Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i1.13055

Biochemical changes in *Salminus brasiliensis* due to successive captures and stocking densities

Neiva Braun[1*,4], Ronaldo Lima de Lima[1,4], Bernardo Baldisserotto[2], Alcir Luiz Dafre[3] and Alex Pires de Oliveira Nuñer[1]

*[1]Laboratório de Biologia e Cultivo de Peixes de Água Doce, Centro de Ciências Agrárias, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Rod. 406, 3532, 88066-000, Florianópolis, Santa Catarina, Brazil. [2]Departamento de Fisiologia e Farmacologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Campus Camobi, Santa Maria, Rio Grande do Sul, Brazil. [3]Laboratório de Defesa Celular, Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil. [4]Programa de Pós-graduação em Aquicultura, Departamento de Aquicultura, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil. *Author for correspondence. E-mail: neivabraun@gmail.com*

ABSTRACT. This study evaluated the biochemical changes associated to successive captures of *Salminus brasiliensis* reared at different stocking densities. The experiment was conducted in tanks with a capacity of 1,000 L, connected to a recirculating system with temperature control and continuous aeration. Fish were stocked at densities of 30, 150 and 300 fish m^{-3} and the successive catches were applied at the end of the experimental period of 80 days. The biochemical changes were evaluated at the end of the trial through the parameters: hematocrit, hemoglobin, glucose and lactate in plasma and Na^+, K^+ and Cl^- and Ca^{2+} in the gills. The concentration of glucose increased with the increase of stocking density as also registered for lactate concentration, except for fish captured twice. Hemoglobin concentration increased with the increase of stocking density only in fish captured three times. The hematocrit and the ions in the gills had no variation under any condition and therefore, the ionic alterations characteristic of a stress response, were not found in this species. The successive captures associated to stocking density were related to biochemical changes in *S. brasiliensis*, but ion concentrations in gills were not indicators of this condition.

Keywords: dourado, hematocrit, hemoglobin, glucose, lactate, ions.

Alterações bioquímicas em *Salminus brasiliensis* decorrentes de capturas sucessivas e da densidade de estocagem

RESUMO. O objetivo deste estudo foi avaliar as alterações bioquímicas relacionadas a capturas sucessivas em *Salminus brasiliensis* criados em diferentes densidades de estocagem. O experimento foi conduzido em tanques com capacidade de 1.000 L, conectados a um sistema de recirculação de água com temperatura e aeração contínua. Os peixes foram estocados nas densidades de 30, 150 and 300 peixes m^{-3}, com aplicação de capturas sucessivas ao término do período experimental de 80 dias. As alterações bioquímicas foram avaliadas pela análise do hematócrito, hemoglobina, glicose e lactato plasmático e Na^+, K^+ e Cl^- e Ca^{2+} branquial. A elevação da concentração de glicose esteve relacionada ao aumento da densidade de estocagem nos peixes submetidos a uma, duas e três capturas. A concentração de lactato apresentou resposta semelhante à registrada para a glicose, com exceção de peixes capturados por duas vezes. A elevação da concentração de hemoglobina esteve relacionada ao aumento da densidade de estocagem nos peixes capturados três vezes. Não foram observadas variações no hematócrito e nos íons após as capturas. As capturas sucessivas associadas à densidade de estocagem interferiram na resposta bioquímica em *S. brasiliensis*, mas a concentração de íons nas brânquias não foram indicadores dessa condição.

Palavras-chave: dourado, hematócrito, hemoglobina, glicose, lactato, íons.

Introduction

The stress response is the reaction of an organism to various stress agents. It includes biological processes, usually classified as primary, secondary or tertiary responses, which are coordinated by the hypothalamic-pituitary-adrenal axis (IWAMA et al., 2005). The stress response represents a level of tolerance that differs among fish species depending on the nature and intensity of the stress agent and on the time that the fish remains under the stress effect (WEDEMEYER, 1996).

The primary stress response is related to neuroendocrine activation, which increases the concentrations of catecholamines and corticosteroids in the blood. These hormonal responses control a series of physiological variables known as secondary

stress responses including metabolic activity and hydrosaline balance as well as immunological, cardiovascular and respiratory functions. The tertiary response is related to changes in the fish body, such as growth inhibition and disturbances in the reproductive cycle and in the immunological state (IWAMA et al., 2005).

Acute stress is generally defined as the condition resulting from the presence of an intense stressor of short duration; chronic stress can result from a wide range of long term disturbances that act as intense or mild stressors. The point at which an acute stressor becomes a chronic stressor is arbitrary and usually defined according to the individual situation (RUANE et al., 2002).

In fish culture, the major acute stressors are capture and environmental alterations such as water temperature changes, confrontations with predators, transport, capture and containment. The major chronic stressors are overpopulation, frequent and repetitive disturbances and poor water quality (WEDEMEYER, 1996; SANTOS et al., 2010). However, acute and chronic stress are often superimposed during the life span, and the consequences of this overlap can include a modulation of the hypothalamic-pituitary-adrenal axis by chronic stress that can interfere with the acute stress response (BARCELLOS et al., 2006). Pursuit, capture and air exposure (BRYDGES et al., 2009; HOSHIBA et al., 2009) and the stocking density (CHATTERJEE et al., 2006) are important and unavoidable stress factors in intensive fish culture.

Stress levels can be measured through physiological and metabolic responses (BARCELLOS et al., 2006; ROCHA et al., 2004). Analysis of secondary stress response can be performed using simple and inexpensive methods for the determination of glucose, lactate and ion levels, along with hematological studies (WEDEMEYER, 1996). Blood glucose concentrations can be used as an indirect method to detect cortisol release. These analyses can provide information about fish health and can became indicators of welfare during stocking, allowing the development of management protocols that reduce aquaculture stress and contribute to increased production (CONTE, 2004; RUANE et al., 2002).

Information on the stress response in tropical species employed in aquaculture is very limited, however, studies on curimbatá, *Prochilodus lineatus*, matrinxã, *Brycon amazonicus*, mandi, *Pimelodus maculatus*, surubim, *Pseudoplatystoma corruscans*, and matrinxã, *Brycon cephalus* (FAGUNDES; URBINATI, 2008; GONÇALVES et al., 2010; HOSHIBA et al., 2009; JERÔNIMO et al., 2009; URBINATI, 2004; ROCHA et al., 2004) have already been reported.

The dourado *S. brasiliensis* has good potential to become commercially cultivated as a table fish because it grows quickly and provides a good quality meat (BORGHESIS et al., 2008; MAI; ZANIBONI-FILHO, 2005). Growth and biochemical parameters have been analyzed for this species (BRAUN et al., 2010); however, there have been no studies on the effect of successive captures on *S. brasiliensis*.

Once capture and stocking density can have deleterious effects on fish physiology and endocrinology (BRYDGES et al., 2009; DI MARCO et al., 2008; VAN DE NIEUWEGIESSEN et al., 2008), factors that could affect *S. brasiliensis* growth under fish culture conditions, and the biochemical changes of this species related to those factors were evaluated in this study.

Material and methods

Animals and experimental design

S. brasiliensis fingerlings used in the experiment were produced by induced breeding of wild broodstock collected in the Uruguay river, Brazil. The fish were stocked at densities 30, 150 and 300 fish m^{-3} (equivalent to 0.18, 0.94 and 2.28 kg m^{-3}) in experimental units distributed in a completely randomized design with three replications. As the optimal stocking density for the growth *S. brasiliensis* in fish culture is not yet determined, we selected densities 30, 150 and 300 to represent the theoretical conditions of fish culture under intensive, highly intensive and super-intensive management, respectively.

The mean ± (SD) initial weight and standard length were 7.61 ± 3.49 g and 8.68 ± 0.17 cm for the density 30 fish m^{-3}, 7.47 ± 2.98 g and 8.73 ± 0.15 cm for 150 fish m^{-3} and 7.58 ± 3.79 g and 8.64 ± 0.96 cm for 300 fish m^{-3}. Before the start of the experiment, the fish were acclimatized in 1,000 L tanks for one week, which were connected to a water recirculating system with constant temperature (25.6 ± 1.7ºC), continuous aeration and biological and mechanical filtration, and a light dark cycle of 12:12h.

The fish were fed with commercial extruded diets containing 42% crude protein offered to apparent satiation twice a day, and the experiment was conducted under these conditions over a period of 80 days.

The concentrations of dissolved oxygen, temperature and pH, measured with HACH

multiparameter proble and the concentrations of total and non-ionized ammonia (KOROLEFF, 1983) were monitored weekly. The means (±SD) of these parameters were 6.84 ± 0.75 mg L^{-1}, 0.14 ± 0.1 mg L^{-1}, 0.0006 ± 0.00 mg L^{-1}, 25.6 ± 1.8ºC and 6.9 ± 0.2, respectively.

The factor capture was applied in samples of fish at the end of the experimental period. Three fishes were simultaneously captured from each experimental unit and one of them was selected for the sampling of biological material used to evaluate the biochemical changes in *S. brasiliensis*. The other fish were transferred to a 10 L tank, connected to the same recirculating system above mentioned, waiting for the subsequent capture. After 10 min. the two remaining fish were captured again, but only one was selected for the sampling of biological material, while the other returned to the 10 L tank. Ten minutes later the last fish was captured for the sampling of biological material. Therefore, one of the fish was captured once, whereas the others were captured for two or three times.

Variables

The biological material for analysis of ionic and hematological and biochemical variables were collected at the end of the experiment.

Blood was obtained from the caudal vein using heparin (Heparin-Cristália, São Paulo State, Brazil) as an anticoagulant and kept on ice until analysis. An aliquot of blood was centrifuged at 3000 x g for 5 min. at 4ºC; the plasma obtained was first stored at 4ºC for glucose and lactate analyses. Another aliquot of blood was centrifuged for 5 min in capillary tubes in a microcentrifuge (Evlab, Paraná State, Brazil) to obtain the hematocrit (Ht).

The concentration of hemoglobin (Hb) was determined using the cyanmethemoglobin method (VAN KAMPEN; ZIJLSTA, 1964). Gill tissues were used for the analysis of Na^+, K^+, Cl^- and Ca^{2+} concentrations. The concentration of glucose and lactate were determined using Biotécnica (Varginha, Minas Gerias State, Brazil) and Kovalente (São Gonçalo, Rio de Janeiro State, Brazil) kits, respectively. For glucose analysis 10 μL of plasma were diluted in 500 μL of reagent (phosphate buffer 182.42 mmol L^{-1}, pH 7.0; Glucose> 15000U L^{-1}; Peroxidase> 1200 U L^{-1}; 4-aminoantipyrine 0.3 mmol L^{-1}; Phenol 10 mmol L^{-1}), 5 μL standard solution (glucose) and 500 μL of reagent; blank 10 μL distilled water and 500 μL of reagent. The samples were incubated at 37°C for 20 min. and the absorbance measured at 505 nm. In the determination of lactate 10 μL of plasma sample and 500 μL of reagent 1 (LDH buffer pH 9.0, 400 mmol L^{-1} ≥ 24KU L^{-1}) were diluted. The samples were incubated at 37°C for 5 min. and absorbance measured at 340 nm. Subsequently 125 μL of reagent 2 (NAD ≥ 4 mmol L^{-1}) were added and the absorbance determined at 340 nm.

The concentration of Cl^- was determined according to Zall et al. (1956), and the concentrations of Na^+, K^+, and Ca^{2+} were determined using a flame photometer (Micronal B260, São Paulo State, Brazil). Nitric acid 2 N was added at a rate of 3.0 mL g^{-1} gill tissue. The tissue was then incubated at 70ºC for 48h, and the supernatant was removed with a pipette for analysis.

Statistical analysis

The biochemical variables were evaluated by regression analysis (ZAR, 2009) at a significance level of 5.0%. For the comparison of regressions that related the stocking densities to the different variables, an analysis of covariance was applied (ZAR, 2009).

Results and discussion

The concentration of glucose increased with the increase of stocking density for fish subjected to one, two or three captures. Despite glucose increase, expressed by the regression coefficient, can be considered the same for those conditions ($b = 0.122$; $p > 0.05$), it was also registered a decrease in glucose concentration with the increase in the number of captures (Figure 1A).

This study indicates that glucose can be seen as an important source of energy for maintaining homeostasis in *S. brasiliensis*. The application of one capture led to a maximum production of glucose at higher densities, followed by a decrease in the second and third captures, indicating a reduction in the ability to provide immediate source of energy or a fast adaptive process to the captures.

The hyperglycemia assists to supply the energy requirements associated to biochemical alteration (WEDEMEYER, 1996) and therefore, the gluconeogenesis is activated and the glycolysis is inhibited, which stimulates the production of glucose. These responses are controlled by complex neuroendocrine mechanisms that include epinephrine and cortisol release. For *B. amazonicus*, blood glucose did not show changes associated with the pursuit after 15, 30 and 60 min. (HOSHIBA et al., 2009), but Fagundes and Urbinati (2008) observed a gradual increase in the concentration of glucose in *P. corruscans* after the first 5 min. of capture.

The influence of density on *S. brasiliensis* biochemical changes associated with capture is corroborated by hemoglobin concentrations in fish captured for three times (Figure 1B). This increased

hemoglobin concentration may be a response to external stimulus and could be related to the increase in oxygen consumption as a way to supply the higher energy demand (NIKINMAA et al., 1983).

During anaerobic glycolysis, glycogen stored in the liver and muscles are used to produce ATP, releasing lactate as a byproduct. The lactate and glucose profiles were similar, except for fish captured twice (Figure 1C). The increase in lactate concentrations are typical of acute stress fish, especially if the stressor causes a reduction in oxygen availability or increased physical activity of the fish (WEDEMEYER, 1996). For *S. brasiliensis* the characteristic response of anaerobic metabolism may have been conditioned by a combination of high density and successive captures with air exposure, which may have triggered the anaerobic response that led to the increase of lactate concentrations, indicating a biochemical change that could be related to the acute stress.

The hematocrit (Figure 1D) and the ions Na^+, K^+, Cl^- and Ca^{2+} in gills (Figure 2) had no variation under any condition. The release of epinephrine changes the ionic flow, leading to an increase in blood pressure and vasodilatation in the gills, which increases the lamellar blood flow (MORALES et al., 2005; WEDEMEYER, 1996). As a result, Na^+ and Cl^- are lost to the surrounding water (HOSHIBA et al., 2009), a condition not found in *S. brasiliensis*. The absence of alteration in the concentrations of Ht and gill ions indicates that capture does not trigger a typical acute stress response in this species.

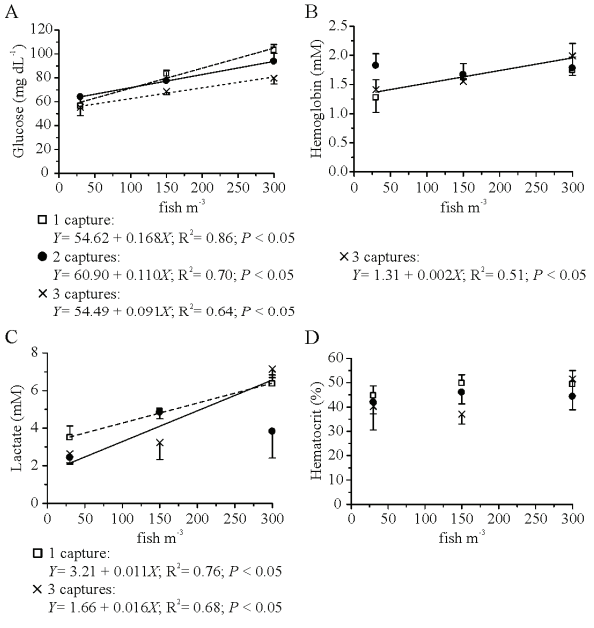

Figure 1. Plasma metabolites and hematological parameters (mean ± standard deviation) in *Salminus brasiliensis* submitted to successive capture stress at different stocking densities. = one capture; • = two captures; × = three captures.

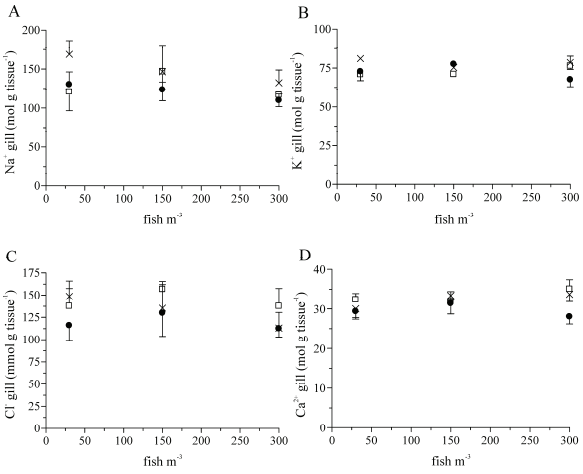

Figure 2. Ion concentration (mean ± standard deviation) in the gills of *Salminus brasiliensis* submitted to successive capture stress at different stocking densities. = one capture; • = two captures; × = three captures.

Conclusion

Stocking density and the successive captures were related to changes in concentrations of glucose, hemoglobin and lactate in the plasma of *S. brasiliensis*. Ion concentrations in the gills were not indicators of biochemical changes for this species, but glucose was a good indicator for this condition.

Acknowledgements

The authors thank to the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) for the scholarship granted to the first author and Barra Grande Energética S. A for the financial support. B. Baldisserotto, A. L. Dafre and A. P. O. Nuñer are research fellows of the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq).

References

BARCELLOS, L. J. G.; KREUTZ, L. C.; QUEVEDO, R. M. Previous chronic stress does not alter the cortisol response to an additional acute stressor in jundiá (*Rhamdia quelen*, Quoy and Gaimard) fingerlings. **Aquaculture**, v. 253, n. 1-4, p. 317-321, 2006.

BORGHESIS, R.; DAIRIKI, J. K.; CYRINO, J. E. P. Apparent digestibility coefficients of selected feed ingredients for dourado *Salminus brasiliensis*. **Aquaculture Nutrition**, v. 15, n. 5, p. 453-458, 2008.

BRAUN, N.; LIMA, R. L.; BALDISSEROTTO, B.; DAFRE, A. L.; NUÑER, A. P. O. Growth, biochemical and physiological responses of *Salminus brasiliensis* with different stocking densities and handling. **Aquaculture**, v. 301, n. 1-4, p. 22-30, 2010.

BRYDGES, N. M.; BOULCOTT, P.; ELLIS, T.; BRAITHWAITE, V. A. Quantifying stress responses induced by different handling methods in three species of

fish. **Applied Animal Behaviour Science**, v. 116, n. 2/4, p. 295-301, 2009.

CHATTERJEE, N.; PAL, A. K.; DAS, T.; MOHAMMED, M. S.; SARMA, K.; VENKATESHWARLU, G.; MUKHERJEE, S. C. Secondary stress responses in Indian major carps *Labeo rohita* (Hamilton), *Catla catla* (Hamilton) and *Cirrhinus mrigala* (Hamilton) fry to increasing packing densities. **Aquaculture Research**, v. 37, n. 5, p. 472-476, 2006.

CONTE, F. S. Stress and the welfare of cultured fish. **Applied Animal Behaviour Science**, v. 86, n. 3/4, p. 205-223, 2004.

DI MARCO, P.; PRIORI, A.; FINOIA, M. G.; MASSARI, A.; MANDICH, A.; MARINO, G. Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. **Aquaculture**, v. 275, n. 1-4, p. 319-328, 2008.

FAGUNDES, M.; URBINATI, E. C. Stress in pintado (*Pseudoplatystoma corruscans*) during farming procedures. **Aquaculture**, v. 276, n. 1-4, p. 112-119, 2008.

GONÇALVES, A. F. N.; TAKAHASHI, L. S.; URBINATI, E. C.; BILLER, J. D.; FERNANDES, J. B. K. Transporte de juvenis de curimbatá *Prochilodus lineatus* em diferentes densidades. **Acta Scientiarum. Animal Sciences,** v. 32, n. 2, p. 205-211, 2010.

HOSHIBA, M. A.; GONÇALVES, F. D.; URBINATI, E. C. Respostas fisiológicas de estresse no matrinxã (*Brycon amazonicus)* após exercício físico intenso durante a captura. **Acta Amazonica**, v. 39, n. 2, p. 445-452, 2009.

IWAMA, G. K.; AFONSO, L. O. B.; VIJAYAN, M. M. Stress in fishes. In: EVANS, D. H.; CLAIBORNE, J. B. (Ed.). **The physiology of fish**. London: Taylor and Francis, 2005. p. 319-342.

JERÔNIMO, G. T.; MARTINS, M. L.; BACHMANN, F.; GREINERT-GOULART, J. A.; SCHMITT-JÚNIOR, A. A.; GHIRALDELLI, L. Hematological parameters of *Pimelodus maculatus* (Osteichthyes: Pimelodidae) from polluted and non-polluted sites in the Itajaí-Açu river, Santa Catarina State, Brazil. **Acta Scientiarum. Biological Sciences**, v. 31, n. 2, p. 179-183, 2009.

KOROLEFF, F. Determination of ammonia. In: GRASSHOFF, K.; EHRDART, M.; KREMLING, K. (Ed.). **Methods for seawater analysis**. Weinheim: Verlag Chemie, 1983. p. 150-157.

MAI, M. G.; ZANIBONI-FILHO, E. The effect of storage age in external tanks in the larviculture performance of *Salminus brasiliensis* (Osteichthyes, Characidae). **Acta Scientiarum. Animal Sciences**, v. 27, n. 2, p. 287-296, 2005.

MORALES, A. E.; CARDENETE, G.; ABELLÁN, E.; GARCÍA-REJÓN, L. Stress-related physiological responses to handling in common dentex (*Dentex dentex* Linnaeus, 1758). **Aquaculture Research**, v. 36, n. 1, p. 33-40, 2005.

NIKINMAA, M.; SOIVIO, A.; NAKARI, T.; LINDGREN, S. Hauling stress in brown trout: physiological responses to transport in freshwater or salt water and recovery in natural brackish water. **Aquaculture**, v. 34, n. 1/2, p. 93-99, 1983.

ROCHA, R. M.; CARVALHO, E. G.; URBINATI, E. C. Physiological responses associated with capture and crowding stress in matrinxã *Brycon cephalus* (Gunther, 1869). **Aquaculture Research**, v. 35, n. 2, p. 245-249, 2004.

RUANE, N. M.; CARBALLO, E. C.; KOMEN, J. Increased stocking density influences the acute physiological stress response of common carp *Cyprinus carpio* (L.). **Aquaculture Research**, v. 33, n. 10, p. 777-784, 2002.

SANTOS, G. A.; SCHRAMA, J. W.; MAMAUAG, R. E. P.; ROMBOUT, J. H. W. M.; VERRETH, J. A. J. Chronic stress impairs performance, energy metabolism and welfare indicators in European seabass (*Dicentrarchus labrax*): the combined effects of fish crowding and water quality deterioration. **Aquaculture**, v. 299, n. 1-4, p. 73-80, 2010.

URBINATI, E. C.; ABREU, J. S.; CAMARGO, A. C. S.; LANDINES, M. A. P. Loading and transport stress of juvenile matrinxã (*Brycon cephalus*, Characidae) at various densities. **Aquaculture**, v. 229, n. 1-4, p. 389-400, 2004.

VAN DE NIEUWEGIESSEN, P.; BOERLAGE, A.; VERRETH, J.; SCHRAMA. J. Assessing the effects of a chronic stressor, stocking density, on welfare indicators of juvenile African catfish, *Clarias gariepinus* Burchell. **Applied Animal Behaviour Science**, v. 115, n. 3-4, p. 233-243, 2008.

VAN KAMPEN, E. J.; ZIJLSTA, W. G. In: BOROVICZÉNY, C. H. G. (Ed.). **Erythrocytometric methods and their standardization**. Lisbonne: Bibliotheca Haematologica, 1964. p. 68-72.

WEDEMEYER, G. A. **Physiology of fish in intensive culture systems**. New York: Chapman and Hall, 1996.

ZALL, D. M.; FISHER, M. D.; GARNER, Q. M. Photometric determination of chlorides in water. **Analytical Chemistry**, v. 28, n. 11, p. 1665-1668, 1956.

ZAR, J. H. **Biostatistical analysis**. 4th ed. New Delhi: Pearson Education, 2009.

Received on April 3, 2011.
Accepted on February 28, 2012.