Physiological response to caught and handling in *Gobius niger*

Francesco Fazio[1], Caterina Faggio[2], Simona Marafioti[1], Agata Torre[2], Marilena Sanfilippo[3] & Giuseppe Piccione[1]

[1] Department of Experimental Science and Applied Biotechnology, Faculty of Veterinary Medicine, Polo Universitario Annunziata, University of Messina - 98168 Messina, Italy; *francesco.fazio@unime.it*
[2] Department of Life Science "M. Malpighi", Faculty of Science MM.FF.NN., University of Messina - Viale Ferdinando Stagno d'Alcontres, 31 - 98166 S. Agata - Messina, Italy
[3] Department of Animal Biology and Marine Ecology, Faculty of Science MM.FF.NN., University of Messina - Viale Ferdinando Stagno d'Alcontres, 31 - 98166 S. Agata - Messina, Italy

Abstract. The authors assessed significant changes in glucose, lactate, MCH and MCHC in *Gobius niger* due to capture and handling. Ours results suggest that these parameters are useful indicators in assessing the stress of capture and handling of fish in order to improve their welfare and reduce the mortality rate.

Keywords: glucose, lactate, haematological parameters, caught, *Gobius niger*, Pisces.

INTRODUCTION

In recent years the concept of biological stress applied to fishes has attracted considerable attention among scientists dedicated to the research of environmental influences on health (Barreto & Volpato, 2006). Stress in fish results from real or perceived challenges or conditions that extend homeostatic processes beyond their ability to control routine physiological functions (Wedemeyer et al., 1990). Whilst there is enormous variety in the types of stressors, it is generally agreed that the exposition to a stressor produces a cascade of events, which develop through different stages evoking an ordered sequence of well-defined physiological changes (Barton & Iwama, 1991; Sumpter, 1991). Fish capture, handling, confinement, crowding and transport can be typical stressors (Carragher & Rees, 1994; Gomes et al., 2003). The physiological response to stress can be divided into primary, secondary and tertiary components, according to the levels of organisation at which it operates (Wendelaar Bonga, 1997).

The primary response is characterized by neuroendocrine modifications such as increases in plasma levels of the catecholamines adrenaline and noradrenaline, and slower but more sustained increases in plasma levels of the corticosteroid cortisol (Sumpter, 1997). As a direct consequence of their high levels in the circulatory system, a wide range of secondary stress responses can be observed, such as the increase in blood glucose (Roncarati et al., 2006) with the consequent decrease of hepatic glycogen that is quickly converted to glucose. Regardless of the wide use of glucose as an indicator of stress, some authors emphasized that plasma glucose could be used as the only index (Mommsen et al., 1999; Flodmark et al., 2002). Usually the increase of glucose in blood is not as rapid as for cortisol. Many authors showed an increase of glucose levels after minutes or days from the stress stimulus because cortisol triggers glucose production (Pratap & Wendelaar-Bonga, 1990; Hemre & Krogdahl, 1996; Barcellos et al., 1999; Falahatkar & Barton, 2007).

The secondary response includes changes in a range of physiological, haematological, immunological parameters (Barton & Iwama, 1991), changes in behaviour and in particular increase in swimming activity (Xu et al., 2006). If the stress is severe or prolonged, tertiary responses that include alterations in disease resistance, growth, reproductive output (Sumpter, 1991; Lowe et al., 1993) occur. The responses examined in this study included evaluation of blood glucose, lactate concentrations and haematological parameters which are all commonly used to assess the degree of acute stress experienced by fishes (Wedemeyer et al., 1990). The aim of our research is to characterize the secondary responses to stress due to capture in *Gobius niger*, through the evaluation of some haematochemical parameters (blood glucose and blood lactate) and haematological profile that are easily assessed and useful for monitoring of fish welfare.

MATERIALS AND METHODS

Twenty *Gobius niger* (Fig. 1), divided in two equal groups (control group and test group) and caught in three different sites in Faro pond, were used in this study (Fig. 2). 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 500m diameter, and is deeper in its central part (30 m), whereas its mean depth ranges from 0.5-5m (Manganaro et al., 2009).

The fish were caught with bottom-set nets placed on bottom for a period between 5-7 hours during May 2010. All animals captured were considered as healthy on the basis of their external appearance and the absence of obvious sign of disease. During the fishing operations, chemical and physical parameters (temperature, pH and salinity) of water were measured in three sampling stations with a multiparametric probe YSI 85 System (YSI Incorporated USA).

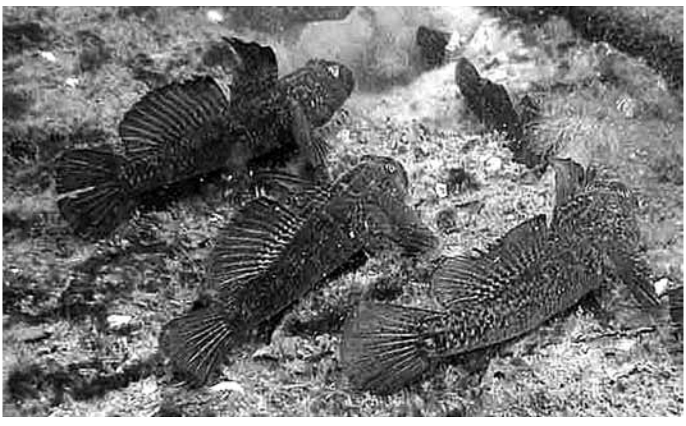

Fig. 1 – Image of *Gobius niger* (Black goby), a marine teleost.

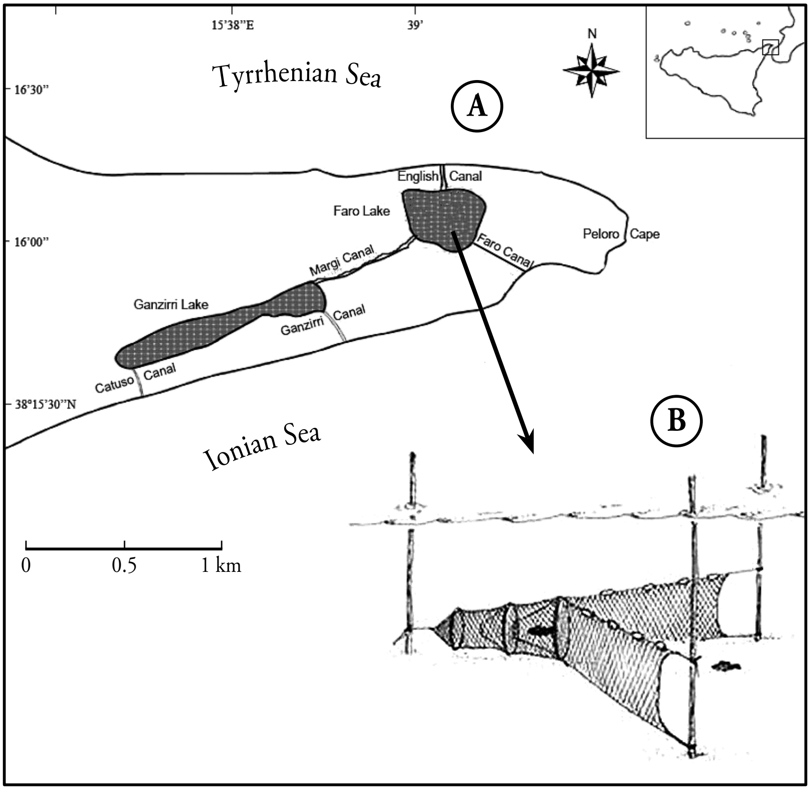

Fig. 2 – Map of the study site (A) and fishing method (B).

After capture, ten *G. niger* were assigned to control group (Group A 17.27±2.40 cm of length and 70.23±2.40 g of weight) and were transported in tank containing 40L of pond-water which was equipped with an aerator. The time needed to transport the fish from the site of capture to the laboratory was approximately 10 min. In the laboratory, the fish were transferred in 150 L tank containing pond-water in order to maintain the same physical and chemical conditions of the water observed at site of capture (Tab. 1). In this tank, the fish were acclimated for a period of 7 days to restore from the effects of capture, handling and transport. During this time fish were kept under natural photoperiod (sunrise at 06:00, sunset at 19:00) 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 haematochemical parameters. Feeding was stopped at least 12 h before blood collection. Kieffer (2000) showed that physiological changes that occur during handling for most fish species to fully recover take approximately 8-12 h. On the contrary other authors have shown that only 4 h are enough to recover the levels of haematological parameters (Frisch & Anderson, 2000, 2005). Our protocol provided 7 day as an acclimation period for Group A, on the basis of this knowledge and in absence of data related to recover of haematological and haematochemical parameters after capture and handling in *G. niger*.

Tab. 1 – Chemical and physical parameters of water investigated in natural habitat and in tank.

	Experimental protocol	
Parameters	**Natural Habitat** (Faro pond) (M ± SD)	**Tank**
T (°C)	25.30 ± 0.88	25.10
pH	8.11 ± 0.03	8.0
Sal (%)	33.43 ± 0.81	33.40

The other ten fish, were assigned to test group (Group B 16.50±2.00 cm of length and 69.00±3.00 g of weight).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.

For Group A, blood samples were taken after 7 days of acclimation period. In order to avoid changes in the variables induced by the manipulation during sampling, fish were anesthetized with 0.5 ml/L of 2-phenoxyethanol that have no effect on haematological profile (Velisek et al., 2007).

For Group B, blood samples were taken after capture and handling of 1 minute. 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 Vet C, SEAC, Florence, Italy) with special lysing reagent for fish (SEAC, Code 71010460) was used to assess the complete haematological profile that involves the determination of the Red Blood Count (RBC), Hematocrit (Hct), Hemoglobin concentration (Hgb), White Blood Cell Count (WBC), Thrombocytes Count (TC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC).

An unpaired t-test was used to determine significant differences in blood glucose, blood lactate and haematological parameters between Group A and Group B. P value < 0.05 was considered statistically significant. Data were analyzed using statistical software Prism v. 4.00 (Graphpad Software Ldt., USA, 2003).

RESULTS

In *G. niger*, glucose levels were significantly lower ($P<0.0003$; $t_{(18)}= 5.481$) in Group A than in Group B, with a difference of 111.87 mg dL^{-1}. Blood lactate exhibited decrease ($P<0.0001$; $t_{(18)}= 12.84$) in Group A compared to that of Group B (difference of 2.68 mmol L^{-1}). Significantly higher levels of MCH and MCHC were recorded for the control group compared with the test group ($P<0.003$; $t_{(18)}= 3.31$) with an increase of 7.55 Pg and 4.04 g dL^{-1} respectively (Fig. 3). All obtained values of haematological parameters were within the physiological range (Katalay & Parlak, 2004). Mean values ± SD of haematological parameters, glucose and lactate are reported in Fig. 3.

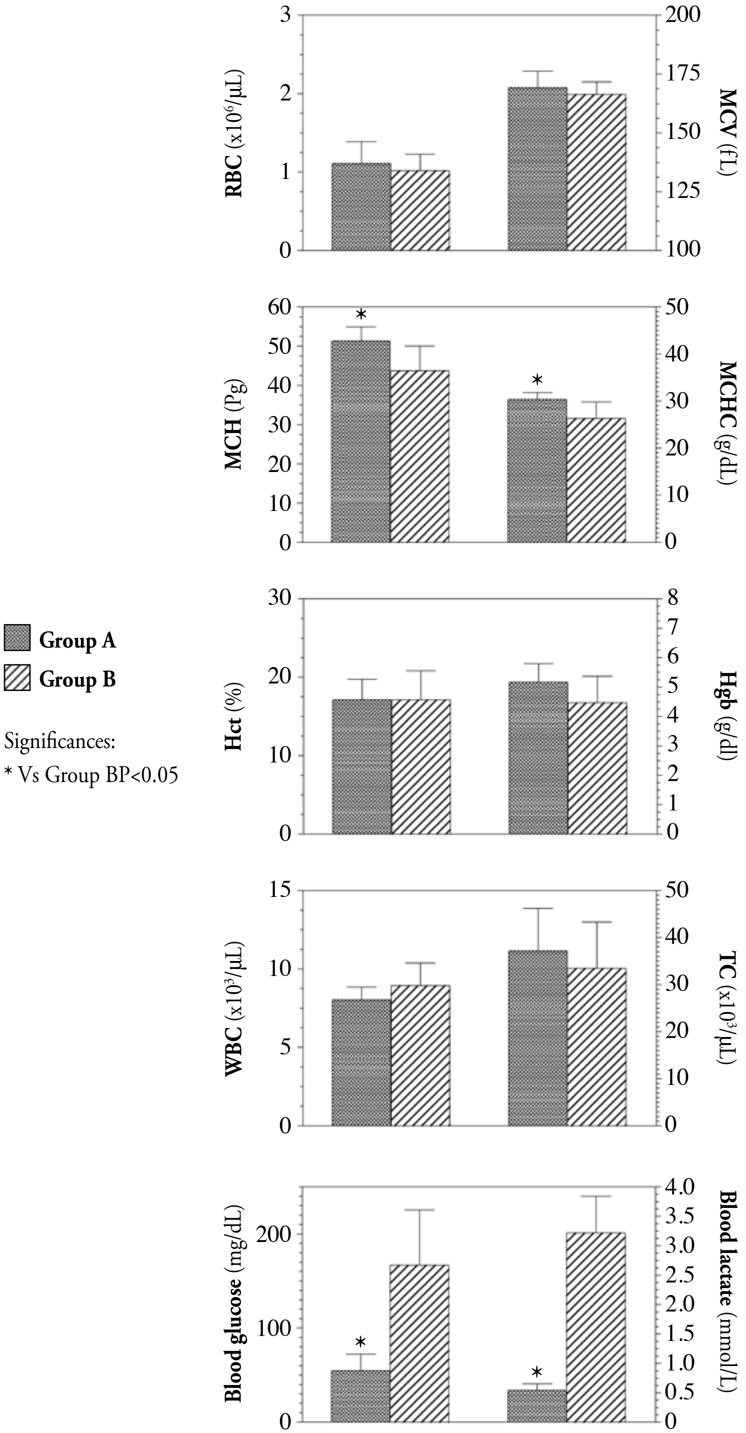

Fig. 3 – Mean value ± SD of studied parameters for Group A and Group B with the statistical significances.

DISCUSSION

The results of this study showed a significantly increase in blood glucose in Group B compared to Group A confirming that this parameter undergoes changes during fish capture and handling. In agreement with our results, Frisch and Anderson (2005) have found that in the coral trout there is an increase in plasma glucose concentration within 15 minutes of capture from the wild or in response to shallow water stress. The rise in plasma glucose levels can be presumed to indicate the occurrence of a classic stress response because following the initiation of the stress response there is mobilisation of energy by glycogenolysis and gluconeogenesis; it is known that both processes result in elevated blood glucose levels (Haux et al., 1985; Laidley & Leatherland, 1988).

Sometimes no significant changes in blood glucose may be observed, because under stress the fish is rapidly consuming the energetic substrates generated (glucose) since the main function of the central nervous system is to maintain homeostasis (Mazeaud et al., 1977; Pickering, 1981). Fast et al. (2008) did not observe a glucose rise in the Atlantic salmon during acute stress, but reported an increase when the fish were exposed to prolonged stress.

Blood lactate concentration obtained on two groups of *G. niger* showed that this parameter similarly to blood glucose exhibited increased values in fish just caught (Group B) respect to fish kept in tank (Group A). This increase may be due to attempt of fish to wriggle free from the nets and from hands of an operator. Movements at high speed requires large increase in muscular activity, so, when this exceeds the ability of the circulatory system to transport oxygen to the active tissues, anaerobic metabolism supplements the aerobic metabolism (Heath & Pritchard, 1962; Brett, 1972). The capacity for anaerobic energy production has been estimated from decreases in substrate reserves and products accumulation with a conversion of glucose to lactate followed by stimulation of piruvate metabolism and oxygen debt (Puckett & Dill, 1984, 1985; Kauffman, 1990; Goolish, 1991). Lactate produced in muscle fibers is released into the circulatory system following bursts of anaerobic activity. Thus, changes in lactate concentration are explained as a strategy to increase oxygen carrying capacity of blood or as a consequence of increased swimming activity during a periods of high energy demand (Ortuno et al., 2001). Acerete et al. (2004) established that lactate concentration increases significantly in several flowing severe exercise or as a result of hypoxia. In some studies, lactate is reported to keep rising for about half an hour following exercise (Richards et al., 2002), whereas in others, lactate reaches its maximum at the end of the exercise (Milligan et al., 2000).

In the values obtained on the haematological parameters of *G. niger*, no significant changes were recorded except for MCH and MCHC that resulted higher in Group A than in Group B. The most important changes in the red blood cell count, haematocrit and MCV values, but in particular a decrease in MCHC value were observed also in carp during harvest (Svobodova et al., 2006). Obviously animals did not show a consistent response to all stressors, and the physiological changes vary with the species and the stressor (López-Olvera et al., 2006), so stress indicators must be established for each species and circumstance (Moberg, 1987).

A study on *Tilapia zillii* and *Clarias gariepinus*, that as *G. niger* are euryhaline teleost fish and live in brackish waters, showed a different response to stress. In particular in *T. zillii* Ht, RBC, MCV and MCHC decreased following the exposure to handling, Hb values increased significantly and the other haematological parameters increased but not significantly after stress; in *C. gariepinus* Ht, RBC, Hb values decreased following the exposure to stress, while MCV, MCH and MCHC increased after the stress (Gbore et al., 2006). Mean cell haemoglobin concentration measure was used to assess the amount of red cell swelling (decreased MCHC) or shrinkage (increased MCHC) present (Milligan and Wood, 1982). The present study shows that capture and handling induced marked red cell shrinkage (increased MCHC). The significant change in MCH may be due the reduction in cellular blood iron, resulting in reduced carrying capacity of blood and eventually stimulating erythropoiesis (Hodson et al., 1978). All haematological and haematolchemical parameters tested are to be referred to the chemical-physical parameters of Faro lake, but are closely related with capture and handling stress. This study supports earlier findings showing that the rise in circulating glucose and lactate following a stressful conditions such as capture and handling are higher rather than in rest condition fish. It is important to establish the most accurate method to measure stress of capture and handling to improve the welfare and reduce the mortality rate of fish.

Acknowledgements. The authors thank the members of SEAC diagnostic group for their scientific, technical and logistic support.

RIASSUNTO

Risposte fisiologiche alla cattura e alla manipolazione in *Gobius niger*

Gli autori studiano la risposta fisiologica indotta dallo stress da cattura e manipolazione in *Gobius niger,* teleosteo marino presente nel lago di Faro (Messina), un piccolo pantano meromittico marino, situato nella punta nord-est della Sicilia vicino al promontorio di Capo Peloro (38°15'57" N; 15° 37' 50" E). Allo scopo 20 soggetti, divisi in due gruppi, sono stati utilizzati nella presente indagine. Dieci soggetti (Gruppo A) che rappresentavano il gruppo controllo, dopo la cattura e la conseguente manipolazione, sono stati trasportati in laboratorio e acclimatati per sette giorni in un acquario di 150 litri. Gli altri dieci soggetti, assegnati al gruppo sperimentale (Gruppo B), sono stati sottoposti a prelievo di sangue subito dopo la cattura e la conseguente manipolazione. Inoltre, nei siti di cattura sono stati effettuati campionamenti di acqua al fine di valutarne i principali parametri chimico- fisici (temperatura, pH e salinità) mediante l'utilizzo di una sonda multiparametrica (YSI 85 System). Su tutti i campioni di sangue, prelevati dalla vena caudale, sono state determinate le concentrazioni di glicemia mediante un glucosimetro (ACCU-Chek Active, Roche Diagnostics) e lattatemia mediante un lattacidometro (Accusport, Boehringer), mentre in laboratorio è stato valutato il profilo ematologico utilizzando un contaglobuli elettronico (HeCo Vet C, SEAC, Firenze). L'analisi statistica dei dati ha mostrato una significativa diminuzione nelle concentrazioni di glucosio e di lattato nel Gruppo A rispetto al Gruppo B con significatività di $P < 0.0001$ e $P < 0.0003$, mentre i valori del contenuto emoglobinico corpuscolare medio (MCH) e della concentrazione emoglobinica corpuscolare media (MCHC) hanno presentato un aumento significativo nel Gruppo A rispetto al Gruppo B con valori di $P < 0.003$. I risultati ottenuti suggeriscono che i parametri valutati nella presente indagine possono essere considerati utili indicatori nella valutazione della risposta secondaria indotta dallo stress da cattura e conseguente manipolazione ed inoltre forniscono valide informazioni per migliorare le condizioni di benessere in questa specie.

REFERENCES

Acerete, L., Balascha, J.C., Espinosab, E., Josab, A. & Tort, L. (2004). Physiological responses in Eurasian perch (*Perca fluviatilis,* L.) subjected to stress by transport and handling. Aquaculture **237**: 167-178.

Al-Kahem, H.F. (1995). Behavioural Responses and changes in some Haematological parameters of the cichlid Fish *Oreochromis Niloticus*, Exposed to trivalent chromium. Journal of King Abdulaziz Univerity Science **7**: 5-13.

Barcellos, L.J.G., Nicolaiewsky, S., de Souza, S.M.G. & Lulhier F. (1999). Plasmatic levels of cortisol in the response to acute stress in Nile tilapia, *Oreochromis niloticus* (L.), previously exposed to chronic stress. Aquaculture Research **30**: 437-444.

Barreto, R.E. & Volpato, G.L. (2006). Stress responses of the fish Nile tilapia subjected to electroshock and social stressors. Brazilian Journal of Medical and Biological Research **39**: 1605-1612.

Barton, B.A. & Iwama, G.K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects on corticosteroids. Annual Review of Fish Diseases **1**: 3-26.

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.

Brett, J.R. (1972). The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Respiration Physiology **14**:151-170.

Carragher, J.F. & Rees, C.M. (1994). Primary and secondary stress responses in golden perch, Macquaria ambigua. Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology **107**: 49-56.

Cazenave, J., Wunderlin, D. Hued, A. & Bistoni, M. (2005). Haematological parameters in a neotropical fish, *Corydoras paleatus* (Jenyns, 1842) (Pisces, Callichthyidae), captured from pristine and polluted water. Hydrobiologia- Springer **537** (1): 25-33.

Falahatkar, B. & Barton, B.A. (2007). Preliminary observations of physiological responses to acute handling and confinement in juvenile beluga *Huso huso* L. Aquaculture Research **38**: 1786-1789.

Fast, M.D., Hosoya, S., Johnson S.C. & Alfonso, L.O. (2008). Cortisol response and immune- related effects of Atlantic salmon (*Salmo salar* Linnaeus) subjected to short-and long-term stress. Fish & Shellfish Immunology **24**(2): 194-204.

Flodmark, L.E.W., Urke, H.A., Halleraker, J.H., Arnekleiv, J.V., Vøllestad, L.A. & Polé, A.B.S. (2002). Cortisol and glucose responses in juvenile brown trout subjected to a fluctuating flow regime in an artificial stream. Journal of Fish Biology **60**: 238-248.

Franklin, C.E., Davison, W. & Mckenzie, J.C. (1993). The role of the spleen during exercise in the antarctic teleost, pagothenia borchgrevinki. The Journal of Experimental Biology **174**: 381-386.

Frisch, A. & Anderson, T. (2005). Physiological stress responses of two species of coral trout (*Plectropomus leopardus* and *Plectropomus maculatus*). Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology **140**: 317-327.

Gatica, M.C., Monti, G.E., Knowles, T.G., Warriss, P.D. & Gallo, C.B. (2010). Effect of comercial live transportation and preslaughter handling of Atlantic salmon on blood constituents. Archivos de Medicina Veterinaria **42**: 73-78.

Gbore, F.A., Oginni, O., Adewole, A.M. & Aladetan, J.O. (2006). The effect of Transportation and Handling Stress on Haematology and Plasma Biochemistry in Fingerlings of *Clarias gariepinus* and *Tilapia zilli*. World Journal of Agricoltural Science **2** (2): 208-212.

Gomes, L.C., Araujo-Lima, C.A.R.M., Roubach, R., Chippari-Gomes, A.R., Lopes, N.P. & Urbinati, E.C. (2003). Effect of fish density during transportation on stress and mortality of juvenile tambaqui, *Colossoma macropomum*. Journal of the World Aquaculture Society **34**: 76-84.

Goolish, E.M. (1991). Aerobic and anaerobic scaling in fish. Biological Reviews **66**: 33-56.

Hattingh, J. & Van Pletzen, A.J.J. (1974). The influence of capture and tansportation on some blood parameters of fresh water fish. Comparative Biochemistry and Physiology **4**: 607-609.

Haux, C., Sjobeck, M.L. & Larsson, A. (1985). Physiological stress responses in a wild fish population of perch (*Perca fluviatilis*) after capture and during subsequent recovery. Marine Environmental Research **15**: 77-95.

Heath, A.G. & Pritchard, A.W. (1962). Changes in the metabolic rate and blood lactic acid of blue gill sun fish, *Lepomis macrochirus* following severe muscular activity. Physiological Zoology **38**: 767-776.

Hemre, G.I. & Krogdahl, A. (1996). Effect of handling and fish size on secondary changes in carbohydrate metabolism in Atlantic salmon, *Salmo salar* L. Aquaculture Nutrition **2**: 249-252.

Hodson, P.V., Blunt, B.R. & Spray, D.J. (1978). Chronic toxicity of water borne lead and dietary lead to rainbow trout (*Salmo gardneri*) in pond Ontario water. Water Research **12**: 869-878.

Ishibashi, J., Ekawa, H., Hirata, H. & Kumai, H. (2002). Stress response and energy metabolisminvarious tissues of Nile tilapia *Oreochromis niloticus* exposed to hypoxic conditions. Fisheries Science **68**: 1374-1383.

Iwama, G.K., Vijayan, M.M. & Morgan, J.D. (1994). The stress response in fish. In: Sherwood, NM, Hew, CL (Eds), Fish Physiology vol. XIII. Academic Press, London pp. 47-57.

Katalay, S. & 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.

Kauffman, R. (1990). Respiratory cost of swimming in larval and juvenile cyprinids. The Journal of Experimental Biology **150**: 343-366.

Laidley, C.W. & Leatherland, J.F. (1988). Circadian studies of plasma cortisol, thyroid hormone, protein, glucose and ion concentratio, liver glycogen concentration and liver and spleen weight in rainbow trout, *Salmo gairdneri* Richardson. Comparative biochemistry and physiology. Part A, Molecular & Integrative Physiology **89** (3): 495-502.

Leonardi, M., Azzaro, F., Azzaro, M., Caruso, G., Mancuso, M., Monticelli, L.S., Maimone, G., La Ferla, R., Raffa, F. & Zaccone, R. (2009). A multidisciplinary study of the Cape Peloro brackish area (Messina, Italy): characterisation of trophic conditions, microbial abundances and activities. Marine Ecology **30** (1): 33-42.

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

Lowe, T.E., Ryder, J.M., Carragher, J.F. & Wells, R.M.G. (1993). Flesh quality in snapper, Pagrus auratus, affected by capture stress. Journal of Food Science **58**: 770-773.

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

Mazeaud, M.M., Mazeaud, F. & Donaldson, E.M. (1977). Primary and secondary effects of stress in fish: some new data with a general review. Transactions of the American Fisheries Society **106**: 201-212.

Milligan, C.L. & Wood, C.M. (1982). Disturbances in haematology, fluid volume. Distribution and circulatory function associated with low environmental pH in the rainbow trout *Salmo gairdneri*. The Journal of Experimental Biology **99**: 397-415.

Milligan, C.L., Hooke, G.B. & Johnson, C. (2000). Sustained swimming at low velocity following about of exhaustive exercise enhances metabolic recovery in rainbow trout. The Journal of Experimental Biology **203** (5): 921-926.

Moberg, G. (1987). Problems in defining stress and distress in animals. Journal of the Amertican Veterinary Medical Association **191**: 1207-1211.

Mommsen, T.P., Vijayan, M.M. & Moon, T.W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries **9**: 211-268.

Ortuno, J., Esteban, M.A. & Meseguer, J. (2001). Effects of short-term crowding stress on the gilthead seabream (*Sparus aurata* L) innate immune response. Fish & Shellfish Immunology **11**: 187-197.

Pickering, A.D. (1981). Introduction: the concept of biological stress. In: Pickering, A. D. (eds.), Stress and Fish Academic Press, London.

Pratap, H.B. & Wendelaar-Bonga, S.E. (1990). Effects of water-borne cadmium on plasma cortisol and glucose in the cichlid fish *Oreochromis mossambicus*. Comparative Biochemistry and Physiology **95**: 313-317.

Puckett, K.J. & Dill, L.M. (1984). Cost of sustained and burst swimming to juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Sciences **41**:(11) 1546-1551.

Puckett, K.J. & Dill, L.M. (1985). The energetics of feeding territorially in juvenile coho salmon (*Oncorhynchus kisutch*). Behaviour **92**: 97-111.

Richards, J.G., Mercado, A.J., Clayton, C.A., Heigenhauser, G.J. & Wood, C. M. (2002). Substrate utilization during graded aerobic exercise in rainbow trout. The Journal of Experimental Biology **205** (14): 2067-2077.

Roncarati, A. Melotti, P., Dees, A., Mordenti, O. & Angellotti, L. (2006). Welfare status of cultured seabass (*Dicentrarchus labrax* L.) and seabream (*Sparus aurata* L.) assessed by blood parameters and tissue characteristics. Journal of Applied Ichthyology **22**: 225-234.

Sumpter, J.P. (1991). The stress response and its consequences in cultured fish. Bulletin of the Institute of Zoology **16**: 229-236.

Sumpter, J.P. (1997). The endocrinology of stress. In Fish Stress and Health in Aquaculture Iwama, G. K., Pickering, A. D., Sumpter, J. P. & Schreck, C. B. (eds.) Cambridge University Press: 95-118.
Svobodova, Z., Vykusova, B., Modra, H., Jarkovsky, J. & Smutna, M. (2006). Haematological and biochemical profile of harvest-size carp during harvest and post-harvest storage. Aquaculture Research **37**: 959-965.
Torres, P., Tort, L., Planas, J. & Flos, R. (1986). Effects of confinement stress and additional zinc treatment on some blood parameters in the dogfish *Scyliorhinus canicula*. Comparative biochemistry and physiology. Part C, Pharmacology, Toxicology & Endocrinology **83**: 89-92.
Van Raaij, T.M., Vianen, G.J. & Van den Thillart, G.E.E. (1996). Blood gas parameters and the responses of erythrocytes in carp exposed to deep hypoxia and subsequent recover. Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology **166**: 453-460.
Velisek, J., Svobodova, Z. & Piackova, V. (2007). Effects of 2-phenoxyethanol anestesia on haematological profile on common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*). Acta Veterinaria Brno **76**: 487-492.
Wedemeyer, G.A., Barton, B.A. & McLeay, D.J. (1990). Stress and acclimation. In: Schreck, C.B., Moyle, P.B. (eds), Methods for Fish Biology American Fisheries Society, Bethesda pp. 451-489.
Wendelaar Bonga, S.E. (1997). The stress response in fish. Physiological Reviews **77**: 591-625.
Xu, J., Liu, Y., Cui, S. & Miao, X. (2006). Behavioural responses of tilapia (*Oreochromis niloticus*) to acute fluctuations in dissolved oxygen levels as monitored by computer vision. Aquacultural Engineering. **35**: 207-217.