169.

Ahamed, N. A., El-Serafy, S. S., El-Shaey, A. A. M. and Abdel-Haimide, N. H., 1992. Effect of ammonia on some haematological parameters of *Orcheomis niloticus. Proceedings of the Zoological Society A.R. Egypt*, 23 (1),155-160.

Arillo, A., Margiocco, C., Melodia, F., Mensi, P. and Schenone, G., 1981. Ammonia toxicity mechanism in fish: studies on rainbow trout (*Salmo gairdneri* Rich.). *Ecotoxicology and Environmental Safety*, 5, 316-328.

Ayoola, S. O., Kuton, M. P., Idowu, A. A. and Adelekun, A. B., 2011. Acute toxicity of nile Tilapia (*Oreochromis niloticus)* juveniles exposed to aqueous an ethanolic extracts of *Ipomoea aquatica* leaf. *Nature and Science*, 9 (**3**), 91-99.

Begum, S. J., 1987. Biochemical adaptive responses in glucose metabolism of fish (*Tilapia mossambica*) during ammonia toxicity. *Current Science*, 56, 705-708.

Benneman, R., 1977. The influence of acclimation and experimental temperature on glutamate dehydrogenase of carp red lateral muscle(*Cyprinus carpio* L.)*Journal of Thermal Biology*,2, 209-214.

Borkovic, S. S., Pavlovic, S. Z., Kovacevic, T. B., Štajn, A. Š., Petrović, V. M. and Saičić, Z. S., 2008. Antioxidant defence enzyme activities in hepatopancreas, gills and muscle of spiny cheek crayfish (*Orconectes limosus*) from the River Danube. *Comparative Biochemisty and Physiology Part C: Toxicology and Pharmacology*,147 (**1**), 122-128.

[DOR: 20.1001.1.15622916.2013.12.1.18.1]

Bouchereau, J. L. and Guelorget, O., 1998. Comparison of three Gobiidae (Teleostei) life history strategies over their geographical range.*Oceanologica Acta,*21,503-517.

Cunha, P. and Antunes, M. M., 1999. Occurrence of vertebral deformities in Gobiidae (Pisces) from the Tagus Estuary. *Aquatic Ecology*, 33, 239-248.

Debala Devi, C. and Usha Anandhi, D., 2010. Studies on the impact of aquatic pollution on haematological parameters of *Cyprinus carpio* (Linn). *Indian Journal of Environmental & Ecoplanning*, 17 (3), 369-374.

Depledge, M.H., Aagaard, A. and Gyorkos, P., 1995. Assessment of trace metal. Toxicity using molecular, physiological and behavioural biomarkers. *Marine Pollution Bulletin*, 31, 19-27.

Elahee, K. B. and Bhagwant S., 2007. Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the west coast of Mauritius. *Ecotoxicology and Environmental Safety*, 68(3), 361-371.

Fazio F., Faggio, C., Marafioti, S., Torre, A., Sanfilippo, M. and Piccione, G., 2011. Physiological response to caught and handling in *Gobius niger*.*Natura Rerum*,1,21-30.

Fazio, F., Faggio, C., Marafioti, S., Torre, A., Sanfilippo, M. and Piccione, G. 2012a. Comparative study of haematological profile on *Gobius niger* in two different habitat sites: Faro Lake and Tyrrhenian Sea. *Cahiers de Biologie Marine*, 53, 213-219.

Fazio, F., Satheeshkumar, P., Senthil Kumar, D., Faggio, C. and Piccione, G., 2012b. A Comparative study of haematological and blood chemistry of Indian and Italian Grey Mullet (*Mugil cephalus* Linneaus 1758). HOAJ Biology 5 (1), 1-5.

Fazio, F., Filiciotto, F., Marafioti, S., Di Stefano, V., Assenza, A., Placenti, F., Buscaino, G., Piccione, G. and Mazzola, S., 2012c. Automatic analysis to assess haematological parameters in farmed gilthead sea bream (*Sparus aurata* Linnaeus, 1758). Mar. Freshwat. Behav. Physiol. 45 **(1)**, 63-65.

Fernandes, C., Fontaìnhas-Fernandes, A., Rocha, E. and Salgano, M. A., 2008. Monitoring pollution in E,smorzi-Paramos lagoon, Portugal: liver histological and biochemical effects in *Liza sapiens. Environmental Monitoring and Assessment*, 145, 315-322.

[DOR: 20.1001.1.15622916.2013.12.1.18.1]

Filiz, H. and Toğulga, M., 2009. Age and growth, reproduction and diet of the black goby, (*Gobius niger*) from Aegean sea, Turkey. *Journal of Fisheries Sciences*,3(3),243-265.

González, J. F., Del Valle, P. L., Thohan, S. and Kane, A. S., 2000. Effects of waterborne nitrite on phase I-II biotransformation in channel catfish (*Ictalurus punctatus*), *Marine Environmental Research*, 50, 29-32.

Innamorati, M., Ferrari, I., Marino, D. and Ribera D'Alcalà, M., 1990. Metodi nell'ecologia del plancton marino, *Nova Thalassia*, 11, 371.

Ishikawa, N. M., Tavares Ranzani-Paiva, M. J., Lombardi, J. V. and Ferreira, C. M., 2007. Haematological parameters in nile Tilapia, *Oreochromis niloticus* exposed to sub-letal concentrations of mercury. *Brazilian Archives of Biology and Technology*, 50, 619-626.

Jee, J. H., Kim, S. G. and Kang, J. C., 2004. Effects of phenanthrene on growth and basic physiological functions of the olive flounder, *Paralichtys olivaceus*. *Journal of Experimental Marine Biology and Ecology*, 304, 123-136.

Jensen, F. B., Andersen, N. A. and Heisler, N., 1987. Effects of nitrite exposure on blood respiratory proprieties, acid–base and electrolyte regulation in carp (*Cyprinus carpio*). *Journal of Comparative Physiology* 157B, 533- 542.

Katalay, S. and Parlak, H., 2004. The Effects of Pollution on Haematological Parameters of Black Goby (*Gobius niger* L., 1758) in Foça and Aliağa Bays. *Journal of Fisheries & Aquatic Sciences*, 21, 113-117.

Kroupovaa, H., Machovaa, J., Piackovaa, V., Blahovab, J., Dobsikovab, R., Novotnyb, L. and Svobodova, Z., 2008. Effects of subchronic nitrite exposure on rainbow trout *(Oncorhynchus mykiss). Ecotoxicology and Environmental Safety*, 71, 813-820.

López-Olvera, J., Marco, I., Montané, J. and Lavín, S., 2006. Transport stress in Southern chamois (*Rupicapra pyrenaica*) and its modulation by acepromazine. *Veterinary Journal*,172(2),347-355.

Maceda-Veiga, A., Monroy, M., Viscor, G. and De Sostoa, A., 2010. Changes in non-specific biomarkers in the Mediterranean

[DOR: 20.1001.1.15622916.2013.12.1.18.1]

barbel (*Barbus meridionalis*) exposed to sewage effluents in a Mediterranean stream (Catalonia, NE Spain). *Aquatic Toxicology*, 100, 229-237.

Manganaro, A., Pulicanò, G., Reale, A., Sanfilippo, M. and Sarà, G., 2009. Filtration pressure by bivalves affects the trophic conditions in Mediterranean shallow ecosystems. *Chemistry and Ecology*, 25 (**6**), 467-478.

McCarthy, J. F. and Shugart, L. R., 1990. Biological markers of environmental contamination. In: McCarthy, J.F., Shugart, L.R. (Eds.), *Biomarkers of Environmental Contamination.* pp.3-14. Lewis Publishers, Boca Raton, FL.

Menezes, S., Soraes, A. M. V. M., Guilhermino, L. and Peck, M. R., 2006. Biomarker responses of the estuarine brown shrimp *Crangon crangon* L. to non-toxic stressor: temperature, salinity and handling stress effects. *Journal of Experimental Marine Biology and Ecology*, 335, 114-122.

Milligan, C. L. and Wood, C. M., 1982. Disturbances in haematology, fluid volume. Distribution and circulatory function associated with low environmental pH in the rainbow trout *Salmo gairdneri. Journal of Experimental Biology* 9, 397-415.

Osmann, A. G. M., Al-Awadhi, R. M., Harabawy, A. S. A. and Mahmoud, U. M., 2010. Evaluation of the use of protein electrophoresis of the African catfish *Clarias gariepinus* (Burchell,1882) for biomonitoring aquatic pollution. *Environmental Research Journal*, 4 **(**3), 235-243.

Saravanan, M., Prabhu, K. and Ramesh, M., 2011. Haematological and biochemical responses of freshwater teleost fish *Cyprinus carpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. *Pesticide Biochemistry and Physiology*, 100, 206-211.

Sinha, A.K, Liew, H. J., Diricx, M., Blust, R. and De Boeck, G., 2012. The interactive effects of ammonia exposure, nutritional status and exercise on metabolic and physiological responses in gold fish (*Carassius auratus L.*) *Aquatic Toxicology*, 109, 33-46.

Svobodova, Z., Machova, J., Drastichova, J., Groch, L., Luskova, V., Poleszczuk, G., Velisek, J. and Kroupova, H., 2005. Haematological and biochemical profile of carp blood following nitrite exposure at different concentration of

[DOR: 20.1001.1.15622916.2013.12.1.18.1]

chloride. *Aquaculture Research*, 36, 1177-1184.

Van Vuren, J. H. J., 1986. The effects of toxicant on the haematology of *Labeo umbratus* (Teleostei:Cyprinidae). *Comparative Biochemical and Physiology*, 93,155-159.

Vedel, N. E., Korsgaard, B. and Jensen, F. B., 1998. Isolated and combined exposure to ammonia and nitrite in rainbow trout (*Oncorhynchus mykiss*): effects on electrolyte status, blood respiratory properties and brain glutamine/glutamate concentrations. *Aquatic Toxicology*, 41, 325-342.

Velíšek J., Svobodová Z. and Piačková V., 2007. Effects of 2-Phenoxyethanol anaesthesia on haematological profile on Common Carp (*Cyprinus carpio*) and Rainbow Trout *(Oncorhynchus mykiss)***.** *Acta Veterinasria BRNO*, 76, 487-492.

[DOR: 20.1001.1.15622916.2013.12.1.18.1]