Can Rodlet Cells Changes in Barbell (*Barbus peloponnesius*) From the River Bregalnica Be Used as Biomarkers of Environmental Contamination?

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Abstract

For evaluating pollution impacts on aquatic ecosystems there is a need to establish early-warning signals (biomarkers) using bioindicator organisms. Some studies suggested that rodlet cells (RCs) changes in fish can be used as pollution biomarkers. Looking for supporting evidence, we quantitatively estimate the RCs amounts in liver, as well as general fish health indicators - the condition factor (CF) and hepato-somatic index (HSI) – in 136 barbell, caught at three locations (reference site and two sites – A and B – suspected to be under pollution impact) from the River Bregalnica. Fishes from site A showed significantly lower values for CF and HSI when compared with the other two locations, and highest average amount of RCs. Both the relative and total volumes of RCs significantly differed from those found at site B (p<0.05), but either site A or B was not statistically different from the reference site. Lower indices of condition (CF and HSI) at polluted localities were only partially associated with higher liver RCs counts. The present data indicated that RCs were a non-efficient bio-monitoring tool. However, further evaluation of these cells as potential biomarkers are necessary, as its behaviour may depend on the type of pollutants. Also, RCs exist in diverse organs and organ-specific responses can thus occur.

Keywords: barbell, rodlet cells, biomarker, pollution

Introduction

Pollution of the aquatic surface waters with man-made substances has been observed throughout the world and has the potential to induce serious harm in wild organisms, to impair their health, condition, reproduction or its habitats (Bresler et al., 1999). In order to evaluate or to predict the pollution impact on the aquatic ecosystems, thereby on aquatic organisms, there is increased need of establishing early-warming signals (biomarkers) using bioindicator organisms (i.e., a biomarker approach). A suite of biomarkers can be used at the various levels of biological organization (Adams and Greeley, 2000). Among others, biomarkers at the individual organism level and cytological-histological level of organization represent rapid and economically worthwhile methods.

At the individual organism level, measures of condition factor and various organo-somatic indices (liver-somatic index, etc.) have proven to be good representative indices of overall organism health (Goede and Barton, 1990). These quantitative fish health indicators have been widely used and discussed in the literature. Although they are not diagnostic tools, they are nevertheless very useful as indicators of trends in fish condition (Gearheardt et al., 1988).

In addition to general condition indices, other non-specific biomarkers have been used to demonstrate the general effects of natural and anthropogenic stressors on fish organism (Blazer et al., 1997; Agius and Roberts, 2003; Adams et al., 2003), especially applicable when looking for integrated effects on individual fish health (Thilakaratne et al., 2007). In recent years, there is increased evidence that Rodlet cells are included in the fish immune system, as a part of un-specific second line of defence (Reite, 1997; Dezfuli et al., 2000; Leino, 2001; Kramer et al., 2005). Although the function of these intricate cells still remains unsolved, many data address on involvement of RCs in stress alarm response on different stress conditions.

An increased number of these cells in different tissues of teleosts have been recorded in relation to exposure to heavy metals (Giari et al., 2007, 2008), herbicides (Dezfuli et al., 2003; Manera and Dezfuli, 2004), parasitic infestations (Reite, 1997, 2005; Dezfuli et al., 2000), physical, mechanical and chemical injuries (Manera and Dezfuli, 2004; Giari et al., 2006). These alterations in the number of the RCs support the hypothesis of their role in host defence, and contribute to their use as useful biomarkers of environmental pollution. Moreover, some authors consider the use of RCs changes as useful biomarkers of exposure to stressors or as a stress response, based on their occurrence in fish following osmotic challenges (Giari et al., 2006), variation of season (Leino, 2001), overcrowding (Poltronieri et al., 2009). But, considering that several endogenous factors can also evoke RCs responses (Rocha et al., 1994; Koponen and Myers, 2000; Figueiredo-Fernandes et al., 2006; Jordanova et al., 2007), as well as the lack of specificity of the response, studying RCs could be a reliable method for the assessment of the fish health status especially when in association with other known biomarkers (Manera and Dezfuli, 2004).

Pollution problems are occurring in Macedonian water bodies. Surface water quality is below regulation standards in the major rivers, including River Bregalnica - the biggest left tributary of the river Vardar, principal source of water for human consumption (e.g., in the city of Stip), agricultural irrigation and also a source of fish for local consumption (Alderton et al., 2005). Beside all this, the health status of the fishes in this river has never been examined. This work is a first attempt to study the influence of environmental contamination on fish health using *Barbus peloponnesius* as a target fish and to establish baseline information and a strategy for helping biomonitoring the river using fish.

For this purpose, fish responses at two levels of biological organization were evaluated with particular attention to RCs and their potential use as biomarkers. Thus, the main objectives of this study were: 1) to evaluate the overall organism health via CF and HSI, as biomarkers at the organism level of organization; and 2) to establish the presence and alterations in RCs occurrence in the liver of the *B. peloponnesius*, as biomarkers at the cytology/histology level. In such way, the results from this preliminary study will contribute in evaluating the usefulness of the selected histological biomarkers, evaluating their usefulness for introduction in a future and larger biomonitoring study in Macedonia and Portugal.

Materials and methods

The fish used in this study is the barbell (*Barbus peloponnesius*), a typical benthophagous fish that colonize special regions of the river. A total of 136 barbells were collected by electrofishing from October 2007 until March 2008, on 3 sampling spots along the river: first spot - reference site, is near the town Berovo, the first upstream site in which barbells can be found; the second located in the middle part of the Bregalnica River - site A (near Kocani) – suspected to be on strong pollution from aquaculture and mining; and the third sampling spot – site B (near the sity of Stip), under both sewage and household water influences.

After captured, the fish were held in aerated containers until processed. The animals were killed with a blow to the head, weighted and measured. Condition factor (CF) was calculated as:

$CF = \text{somatic weight} \times 100 / \text{fork length}^3$.

Fish were dissected afterwards. Tthe liver was removed, weighed, fixed in Bouin's fixative for 48 h, routinely processed to paraffin, sectioned at 5 µm thin sections and stained with haematoxyline – eosin (H&E). Hepatosomatic indices (HSI) were calculated with the following formula:

$HSI = \text{somatic weight} \times 100 / \text{liver weight}$.

Five randomly selected liver sections were analyzed (final magnification: 400 x) with standard stereological point counting techniques (Feere and Weibel, 1967), and according detailed protocols applied to other fish (Jordanova et al., 2007). Following such techniques, the relative volume of the RCs in the liver (expressed as %) was computed according to the formula:

$V_v (\text{structure, reference}) = [P(s) \times 100] / P(r)$

where V_V (structure, reference) is the percentage of the total volume of a reference space occupied by structure of an interest within that space, P(s) is the total number of points falling over the structure of an interest (RCs), and P(r) is the total number of points falling over the reference space that contains it; in our case all the liver tissue. From this calculations and the liver weight (LW), the absolute volume of the RCs expressed in mm^3 was estimated:

$V (\text{structure}) = V_V (\text{structure, reference}) \times LW$

All results are presented as mean values accompanied by the coefficient of variation (CV=SD/Mean). Statistical analysis was performed using Statistica 6.0 for Windows. Normality and homogeneity of variance of the data sets were first checked by the Levene's test. Whenever data distribution was normal, the differences between groups were evaluated by using one-way ANOVA, followed by the Tukey test whenever the ANOVA revealed differences. When we got no homogeneity of variance non-parametric Mann-Whitney *U* test was applied. Differences were considered significant when $p < 0.05$.

Results and Discussion

The aim of the present pilot study was to evaluate RCs values as biomarkers of environmental pollution. For this aim, the RCs amount in the liver of barbell, collected from a reference site and two locations suspected to be under pollution impact, were investigated. In addition to quantitative estimation of RCs amount, general fish health indicators were also included. The data obtained from the general morphometric measurements of the fish weight, length and condition factor are presented in Table 1. Small but significant differences were seen in the mean total length of barbell captured at site A compared with referent site. The CF of animals caught at site A and site B was significant lower compared with referent site. A significant difference was also observed between fish caught at site A and those at site B. The CF values in examined fish indicated lower condition of the fish at site A and B, compared with the reference. The data are in agreement with other field studies in which decrease of the condition factor was identified in fish from polluted areas (Eastwood and Couture, 2002; Hinck et al., 2007; Carrola et al., 2009). The reduced fish size and condition in wild population can also occur in response to many environmental variance, for example water temperature, food availability and many other factors (Swansburg et al., 2002, van der Oost et al., 2003).

Table 1. Body weight (BW), total length (TL) and condition factor (CF) of the barbell[1] sampled at the reference site and two sites (A and B) suspected to be under pollution impact*

Parameters	BW (g)	TL (cm)	CF (%)
Reference site	23,19 (0,51)	12,55 (0,16)[a]	1,34 (0,13)[a]
Site A	28,46 (0,72)	14,43 (0,19)[b]	1,05 (0,14)[b]
Site B	25,00 (0,64)	13,26 (0,19)[ab]	1,19 (0,11)[c]

[1] Values are expressed as: mean (coefficient of variation).
*Lower case subscript letters represent differences among sampling sites. Values with different letters are significantly different (p < 0.05), according to the post-hoc Tukey test.

Table 2. Hepato-somatic indices (HSI), relative volume (V_V) and total volume (V) of the rodlet cells (RCs) in the liver of the barbell[1] sampled at the reference site and two sites (A and B) suspected to be under pollution impact

Parameters	HSI %	V_v (RCs/L) %	V_{liver} (RCs) mm^3
Reference site	2,33 (0,25)[a]	0,03 (1,25)[ab]	0,01 (1,42)[ab]
Site A	1,63 (0,20)[b]	0,09 (1,24)[a]	0,03 (1,07)[a]
Site B	2,16 (0,38)[a]	0,03 (2,12)[b]	0,02 (3,03)[b]

[1] Values are expressed as: mean (coefficient of variation).
*Lower case subscript letters represent differences among sampling sites. Values with different letters are significantly different (p < 0.05), according to the Mann-Whitney *U* test.

The organ specific metrics: hepato-somatic index, relative and absolute volumes of the RCs are summarised in Table 2. In our investigations, values for HSI obtained for all fishes show fluctuations similar as CF. Namely, significant lowest value for HSI were recorded at site A when compared with both reference and B sites. The liver is important organ in detoxification, so it is expected that exposure to contaminants lead to changes in liver size (Goede and Barton, 1990). The exposure on some kinds of pollutants can result in an increase of the HSI of fish, although exposure to other toxicants can have opposite effect, to reduce the HSI. Enlarged livers or increased HSI are reported in fishes exposed to several kinds of effluents and water-toxic substances (Billiard and Khan, 2003; Hinck et al., 2006), or increasing rearing density (Papoutsoglou et al., 2006). Decreased liver size has been reported in various fish species after exposure to contaminants including metals (Adams et al., 1992; Lanno and Dixon, 1996), as well as feed deprivation (Shoemaker et al., 2003). These leads to suppose that HSI variations (increasing or decreasing), probably depends from the type of the pollution.

In the liver of barbell, RCs were regularly present. They were noticed among epithelial cells of the bile ducts, perpendicular to the lumen, with the nucleus oriented towards the basal lamina (Fig. 1). This location of the RCs in the liver is also reported for some other fish species (Rocha et al., 1994; Dezfuli et al., 2000; Jordanova et al., 2007). Although the exact nature of RCs is still unknown, many authors favoured the hypothesis that they represent inflammatory cells, involved in host defence (Manera and Dezfuli, 2004; Reite and Evensen 2006). Increased counts of RCs after infection by parasites, and exposure to diverse chemicals and environmental stressors supported the hypothesis that RCs might be considered “stress- or toxicant-induced type of fish cell produced in response to sub-lethal environmental conditions (Dezfuli et al., 1998, 2003; Manera et al., 2001; Giari et al., 2007). These responses of RCs to chemical substances are promising evidence of the potential use of these cells as biomarkers of pollution.

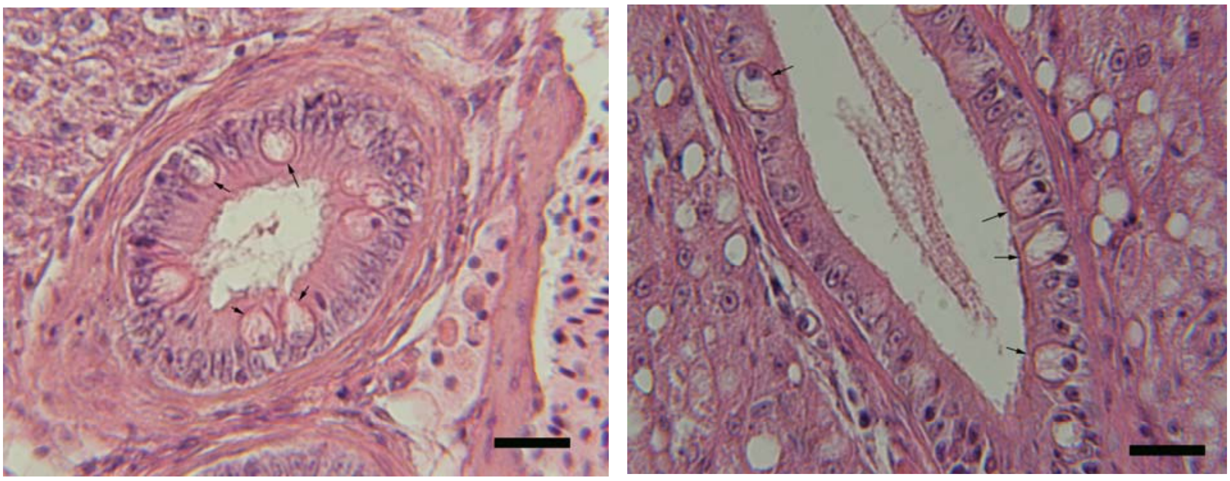

Fig. 1. Light micrographs of the liver from a barbell presenting stromal tracts with bile duct. Rodlet cells (arrows) are located among the epithelial cells of a bile duct. H&E. Bar = 20 µm

Looking for supporting evidence, we quantitatively evaluated RCs in the liver of the wild barbell population. Mean values from stereological measurements of the RCs are presented in Table 2. Similar patterns in the changes of relative and absolute volume of the RCs in the liver of barbell from the investigated localities were recorded. Namely, fish from site A also showed the highest average amount of RCs, being both the relative and total volumes of RCs significantly different from those found at site B. However, the amount of RCs in fish of either site A or B was not statistically different from the reference site. RCs exist in diverse organs and organ-specific responses can thus occur. Dezfuli et al. (2003) found that exposure of the fish to an herbicide provoked increasing in RCs number in bulbus arteriosus and gills, but not in kidney, liver and intestine. This suggests that maybe hepatic RCs are not good biomarker to assess pollution stress, at least in our case. On the other hand in addition to organ-specific responses, RCs behaviour may depend on the type of pollutants or stressors. Studies by Mazon et al (2006) showed that, in carp, the gills number of RCs increased after infection but did not change after stress, while in the kidney their number increased after stress and no significant changes were observed after infection. The noted increase in the RCs amount in our site A compared with site B could be result of different type of pollutions; from aquaculture and mining at site A versus sewage and household water at site B. However, although there are statistical studies showing an increase of these cells under the influence of quite different stressors, ranging from parasites, toxins, general tissue damage to adverse environmental influences (Manera and Dezfuli, 2004), other factors not connected with environmental pollution, as age, season and reproductive cycle, were also involved in RCs response (Manera and Dezfuli, 2004; Jordanova et al., 2007)

From the presented results, it is apparent that the gross indicators are suggestive of pollution impacts at site A. The microscopy data, however, is only partially indicative of stress effects at site A, as so it was not able to clearly distinguish the reference from the site where gross indicators were poorer. Even if our data indicate that pollution may raise the RCs pool, its accumulation, at least in our context, resulted in a non-efficient bio-monitoring tool, due to its apparent insensitivity. However, this potential biomarker merits further scrutiny, as its behaviour may depend on the type of pollutants. Also, RCs exist in diverse organs and organ-specific responses can thus occur. Finally, from this study we suggest that RCs changes must not be viewed alone but rather as part of multi-biomarker approaches, a fact that we will further explore.

References:

Adams, S.M., Crumby, W.D., Greeley, Jr.M.S., Ryon, M.G., Schilling, E.M. (1992): Relationships between physiological and fish population responses in a contaminated stream. Environmental Toxicology and Chemistry, 11: 1549–1557.

Adams, S.M., Greeley, M.S. (2000): Ecotoxicological indicators of water quality: using multi-response indicators to assess the health of aquatic ecosystems. Water, Air and Soil pollution, 123: 103-115.

Adams, S.M., Greeley, M.S., Law, J.M., Noga, E.J., Zelikoff, J.T. (2003): Application of multiple sublethal stress indicators to assess the health of fish in Pamlico Sound following extensive flooding. Estuaries, 26: 1365–1382.

Agius, C., Roberts, R.J. (2003): Melano-macrophage centres and their role in fish pathology. Journal of Fish Diseases, 26: 499–509.

Alderton, D.H.M., Serafimovski, T., Mullen, B., Fairall, K., James, S. (2005): The Chemistry of Waters Associated with Metal Mining in Macedonia. Mine Water and the Environment, 24: 139–149.

Billiard, S.M., Khan, R.A. (2003): Chronic stress in cunner, *Tautogolabrus adspersus*, exposed to municipal and industrial efluents. Ecotoxicology and Environmental Safety, 55: 9-18.

Blazer, V.S., Fournie, W.K. and Weeds, B.A. (1997): Macrophage aggregates: biomarker for the immune functions of fishes. In Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment (Dwyer, F.G., Doane, T.T. and Hinmman, M.L., eds), pp: 360–375.

Bresler, V., Bissinger, V., Abelson, A., Dizer, H., Sturm, A., Kratke, R., Fishelson, L., Hansen, P.D. (1999): Marine molluscs and fish as biomarkers of pollution stress in littoral regions of the Red Sea, Mediterranean Sea and North sea. Helgoland Marine Research, 53: 219-243.

Carrola, J., Fontaínhas-Fernandes, A., Matos, P., Rocha, E. (2009): Liver Histopathology in Brown Trout (*Salmo trutta* f. fario) from the Tinhela River, Subjected to Mine Drainage from the Abandoned Jales Mine (Portugal). Bulltein of Environmental Contamination and Toxicology, 83: 35–41.

Dezfuli, B.S., Capuano, S., Manera, M. (1998): A description of rodlet cells from the alimentary canal of *Anguilla anguilla* and their relationship with parasitic helminths. Journal of Fish Biology, 53: 1084-1095.

Dezfuli, B.S., Giari, L., Simoni, E., Palazzi, D., Manera, M. (2003): Alteration of rodlet cells in chub caused by the herbicide Stam M-4 (Propanil). Journal of Fish Biology, 63: 232-239.

Dezfuli, B.S., Simoni, E., Rossi, R., Manera, M. (2000): Rodlet cells and other inflammatory cells of *Ploxinus ploxinus* infected with *Raphidascaris acus* (Nematoda). Disease of Aquatic Organisms, 43: 61-69.

Eastwood, S., Couture, P. (2002): Seasonal variations in condition and liver metal concentrations of yellow perch *(Perca flavescen*s) from a metal-contaminated environment. Aquatic Toxicology, 58: 43–56.

Feere, H., Weibel, E.R. (1967): Stereologic techniques in microscopy. Journal of the Royal Microscopical Society, 87: 25-34.

Figueiredo-Fernandes, A.M., Fontainhas-Fernandes, A.A., Monteiro, R.A.F., Reis-Henriques, M.A., Rocha, E. (2006): Temperature and gender influences on the hepatic stroma (and associated pancreatic acini) of nile tilapia, *Oreochromis niloticus* (Teleostei, Cichlidae): A stereological Analysis by Light Microskopy. Journal of Morphology, 267: 221-30.

Gearheard, J., Kerwin, J., Roberts, S., Bolding, B. - Washington Department of Wildlife, Augmented Fish Health Monitoring For Washington Department Of Wildlife, Annual Report 1988, Report to Bonneville Power Administration, Contract No 1986BP64344, Project No. 198601300, 33 electronic pages (BPA Report DOE/BP-64344-2)

Giari, L., Manera, M., Simoni, E., Dezfuli, B.S. (2006): Changes to chloride and rodlet cells in gills, kidney and intestine of *Dicentrarchus labrax* (L.) exposed to reduced salinities. Journal of Fish Biology, 69: 590-600.
Giari, L., Manera, M., Simoni, E., Dezfuli, B.S. (2007): Cellular alterations in different organs of European sea bass *Dicentrarchus labrax* (L.) exposed to cadmium. Chemosphere, 67: 1171-1181.
Giari, L., Simoni, E., Manera, M., Dezfuli, B.S. (2008): Histo-cytological responses of *Dicentrarchus labrax* (L.) following mercury exposure. Ecotoxicology and Environmental Safety, 70: 400-410.
Goede, R.W., Barton, B.A. (1990): Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. American Fisheries Society Symposium, 8: 93–108.
Hinck, J.E., Blazer, V.S., Denslow, N.D., Echols, K.R., Gross, T.S., May, T.W., Anderson, P.J. (2007): Chemical contaminants, health indicators, and reproductive biomarker responses in fish from the Colorado River and its tributaries. Science of the Total Environment, 378: 376–402.
Hinck, J.E., Schmitt, C.J., Blazer, V.S., Denslow, N.D., Bartish, T.M., Anderson, P.J., Coyle, J.J., Dethloff, G.M., Tillitt, D.E. (2006): Environmental contaminants and biomarker responses in fish from the Columbia River and its tributaries: Spatial and temporal trends. Science of the Total Environment, 366: 549–578.
Jordanova, M., Miteva, N., Rocha, E. (2007): A quantitative study of the hepatic eosinophilic granule cells and rodlet cells during the breeding cycle of Ohrid trout, *Salmo letnica* Kar. (Teleostei, Salmonidae). Fish and Shellfish Immunology, 23: 473-478.
Koponen, K., Myers, M.S. (2000): Seasonal changes in intra- and interorgan occurrence of rodlet cells in freshwater bream. Journal of Fish Biology, 56: 250-263.
Kramer, C.R., Kramer, A.J., Konovalov, A. (2005): Rodlet cell distribution in the gall bladder epithelium of *Fundulus heteroclitus*. Journal of Fish Biology, 67: 555-560.
Lanno, R.P., Dixon, D.G. (1996): The comparative chronic toxicity of thiocyanate and cyanide to rainbow trout. Aquatic Toxicology, 36: 177-187.
Leino, R.L. (1996): Reaction of rodlet cells to a myxosporean infection in kidney of the bluegill, *Lepomis macrochirus.* Canadian Journal of Zoology**, 74:** 217–225.
Leino, R.L. (2001): Phylogenetic Differences in Tissue Distribution of Rodlet cells. Proceeding of the First International Rodlet Cell Workshop, June 14-16, Ferrara, Italy.
Manera, M., Dezfuli, B.S. (2004): Rodlet cells in teleosts: a new insight into their nature and function. Review paper. Journal of Fish Biology, 65: 597-619.
Manera, M., Simoni, E., Dezfuli, B.S. (2001): The effect of dexamethasone on the occurrence and ultrastructure of rodlet cells in goldfish. Journal of Fish Biology, 59: 1239-48.
Mazon, A.F., Huising, M.O., Tavernr-Thiele, A.J., Bastiaans, J., Verburg-van Kemenade, B.M.L. (2006): The first appearance of Rodlet cells in carp (*Cyprinus carpio* L) ontogeny and their possible roles during stress and parasite infection. Fish and Shellfish Immunology, 22 (1-2): 27-37.
Montero, D., Blazer, V.S., Socorro, J., Izquierdo, M.S., Tort, L. (1999): Dietary and culture influences on macrophage aggregate parameters in gilthead seabream (*Sparus aurata*) juveniles. Aquaculture, 179: 523-534.
Papoutsoglou, S.E., Karakatsouli, N., Pizzonia, G., Dalla, C., Polissidis, A., Papadopoulou-Daifoti, Z. (2006): Effects of rearing density on growth, brain neurotransmitters and liver fatty acid composition of juvenile white sea bream *Diplodus sargus* L. Aquaculture Research, 37: 87-95.
Poltronieri, C., Laurà, R., Bertotto, D., Negrato, E., Simontacchi, C., Guerrera, M.C., Radaelli, G. (2009): Effects of exposure to overcrowding on rodlet cells of the teleost fish *Dicentrarchus labrax* (L.). Veterinary Research Communications, 33: 619-629.
Reite, O.B. (1997): Mast cells/eosinophilic granule cells of salmonids: staining properties and responses to noxious agents. Fish and Shellfish Immunology, 7: 567–584.
Reite, O.B. (2005): The rodlet cells of teleostean fish: their potential role in host defence in relation to the role of mast cells/eosinophilic granule cells. Fish and Shellfish Immunology, 19: 253-267.
Reite, O.B., Evensen, O. (2006): Inflamatory cells of teleostean fish. A review focusing on mast cells/eosinophilic granule cells. Fish and Shellfish Immunology, 20: 192-208.
Rocha, E., Monteiro, R.A.F., Pereira, C.A. (1994): Presence of rodlet cells in the intrahepatic biliary ducts of the brown trout, *Salmo trutta fario* Linnaeus, 1758 (Teleostei, Salmonidae). Canadian Journal of Zoology, 72: 1683–1687.
Shoemaker, C.A., Klesius, P.H., Lim, C., Yildirim, M. (2003): Feed deprivation of channel catfish, *Ictalurus punctatus* (Rafinesque), influences organosomatic indices, chemical composition and susceptibility to *Flavobacterium columnare*. Journal of Fish Diseases, 26: 553–561.
Swansburg, E., Chaput, G., Moore , D., Caissie, D., El-Jabi, N. (2002): Size variability of juvenile Atlantic salmon: links to environmental conditions. Journal of Fish Biology, 61: 661-683.

Thilakaratne, I.D.S.I.P., McLaughlin, J.D., Marcogliese, D.J. (2007): Effects of a pollution and parasites on biomarkers of fish health in spottail shiners *Notropis hudsonius* (Clinton). Journal of Fish Biology, 71: 519-538.
van der Oost, R., Beyer, J., Vermeulen, N.P.E. (2003): Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environmental Toxicology and Pharmacology, 13 (2): 57-149.