MACUB

# IN VIVO

The Publication of the Metropolitan Association of College and University Biologists

Spring 2013 Volume 34, Issue 3

# BERGEN COMMUNITY COLLEGE

# TO HOST THE

# 46th ANNUAL MACUB

# FALL CONFERENCE

# SATURDAY, OCTOBER 26, 2013

## Instructions for Authors

***IN VIVO*** is published three times yearly during the Fall, Winter, and Spring. Original research articles in the field of biology in addition to original articles of general interest to faculty and students may be submitted to the editor to be considered for publication. Manuscripts can be in the form of a) full length manuscripts, b) mini-reviews or c) short communications of particularly significant and timely information. Manuscripts will be evaluated by two reviewers.

Articles can be submitted electronically to *invivo@mec.cuny.edu* or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - [1]Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* **32:** 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

# In This Issue:

# The Effects of Environmental Toxicants on Gill Histopathology of Two Populations of the Killifish *Fundulus heteroclitus* (L.)

**Hugh Potter[1]* and Charles R. Kramer[2]**

**[1]Department of Biology, Union County College, Cranford New Jersey 07106**
**[2]Department of Biology, College of Staten Island, Staten Island, New York 10314**

***Corresponding author**

Abstract

The teleost gill is very susceptible to damage due to the presence of heavy metals and hydrocarbons in the marine environment. The histopathological impact of these toxins on the gill was studied in a population of common killifish, *Fundulus heteroclitis* (L.), collected from Saw Mill Creek, a tidal tributary of the Arthur Kill between Staten Island and New Jersey. The gills of these fish also exhibited a high level of infestation with trematode larvae and myxosporidial parasites. This impacted population was compared with *F. heteroclitis* collected from Lemon Creek, a low impact site on the south shore of Staten Island. The Lemon Creek killifish showed much less of the gill histopathology and parasite load seen in the Saw Mill Creek fish. However, some of these changes were present in aged killifish originally collected from this same site and maintained in captivity for four years in a toxin-free environment. This indicates that the pathologies may exist permanently after these fish are removed from a toxic environment. The Saw Mill killifish demonstrated increased numbers of mucous cells in the gill epithelium and an amplification of rodlet cells in the cartilaginous tissue at the base of the gill filaments. The rodlet cells were also found within the endothelial linings of the afferent and efferent brachial arteries. Melanocytes were observed in the gill filaments and associated structures of the gill. The role played by these cellular components in relation to inflammatory processes and parasite infestation in the gills of fish is discussed.

Key words: gills, branchial skeleton, pollutants, heavy metals, histopathology

## Introduction

The fish gill is a vital multifunctional organ. It is responsible for gas exchange, osmoregulation, acid-base balance and excretion of nitrogenous wastes[1,2]. In addition to the above functions, the epithelium of the gill filaments is richly endowed with mucous cells. The mucus produced by these cells has an important role in protecting the fish from waterborne toxins and pathogens[1,3]. The number of these cells and the amount of mucus produced will vary depending on the conditions of the environment.

In most bony fish, including *Fundulus heteroclitus*, there are four pairs of gills. Each consists of two hemibranchs of primary gill lamellae extending from the gill arch[4]. Each primary lamella consists of a thin filament covered with a squamous epithelium and a cartilaginous core. Blood passes down the filament in an afferent and an efferent artery. Uniformly spaced along the primary lamellae are secondary lamellae. These structures are covered by simple squamous epithelium above and below. Within the lamella is a horizontal blood space supported by contractile pillar cells. Chloride and mucous cells are found within the epithelium of the primary lamella in the space between the secondary lamellae[5]. Rodlet cells have been detected in the epithelium of the primary and secondary lamellae of the flower fish, *Pseudophoxinus antalyae*[1].

The gills of fish are often described, along with skin, as the structures that are most affected histologically by waterborne pollutants[2,3]. This is a logical statement since the gill chambers, pharynx and skin have the most direct and continuous contact with the aquatic environment[5].

The primary histopathological changes that occur in the fish gill are generally common to all of the stressors, biological, chemical and physical, that have been reported in the literature[1,3,6-17]. The most commonly described alterations in gill histology are seen in the lamellar epithelium which undergoes detachment and hyperplasia leading to a fusion of the secondary lamellae of the gill filaments. In a condition termed telangiectasia, the blood vessels of the primary lamellae and the blood spaces of the secondary lamellae become congested with blood and swollen. There is also a very significant increase in the number of mucous cells and mucus production on the respiratory surfaces.

Some studies[3,18,19] have provided evidence that stressors not directed to the gills, such as stressing fish by handling, diets with vitamin deficiencies and giving fish intraperitoneal injections of saline can produce many of the changes in gill histology produced by waterborne toxins and gill specific parasites. This suggests that the autonomic nervous system and/or the hypothalamic-hypophyseal axis may play a role in gill histopathology.

It was the purpose of this study to investigate the effects of a major oil spill that took place in the Arthur Kill (NY/NJ) in 1990[20] on the gills of the estuarine killifish *F. heteroclitus*. The fish were collected with a common minnow trap from an area called Saw Mill Creek, an oil spill site where previous studies have shown the presence of high levels of heavy metals Fe, Cu, Zn, Cr, Ni, Ag, Cd and Hg as well as organic pollutants[20,21]. The gill responses were compared to those of fish collected from Princes Bay, a non impacted site located on the south shore of the island and an 'aged' fish maintained in the laboratory for over four years.

## Materials and Methods

The wild caught fish were collected during the summer of 2010. The members of each group, Saw Mill Creek (N=8; St.L 28.7-62.4 mm) and Princes Bay, Lemon Creek (N=6; St.L 45.0-52.4 mm), consisting of a mixture of sexually mature males and females, were immediately placed into separate 20-gallon aquaria and kept overnight under conditions previously described[22,23]. The 'old' fish, a leftover from a previous study[22], was collected from the same Lemon Creek site. This fish was maintained for four plus years under conditions described above.

All fish were sacrificed by over anesthetizing them in a solution of MS-222 (tricaine methanesulfonate (Sigma)). Under a dissection microscope, the opercular plates were carefully cut away and the individual gill arches were removed and the hemibranchs were separated and placed in Bouin's fixative for 24 hours. The tissues were subsequently decalcified in commercial 'decal' solution and processed for paraffin according to previously described procedures[22]. Sections, 6 μm in thickness, were cut on a rotary microtome and stained with Masson' trichrome. Observations focused on the histopathological responses of the primary and secondary lamellae of the gill hemibranch.

## Results

The waters of Princes Bay/Lemon Creek are less impacted by industrial pollutants than the Arthur Kill. Microscopic examination of the gills of *F. heteoclitus* from Lemon Creek showed a normal appearance of gill filaments with well defined secondary lamellae (Fig. 1). Melanocytes can be seen within the filament. Cartilaginous knobs seemed to provide a support for the gill filaments and skeletal muscle elements which represented the abductor and adductor muscles (Fig. 1). Rodlet cells were seen forming a single layer in the perichondrium of the cartilaginous knobs. Each rodlet cell was oriented so that the rodlets faced the lumen of the afferent branchial artery (ABA) (Fig. 2). Rodlet cells were also observed within the endothelia of the ABA and the efferent branchial artery (EBA). In addition, melanocytes were seen within the endothelia common to the ABA and the EBA (Fig. 3).

*F. heteroclitus* collected from the more heavily impacted Saw Mill creek showed the histopathology in gill tissues generally reported in previous studies. The gill filaments exhibited the extensive hyperplasia of interlamellar epithelium leading to fusion of secondary lamellae and a clubbing of the terminus of the gill filaments (Fig. 4). Cysts of myxosporidial parasites (Fig. 5) and trematode larvae (Fig. 6) were often seen within the gill filaments and were associated with pronounced hyperplasia (Fig. 7). Mucous cells were abundant in the hyperplastic epithelium of the filaments (Fig. 8). Epithelial detachment, a frequently reported phenomenon in previous studies, was commonly seen in the secondary lamellae of the gill filaments of Saw Mill Creek killifish (Fig. 9). This effect was produced by an intralamellar edema. Melanocytes and melanin deposits were more commonly found and extensively distributed in the gills of these fish.

As in the Lemon Creek killifish, rodlet cells could be found within the perichondrium of cartilaginous knobs anchoring muscular elements of the gill arches. However, in the Saw Mill Creek fish, the rodlet cells were much more abundant, forming several layers (Fig. 10).

The specimens of *F. heteroclitus* maintained in our lab for four years, were lacking in the perichondrial rodlet cells (Fig. 11) despite showing many of the gill modifications, such as hyperplasia and lamellar fusion associated with exposure to environmental contaminants and pathogens (Fig. 12).

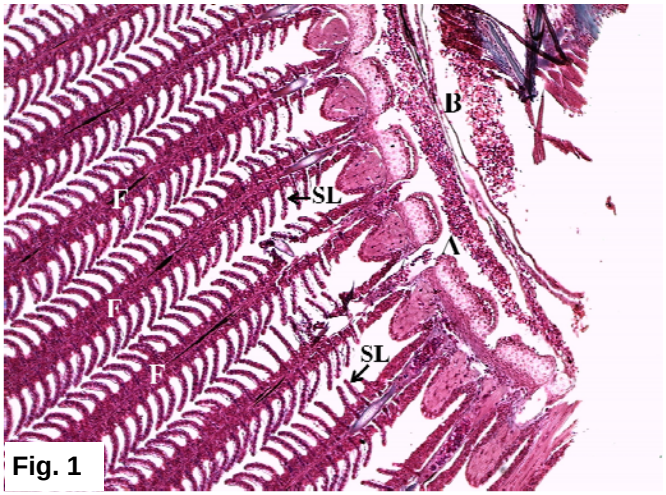

Fig. 1

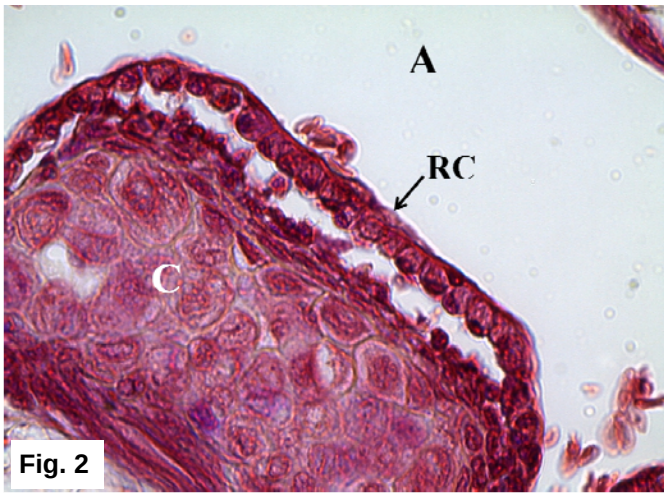

Fig. 2

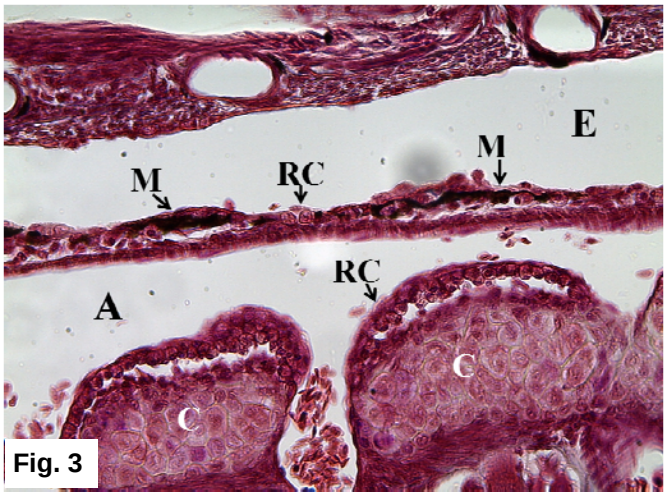

Fig. 3


Fig. 4

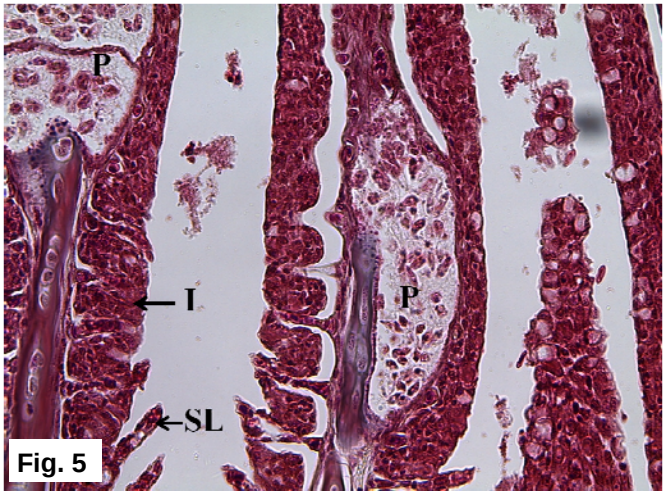

Fig. 5

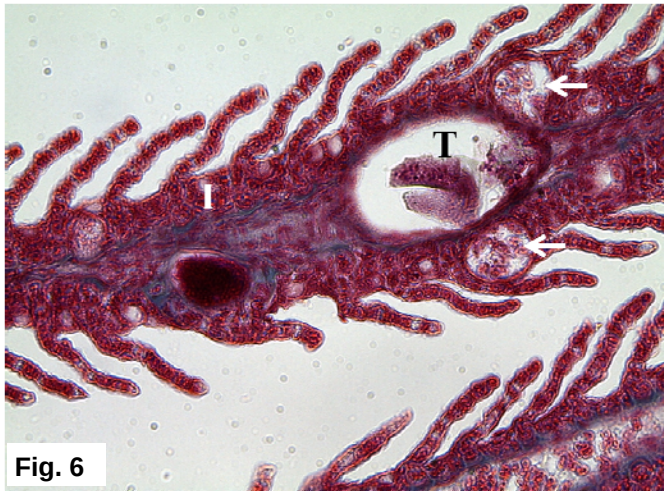

Fig. 6

Fig. 1. An L.S. through the hemibranch of a gill of *Fundulus heterolitus* from the non-impacted site, Lemon Creek showing structure of the filaments (F), secondary lamellae (SL), afferent (A) and efferent (E) branchial arteries, muscle (M) and cartilaginous knobs (C). Masson's trichrome, x100.

Fig. 2. A section through the cartilaginous knob (C) of a gill from a non-impacted fish showing a single layer of rodlet cells (RC) within the endothelium of the perichondrium. The apical ends of the RCs are facing the lumen of the afferent artery (A). Masson's trichrome, x1000.

Fig. 3. A section through the cartilaginous knobs (C) of a gill from a non-impacted fish. RCs are seen in the perichondrium associated with the endothelium of the afferent artery (A) as well as the efferent (E) arteries. Melanocytes (M) were seen in the endothelium common to both arteries. Masson's trichrome, x400.

Fig. 4. A low mag. section through the gill of *F. heteroclitus* collected from highly contaminated Saw Mill Creek. Note the obliteration of the secondary lamellae caused by hyperplasia of the interlamellar epithelium (arrow), clubbing of the terminus of the filaments and telengiectasia, dilation of the central sinus (*). Myxosporidial and trematode cysts are also apparent in some of the filaments. Masson's trichrome, x40.

Fig. 5. A section through the hemibranch of a SMC fish. Note the extensive hyperplasia of the interlamellar zone (I), the fusion of the secondary lamellae (SL) and the presence of myxosporidial cysts (P). Masson's trichrome, x400.

Fig. 6. A section through the filament of an SMC fish. Note the extensive interlamellar hyperplasia (I) and the presence of myxosporidial (arrow) and trematode (T) cysts. Masson's trichrome, x400.

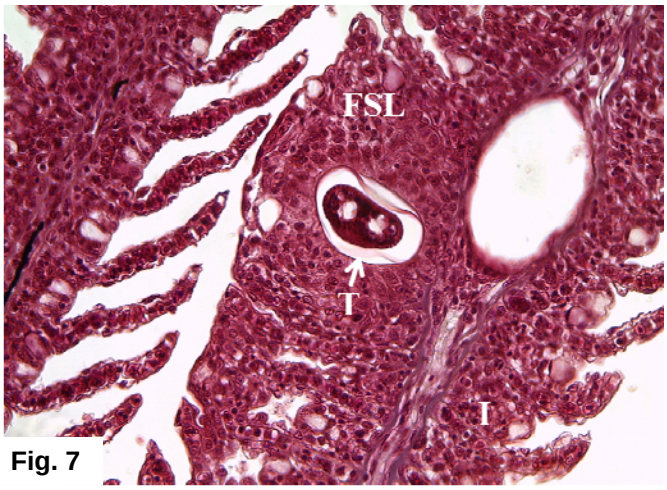


Fig. 7

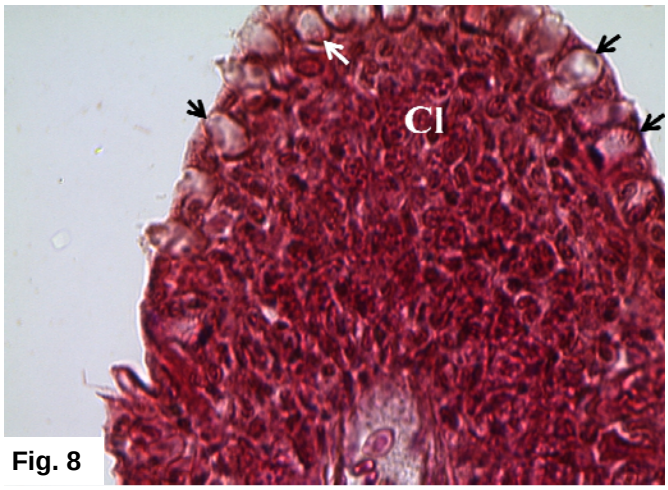


Fig. 8


Fig. 9

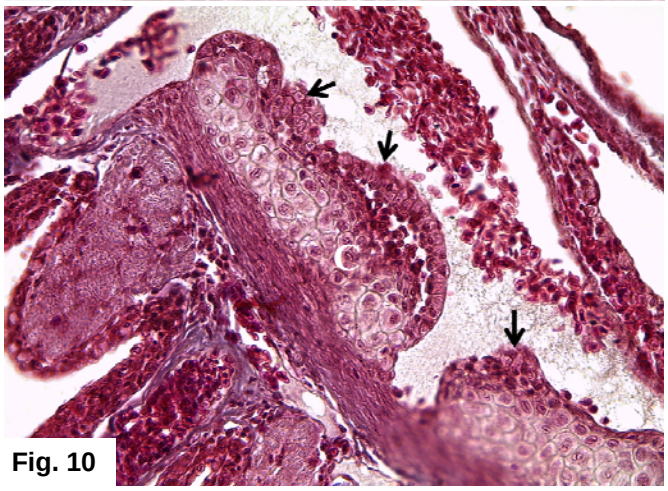

Fig. 10

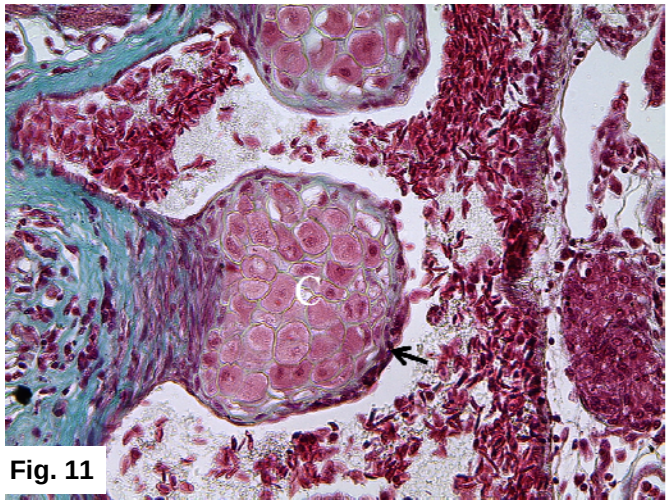


Fig. 11

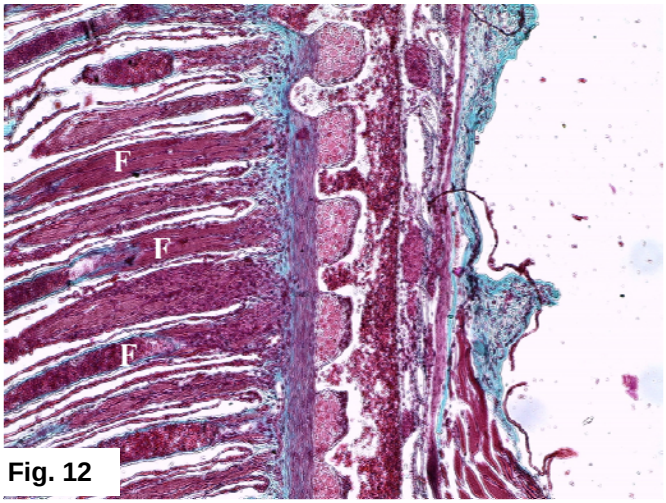


Fig. 12

Fig. 7. The gill of an SMC fish showing marked interlamellar hyperplasia (I) and fusion of the secondary lamellae (FSL). A trematode cyst (T) is also conspicuous. Masson's trichrome, x400.

Fig. 8. A filament tip of an SMC fish showing marked cellular hypertrophy leading to clubbing (Cl) and the abundance of mucous cells (arrows). Masson's trichrome, x1000.

Fig. 9. A section of the gill filament of an SMC fish showing marked detachment of the overlying epithelium (arrow) of the secondary lamellae. Marked interlamellar hyperplasia (I) is also apparent. Masson's trichrome, x1000.

Fig. 10. The cartilaginous knob of the gill of an SMC fish showing abundant multiple layers of RCs (arrow) in the perichondrium associated with the endothelium of the afferent branchial artery. Masson's trichrome, x400.

Fig. 11. A section through the cartilaginous knob (C) of an 'old', laboratory-maintained fish. Note the lack of rodlet cells within the perichondrium (arrow). Masson's trichrome, x400.

Fig. 12. The gill filament (F) of an 'old', laboratory-maintained fish. Note, some changes associated with toxins, heavy metals and parasites still persist such as hyperplasia and lamellar fusion. Masson's trichrome, x100.

## Discussion

The fish gill has been widely described as a sensitive monitor of environmental stresses. Many studies have demonstrated the structural impact of parasitic infestation on this organ[6-12]. Physical and chemical factors have been found to induce histopathological changes in gill histology. These factors include increases in water temperature[13], changes in salinity[14] and pH[15]. Heavy metal exposure has been found to be especially damaging to gill tissues[1,3]. Petroleum effluents have also been reported to produce pathological changes in gill tissues[16]. There are a number of characteristic modifications that occur in gill histology as a result of exposure to the biological, chemical and physical stresses mentioned above. Most of these were observed in the gills of *F. heteroclitus* taken from Saw Mill Creek in this study.

Telangiectasia, a dilation of blood vessels within the primary and secondary lamellar blood vessels, leads to congestion of blood and hemostasis[3,5]. This condition was frequently observed in the gill of fish taken from Saw Mill Creek. In our study and others, epithelial lifting is seen generally in the epithelium lining the secondary lamellae of fish exposed to environmental stressors. It is characterized by the detachment of the epithelial cells from the lamina propria of the secondary lamellae[3,5,24]. Two possible reasons have been suggested for this detachment. Damage to the epithelium could allow fluid infiltration from the environment or fluid from the congested blood spaces might be filtering into the tissue spaces[3].

Hyperplasia of epithelial tissue in the interlamellar spaces of the gill filament has been commonly reported in stressed fish[2,3,5,10-12]. In our work as in others, this ultimately leads to a fusion of adjacent lamellae and the production of large tissue masses and filaments with club-shaped ends.

The lifting of the surface epithelial layer that we and others[3,5,24] observed could have provided an added barrier to toxic exposure by increasing the distance the toxicants would have to travel before penetrating the blood. In turn, this could have been effected by the increase in ionocytes that we observed in the interlamellar zone. By secreting chloride, these cells could have produced an osmotic effect that induced the swelling and subsequent lifting of the epithelium. Furthermore, the dilation, telangiectasia, of the central venous sinus of the primary lamellae that we observed could have aided this process by enhancing blood flow to the secondary lamellae. Blood vessel dilation within the gill was observed in the catfish, *Clarias gariepinus,* collected from a polluted aquatic system[5].

Along with epithelial cells, the hyperplastic areas also contain increased numbers of mucous cells[1-3,5,9-12,24,25]. Significant increases in the mucous cell population in the gill tissues occur due to exposure to parasites, heavy metals, increases in water temperature, changes in salinity and pH[1]. In our work, this effect was seen to occur more in the gill tissues of *F. heteroclitus* from Saw Mill Creek than in fish from Lemon creek. Mucus has been described as having an important role in moderating the damage to gill integrity due to waterborne toxins. Mucus can trap or chelate heavy metal cations[3]. A thick layer of mucus can retard the diffusion of toxins across the gill epithelium from the surrounding water. However, this may also reduce gas exchange across the respiratory surface and force behavioral changes in the fish, i.e., swimming near the surface, which might increase predation[3,26].

Rodlet cells are not as consistent a presence in healthy or stressed gill tissues of fish as mucous cells. Rodlet cells have been found in the epithelium of the lamellae of *Odontenthes bonariensis*[27] and *Pseudophoxinus antalyae*[1]. In the European sea bass, *Dicentrarchus labrax*, increased numbers of rodlet and mucous cells were seen in gills infected with the trematode, *Diplectanum aequans*[10]. Increases in rodlet cells were observed in the primary and secondary lamellae of the gills of the bream, *Abramis brama*, parasitized by the copepod, *Ergasilus siebaldi*[28]. However, in a number of studies involving gills impacted by a variety of toxins, no mention was made of any change in rodlet cell numbers[5,9,12,24,25].

RCs have been said to be mediators of inflammatory processes in fish. This is supported by the significant number of RCs near areas of parasitic infestation in fish gills[10,11]. The experimental infection of carp, *Cyprinus carpio*, with *Trypanoplasma borreli* led to inflammation of the gill tissues. This treatment also produced an increase in RCs in these tissues[29]. Additionally, stressing fish by restraining them in a hand held net reduced the number of RCs in the gill tissue. This result implies an anti-inflammatory role for the RC. Manually stressing the fish would lead to the release of cortical steroids which would reduce inflammation and thereby obviate the need for increased numbers of RCs[29].

In our study, RCs were not apparent in the gill filaments of *F. heteroclitus* from Lemon Creek or Saw Mill Creek. In a previous study[22] RCs were observed in aggregations within the thin perichondrium surrounding a knob of cartilage at the base of gill filaments. In the current study, RCs were found associated with these cartilaginous knobs. In the Lemon Creek fish, the RCs formed a single layer on the surface of the cartilage facing the afferent branchial artery. In the killifish from Saw Mill Creek, there were a number of layers of RCs in a less organized arrangement. The RCs in both groups of *F. heteroclitus* were oriented facing the lumen of the afferent branchial artery which would facilitate the release of rodlets into the circulation.

In the fish from Saw Mill Creek, intact RCs were not found in the vicinity of parasitic cysts in the gill or in any other portion of the filament. The same was true for fish collected from Lemon Creek. However, the presence of RCs adjacent to the afferent branchial artery could represent a location from which rodlets could be released into this blood vessel and then enter the filament circulation to participate in an anti-inflammatory process near areas of tissue damage and parasitic infestation. The presence of a larger RC population within gill cartilage of fish from the much more contaminated water of Saw Mill Creek supports this role of the RCs.

A response to toxic exposure that we observed that was lacking in other studies was the increase in melaninization that took place within the gill filaments. Melanin deposition in response to different stressors has been reported in invertebrates[30,31] and vertebrates alike[32] including humans[33,34]. The protective function of melanin in tissues has been associated with antimicrobial activity, wound healing, uv-light exposure and activation of the innate immune response[31-35]. In some instances, it has been suggested that melanin can actually add to the virulence of microbes by imparting a defensive action against the host's immune response[36]. The primary protective function associated with melanin deposition, however, is that of an antioxidant that modulates the activity of antioxidant enzymes such as SOD and catalase, thus reducing the effects of exposure to stressors such as the heavy metals[34,37]. Heavy metals, especially mercury[2], are known to produce oxidative stress by both producing oxygen reactive species (ROS) in the tissues and inactivating the cell's defenses against ROS2. Pandey *et al.*[2] measured a significant decline in antioxidant enzymes superoxide dismutase (SOD), glutathione-s-transferase (GSI) and catalase in the gills of the exposed fish. In view of the effects of oxidative damage by heavy metals, our observation of increased melaninization is an expected response. Increased melanin formation often follows tissue injury and is localized to an area of inflammation. Chemical mediators of inflammation such as arachidonic acid have been found to increase melanin formation and melanocyte proliferation[38].

In the current study, melanocytes were observed in the gill filaments just below the epithelium. These cells are often closely associated with encysted trematodes. In many species of freshwater fish, encystment of skin, muscle and gills by the trematode, *Neascus* sp. (Strigeidae) leads to formation of melanin surrounding the cyst. In this "black spot disease", the melanin is manufactured by the host tissue and is believed to be a response to inflammation[39].

Other studies have described the presence of melanomacrophages, melanosome-engorged macrophages (MM), in the vicinity of cysts of the nematode, *Anisakis simplex* in the gut of the eel, *Anguilla anguilla*[11] and in the spleen and head kidney of the turbot, *Psetta maxima*, infected with a myxosporean parasite[40]. Bermudez *et al.*[40], noted that during the early stages of the infection, melanomacrophage centers (clusters of MMs) were commonly seen near the parasitic cysts. In those regions there was little evidence of inflammation. This was interpreted as evidence of an anti-inflammatory function of the MM centers[40]. In the platyfish, *Xiphophorus maculatus*, large clusters of MM centers were found in the liver, head kidney and spleen following hyperthermic treatment of the fish[41]. These organs had atrophied and suffered internal damage due to the higher temperatures. An anti-inflammatory role was attributed to the MM centers[25,40,41].

The advanced degree of gill histopathology that we observed coincident with the high infestation of parasites in the fish from the high impact area suggests a correlation between these two factors. In the present study, the killifish from Saw Mill Creek exhibited a heavy parasite load on the primary and secondary lamellae of their gills, consisting of cysts of trematode larvae and myxosporidians. The fish taken from Lemon Creek, an area that had much lower levels of metals and other toxic chemicals, showed a much more reduced level of parasitism. A number of studies exist in which the degree of gill damage corresponds with the incidence of parasite

infection[6,8,10,11]. Dykova and Lom[6] observed significant hispathology in the gills of three teleost species, perch, *Perca fluviatilis*, ruff, *Acerina cernua* and pike, *Esox lucius* that were heavily infested with myxosporidia. The damage included massive cellular hyperplasia surrounding the cysts, macrophage invasion and granuloma formation. Goulding *et al*.[39] found a high incidence of digenetic trematode cysts in heavily damaged gills of wild-caught *F. heteroclitus*. Santiago Bass *et al.*[7] found extensive gill branching in *F. heteroclitus* heavily infected with digenetic metacercariae. Gobler *et al.*[8] found that the gills of juvenile *Cyprinodon variegates* and adult *F. majales,* and *F. heteroclitus* and adult *Menidia menidia* were significantly damaged in response to harmful algal blooms of *Cochlodinium polykrikoides*. The gills of these fish showed marked epithelial proliferation with foci of secondary lamellae fusion. Pandey's team[2] suggested that the oxidative stress induced by heavy metal exposure could have weakened the fish's immune system which would increase its susceptibility to parasites. Van Dyk *et al.*[5] also suggested that damage to the gills of the catfish, *C. gariepinus* resulted from a decline in the immune response caused by exposure to contaminated effluent from a sewage treatment plant. Thus, in the Saw Mill Creek population, the continuous exposure of the fish to heavy metals and organic pollutants could have contributed to a reduced immune response resulting in a high degree of parasite infection.

It appears that some of the induced pathological changes that we observed were non-reversible. In the aged fish maintained long term in the toxin-free conditions of the laboratory, interlamellar hyperplasia and lamellar fusion persisted even after being removed from the wild for four years.

In conclusion, our study strongly suggests that fish exposed to high levels of organic pollutants and heavy metals suffer deleterious effects to their gills. Furthermore, these effects appear to be two-pronged, direct, via the destruction of key gill elements and indirect via an increase in parasite infestation. The latter most likely results from an opportunistic invasion caused by a compromise of the host's immune system. Ostensibly, the destruction of gill morphology should be accompanied by changes in behavior associated with alteration in respiration. These would include changes in activity, stamina, respiratory rate and positioning in the water column. Behavioral modifications were not a focus in this study, but will be included in future work.

## References


[1]Cinar, K., A. Aksoy, Y. Emre and R.N. Asti, 2009. The histology and histochemical aspects of gills of the flower fish, *Pseudophoxinus antalyae*. Vet. Res. Commun. **33:** 453-460.

[2]Pandey, S., S. Parvez, R. A. Ansari, M. Ali, M. Kaur, F. Hayat, F. Ahmad and S. Raisuddin, 2008. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, *Channa punctata* Bloch. Chem-Biol Interact. **174**: 183-192.

[3]Mallatt, J., 1985. Fish gill structural changes induced by toxicants and other irritants: A statistical review. Can. J. Aquat. Sci. **42**: 630-648.

[4]Wilson, J.M. and P. Laurant, 2002. Fish gill morphology: Inside out. J. Exp. Zool., **293:** 192-213.

[5]van Dyk, J.C., M.J. Marchand, G.M. Pieterse, I.E.J Barnham and M.S. Bornman, 2009. Histological changes in the gills of *Clarias gariepinus* (Teleostei: Clariidae) from a polluted South African urban aquatic system. Afr. J. Aquat. Sci. **34(3):** 283-291.

[6]Dykova, I and J. Lom, 1978. Histopathological changes in fish gills infected with myx-osporidian parasites of the genus *Henneguya*. J. Fish Biol. **12**: 197-202.

[7]Santiago Bass, C., S. Khan and J.S. Weis, 2007. Morphological changes in the gills of killifish associated with severe parasite infection. J. Fish Biol. **71**: 920-925.

[8]Gobler, C., D.L. Berry, O. Roger Anderson, A. Burson, F. Koh, B.S. Rodgers, L.K. Moore, J.A. Goleski, B. Allam, P. Bowser, Y. Tang and R. Nuzzi, 2008. Characterization, dynamics, and ecological impacts of harmful *Cochlodinium polykrikoides* blooms on Eastern Long Island, NY, USA. Harmful Algae, **7:** 293-307.

[9]Naldoni, J., S. Arana, A.A.M. Maia, P.S. Ceccarelli, L.E.R Tavares, F. Borges, C.F. Pozo and E.A. Adriano, 2009. Henneguya pseudoplatystoma sp. causing reduction in epithelial area of gills in the farmed pintado, a South American catfish: Histopathology and untrastructure. Vet. Parasitol. **166(102):** 52-59.

[10]Dezfuli, B.S., L. Giari, E. Simoni, R. Menegatti, A.P. Shinn and M. Manera, 2007. Gill histopathology of cultured European sea bass, Dicentrarchus labrax (L.) with *Diplectanum aequans* (Wagener 1857) Diesing 1958 (Diplectanidae: Monogenea). Parasitol. Res. **100(4):** 707-713.

[11]Dezfuli, B.S., S. Capuano, and M. Manera, 2007. A description of rodlet cells from the alimentary canal of *Anguilla anguilla* and their relationship with parasitic helminthes. J. Fish Biol. **53:** 1084-1095.

[12]Arafa, S.Z., M.M. El-Nagger, S.A. El-Abbassy, 2009. Mode of attachment and histo pathological effects of *Macrogyrodctylus clarii*, monogenean parasite of catfish, *Clarias gariepinus*, with report on host response. Acta Parasitol. **54(2):** 103-112.

[13]Boyd, R.B., A.L. DeVries, J.T. Eastman, G.G. Pietra, 1980. The secondary lamellae of the gills of cold water (high altitude) teleost. A comparative light and electron microscopic study. Cell Tissue Res. **213:** 361-510.

[14]Avella, M., J. Berhaut, M. Bornancin, 1993. Salinity tolerance of two tropical fishes, *Oreochromis aureus* and *O. niloticus*. I. Biochemical and morphological changes in the gill epithelium. J. Fish Biol. **42:** 243-254.

[15]Wilkie, M.P. and C.M. Wood, 1994. The effects of extremely alkaline water (9.5) on Rainbow trout gill function and morphology. J. Fish Biol. **35:** 87-98.

[16]Giari, L., B.S. Dezfuli, M. Lanzoni and G. Castaldelli, 2012. The impact of an oil spill on organs of bream *Abramis brama* in the Po River. Ecotox. Environ. Safe. **77:**18-27.

[17]Oliveira Ribeiro, C.A.,E. Pelletier, W.C. Pfeiffer and C. Rouleau, 2000. Comparative uptake, bioaccumulation, and gill damages of inorganic mercury in tropical and nordic freshwater fish. Environ. Res. **83(3):** 286-292.

[18]Roberts, R.J., 1978. The pathophysiology and systematic pathology of teleosts. In *Fish Pathology*, Bailliere Tindall, London, pp. 55-91.

[19]Scott, A.L. and W.A. Rogers, 1980. Histological effects of prolonged sub lethal hypoxia on channel catfish, *Ictalurus punctatus*. J. Fish Dis. **3:** 305-306.

[20]Paulson, A.j., B. Sharack and V. Zdanowicz, 2003. Trace metals in ribbed mussels from Arthur Kill, New York/New Jersey, USA. Mar. Pollut. Bull. **46**:139-152.

[21]Perez, M.H. and W.G. Wallace, 2004. Differences in prey capture in grass shrimp, *Palaemonetes pugio*, collected along an environmental impact gradient. Arch. Environ. Contami. Toxic. **46**: 81-89.

[22]Qama, E., J. Blaize, W.J. L'Amoreaux and C.R. Kramer, 2009. Rodlet cell distribution and activity in the gills of laboratory-maintained and wild-caught *Fundulus heteroclitus.* In Vivo, **31(1):** 1-7.

[23]Kramer, C.R., A. Kramer and A. Konovalov, 2005. Rodlet cell distribution in the gall bladder epithelium of *Fundulus heteroclitus*. J. Fish Biol. **67(2):** 555-560.

[24]Ramirez-Duarte, W.F., I.S. Rondon-Barragan and P.R. Eslava-Mocha, 2008. Acute toxicity and histopathiological alterations of Roundup® herbicide on "cachama blanca" (*Piaractus brachypomus*). Pesq. Vet. Bras. **28(11**): 547-554.

[25]Andrews, M., S. Battaglene, J. Cobcroft, M. Adams, E. Noga and B.Nowak, 2010. Host response to chondracanthid copepod *Chondracanthus goldsmidi*, a gill parasite of the striped trumpeter, *Latris lineata* (Forster), in Tasmania. J. Fish Dis. **33(3):** 211 -220.

[26]Machado, M.R. and E. Fanta, 2003. Effects of organophosphorous methyl parathion on the branchial epithelium of a freshwater fish. Braz. Arch. Biol. Technol. **46(3):** 361-372.

[27]Vigliano, F.F., R. Bermudez, M.I. Quiroga and J.M. Nieto, 2006. Evidence for melanomacrophage centres of teleost as evolutionary precursors of germinal centres of higher vertebrates: an immunohistological study. Fish Shellfish Immunol. **21(4):** 467-471.

[28]Dezfuli, B.S., L. Giari, R. Konecny, R. Jaeger and M. Manera, 2003. Immunocytochemistry, ultrastructure and pathology of gills of *Abramis brama* from Lake Mondsee, Austria, infected with *Ergasilus sieboldi* (Copepoda). Dis. Aquat. Org. **53(3):** 257-262.

[29]Mazon, A.F., M.O. Huising, A.J. Taverne-Thiele, J. Bastiaans, B.M.L. Verburg van Kemenade, 2007. The first appearance of rodlet cells in carp (*Cyprinus carpio* L). ontogeny and their possible roles during stress and parasite infection. Fish Shellfish Immunol. **22(1-2):** 27-37.

[30]Zahedi, M., D.A. Denham and P.J. Ham, 1992. Encapsulation and melanization responses of *Armigeres-Subalbatus* against inoculated sephadex beads. J. Invertebr. Pathol. **59(3):** 258-263.

[31]Jiang, H., 2008. The biochemical basis of antimicrobial responses in *Manduca sexta.* Insect Sci. **15(1):** 53-66.

[32]Memoli, S., A. Napolitano, M. D'Ischia, G. Misuraca A. Palumbo and G. Prota, 1997. Diffusable melanin-related metabolites are potent inhibitors of lipid peroxidation. Biochim. Biophysic. Acta, **1346(1):** 61-68.

[33]Costin, G-E. and V.J. Hearing, 2007. Human skin pigmentation: melanocytes modulate skin color in response to stress. FASEB J. **21(4):** 976-994.

[34]Baldea, I., T. Mocan and R. Cosgarea, 2009. The role of ultraviolet radiation and tyrosine stimulated melanogenesis in the induction of oxidative stress alterations in fair skin melanocytes. Exp. Oncol. **31(4):** 200-208.

[35]MacKintosh, J., 2001. The antimicrobial properties of melanocytes, melanosomes and melanin and the evolution of black skin. J. Theor. Biol. **211(2):** 101-113.

[36]Nosanchuk, J.D. and A. Casadevall, 2003. The contribution of melanin to microbial pathogenesis. Cell. Microbiol. **5(4):** 203-223.

[37]Panich, U., K. Kongtaphan, T. Onkoksoong, K. Jaemsak, R. Phanungrakwittaya, A. Thawarn, P. Akarasereenont and A. Wongkajornsilp. Modulation of antioxidant defense by *Alpinia galangal* and *Curcuma aromatica* extracts correlates with their inhibition of UVA-induced melanogenesis. Cell Biol. Toxicol. **26(2):** 103-116.

[38]Nordlund, J.J., 1985. Pigmentary system in host defenses. In *Experimental and Clinical Photoimmunology,* Vol. III, CRC Press, pp. 189-202.

[39]Goulding, D.R., T.L. Blankenship-Paris, G.A. Lewbart, P.H. Myers, T.K. Demianenko, J.A. Clark and D.B. Forsythe, 2004. Gill trematodes (flukes) in wild-caught killifish (*Fundulus heteroclitus*). Contemp. Top. Lab. Anim. **43(2):** 32-34.

[40]Bermudez, R., A.P. Losada, S. Vazquez, M.J. Redondo, P. Alvarez-Pellitero and M.I. Quiroga, 2010. Light and electron microscopic studies on turbot *Psetta maxima* infected with *Enteromyxum scophthalmi*: histopathology of turbot enteromyxosis. Dis. Aquat. Org. **89**:209-221.

[41]Perlmutter, A. and H. Potter, 1998. Hyperthermic suppression of a genetically programmed melanoma in hybrids of fishes: genus *Xiphophorus*. J. Cancer Res Clin. **114(4):** 359-362.

# Universal Science Literacy:
# The Benefits and Appeal of Biology Electives to Non-Science Major Students

**Carla Beeber and Carol A. Biermann**
**Department of Biology, Kingsborough Community College, 2001 Oriental Blvd, Brooklyn, NY 11235**

Abstract

A variety of non-science major courses are available at Kingsborough Community College/CUNY so that students can achieve science literacy. The biology courses that were examined in this study were Biology of Women and Marine Biology. Students in each course were surveyed to acquire feedback on their impressions about their experiences in these courses. Overwhelmingly, data indicated that these non-science majors felt that the two courses met the goals of extending their scientific literacy, and that the knowledge acquired related to their every day lives.

Key words: non-science major's courses; science literacy; students' feedback.

## Introduction

Both at the community college and senior college levels, courses for non-science majors are more common than ever before. However, there is a paucity of studies concerning these courses. This investigation was performed in order to extend knowledge concerning the effectiveness of non-science major biology courses. Science educators have recognized "…that science literacy is a professional responsibility of scientists"[1]. Non-science majors are often not happy to be in a science classroom. They may possess a natural aversion to science. Some instructors may consider many of these students "unteacheable". Instructors of these courses need to engage students so that they may overcome their fears of failure that might be the basis for their dislike of science[2]. Non-science majors can be motivated by non-science courses with curricula that enhance the relevance of science to their lives. In a study by Glynn *et al*[3] the authors demonstrated a direct correlation between students' motivation and their performance and their motivation was influenced by their belief in the relevance of science to their careers.

The goals of non-science major courses include: to increase students' scientific literacy, to help them appreciate how science works and to provide them with a knowledge base in science. Science literacy will enable students to grapple with ideas and acquire problem-solving skills[4]. Georgia State University proposed a standard for non-science major biology courses and established the following goals[5]:

1. Appreciation for how science is conducted.
2. To provide a knowledge base in a particular scientific field that students can use as a foundation for life-long learning in the sciences.
3. To introduce students to the process of scientific thinking.

As citizens in today's world, all students will be exposed to science in their everyday experiences. Situations and dilemmas presented to these students will require them to make informed decisions concerning their own wellbeing, as well as the future of our planet. With the advances of genetics and technology, the public needs to be informed on these subjects. Fifty years ago, Linus Pauling[6], recipient of the Nobel prize for chemistry, made a compelling case for non-science majors learning science; "…citizens must have knowledge enough of the world to make the right decisions. In this modern world it means that citizens must have a significant understanding of science".

Bowling *et al*.[7] looked at the current instruction in genetics and tested 300 non-science majors at the undergraduate level using an assessment instrument administered pre-and post-course. Their data demonstrated the need for improving the current courses so that students may achieve scientific literacy in the field of genetics.

College curricula allow a great deal of flexibility to non-science majors in their choices of science courses leading to their respective liberal arts degrees. Non-majors may choose courses in the fields of biological or physical sciences. These courses range from lecture/ demonstration to traditional lecture/laboratory. Science

literacy is of prime importance for all college students, whether they be science majors or in liberal arts curricula. Non-majors may be a difficult group to teach because of lack of interest in science, no experience with learning science and/or a past unpleasant experience with a previous science course.

Faculty view teaching non-science majors in two ways: as a challenging group to teach, or an opportunity to teach innovatively and creatively. Timothy Goldsmith of Yale University[1], states that the instructor needs to identify how much prior knowledge on the subject students are bringing with them to the classroom. "Non-major students need to be offered hooks to hang their new knowledge on," states Edgar Moctezuma, an instructor at the University of Maryland, College Park[8].

A study by Tuberty *et al*.[9], concerned their students' understanding of the nature of science in a non-science major introductory biology course. The authors surveyed 287 freshmen before and after having attended the course. The results demonstrated that, although the course impacted some students negatively, most seemed to view the "explicit approach" (i.e., class discussions) as the major influence on their conceptions of the nature of science.

The authors concluded that, "If the goal of science education is to improve scientific literacy… instructors… should stop focusing only on teaching and learning discreet topics…and design these courses in such a way that students encounter, experience, and understand the contemporary nature of science"[9].

The instructor also has to become an exemplary communicator, while at the same time teaching a rigorous, non-watered down science course. "Conveying the importance of science, the excitement of science, and some real scientific knowledge to students with no vocational interest in science requires a blend of particular skills and wide-ranging interests.."[8]. Often, the use of an active learning communicative format, such as an interactive computer program, field experiences or student-created PowerPoint presentations are needed in order to keep students engaged. This investigation was undertaken in order to broaden and extend our knowledge concerning the effectiveness of non-science major's courses.

## Method

Surveys are a useful tool to assess non-science major courses. In this investigation, surveys were used to obtain students' responses concerning these types of courses. Students filled up a consent form where they were advised that their participation was absolutely voluntary and information collected was confidential and no student's name was going to be used in any publication resulting from the study. Assessment involves the measurement of students' progress towards the attainment of stated learning outcomes. In general, assessment has the purpose of supporting and improving students' learning. There are various forms of assessments available to faculty in order to attain these objectives. Assessments may be reflective, comprehensive, or diagnostic. Surveys are vehicles to acquire information and to generate knowledge of *what is*[10]. Surveys, such as the ones used in this study, are a form of reflective assessment of students' experiences in biology courses. The aim of such assessments is to provide faculty with feedback in order to improve course curricula. When students filled out survey questionnaires, their responses were an essential form of feedback. Students' Impressions about their experiences in these courses were ascertained. As part of the core curriculum, non-science majors (Liberal Arts students) are often required to take science classes for the purpose of acquiring and developing scientific literacy. At Kingsborough Community College/ CUNY, there are several biology courses that meet these students' degree requirements. Two of these classes are 3 credits, 3 hours lecture blocks: Biology of Women (Bio 28) and Marine Biology (Bio 25). The Biology of Women course consisted of two sections for a total of 65 students, while the Marine Biology course consisted of one section of 18 students.

## Description of the Courses

### Marine Biology -Bio 25:

A description of activities performed in the Marine Biology course is shown in Table 5. The instructor attempted to make each one of the 12 activities meaningful, interesting and stimulating. The instructor performed dissections as demonstrations. Students performed the "Prey-Predators" activity[11].

Students viewed several films and analyzed "Finding Memo" by listing ocean organisms in the film and analyzing their natural behavior. Each week students were required to comment in writing on a particular statement or question pertaining to the material. They were also required to collect organisms and present their collections to the class. The instructor viewed their work and returned it with comments.

**Biology of Women- Bio 28:**

The course goal is to engage students in learning the details of female physiology and ailments specific of women. One of the main goals of the course is to teach students methods of prevention and care. The activities of the course are described in Table 6. The concepts covered in the course curricula are:

1. Introduction to the course. Women and their health: from ancient history to present.
2. Reproductive Anatomy; Anatomy and Physiology of the Female and Male Reproductive systems. The sexual response.
3. Women bodies: Muscle, Fat and Health
4. The menstrual cycle and its hormonal interrelationships
5. Menstrual problems: causes and treatments.
6. The biological basis of gender.
7. Pregnancy, labor and delivery. Embryology and fetology.
8. The mammary glands, Lactation, Breast cancer.
9. Control of Fertility.
10. Problems of Fertility.
11. Women & Cancer. HIV & Women.
12. Aging and menopause.

The course is conducted with use of PowerPoint. Students are invited not only to ask questions but to propose specific subjects of their own interest as well. At the beginning of the semester, students are given a list of five movies and asked to choose one to view at home. All five movies deal with subjects of women's concern in society. Students are given a list of specific questions for each movie to answer in writing after having viewed the movie. Some of the papers are discussed during class time. Students are always very enthusiast about this activity and their writing often provides interesting material for class discussion. At the end of the semester students are polled to choose a topic not included in the curriculum that they wish to discuss in the classroom. This particular activity, because it is at the end of the semester, often gives the instructor a sense of how students have understood the importance of learning the science of women.

## Results and Discussion

Students in the two courses were surveyed by asking them to analyze and comment on different questions pertinent to the content and activities of each course. The Likert scale was used to evaluate the data and the results are presented in percentages. Students also had the opportunity to verbalize what they liked most or least about the courses. Tables 7 and 8 depict a selection of students' comments for each course that was analyzed.

Since students in both classes gave very positive feedback concerning the Biology 25 and 28 courses, we believe that non-science major courses are valuable in expanding students' knowledge base. Tables 7 and 8 show selected students' comments. There were no negative comments regarding either course. Students developed science literacy in each course by reading and analyzing the texts and other sources, learning to appreciate and understand more about how science works. They realized how the topics discussed and studied in these courses impinged upon their lives. These surveys proved necessary to tailor these courses to students' needs, thereby helping us improve our teaching methods.

Marine Biology (Bio 25) is a non-science major course that aims at making students aware and appreciative of marine organisms. Furthermore, the course gives students an understanding of human environmental impact. To this end, students were asked to comment on the three field trips that were scheduled during class time. The following were the three trips: Kingsborough Boat on the Jamaica Bay, the NY Aquarium and the Beach on the campus of Kingsborough.

Tables 1, 2 and 3 illustrated the level of appreciation for these experiences and close to 90% of students rated these trips excellent to superior. Students seemed to be especially appreciative of the boat trip. During this time they were able to dredge the ocean floor, collect and identify organisms. They observed the plankton collected under the microscope. The walk on the beach, as shown in Table 3, was not very well received by students, probably because on that day, there were few organisms available on the beach for viewing. Students' feedback on the

aquarium trip was very positive. Some of the spectacular displays made quite an impression on the students.

Table 4 shows the statistical analysis of students' responses to the Biology of Women (Bio 28) questionnaire. A qualitative analysis of the responses is shown below .

1. Biology of women is tailored to the non-science major; it attempts to simplify some of the most difficult biological concepts presented using PowerPoint slides. The instructor always adds verbal explanations and answers to pointed questions. From students' responses it appears that they appreciated and comprehended the concepts presented.

2. The majority of Students appeared to be involved in the learning process, having found PowerPoint presentations to be a useful means to clearly understand the concepts.

3. Students found copies of the PowerPoint slides very helpful as study aids because these slides reiterated the material that was presented in the classroom.

4. The fourth questions contained the word "science" (not used in any of the other previous or subsequent question). The psychological fear of science in non-science majors perhaps played a role in their understanding of the science. Only 31% considered their understanding superior.

5. One of the goals of teaching a biology course to non-science majors is to increase their interest in biology and, given the results of their responses, this course seemed to have accomplished the goal.

6. Initially, when teaching this course, it was apparent how little most young people knew about issues concerning their own health and body physiology. Towards the end of course, most students stated that, in the future, they would be better equipped to take care of themselves, armed with a knowledge that allows them to make educated decisions.

7. Since the sex revolution of the 1970s, one of the foremost goals of education should have been informing young people of the scientific concepts of reproductive biology. Students at the end of this course often stated that they now have the knowledge that will help them in their intimate relations. (Just recently, in NYC public schools there has been a proposal to make sex education mandatory).

8. Students expressed satisfaction with this course. Every semester the enrollment in this course fills every seat available.

One interesting instructor's observation about the course Biology of Women is that, every semester, the student population always included about ten males, while the majority of students were female. Initially, male students viewed the course as "a chick course", however, after the first couple of weeks, the same male students began to relate to the material and see its relevance to their lives. They then decided not to drop the course and stayed to the end.

Since students gave very positive feedback concerning both the Marine Biology and the Biology of Women courses, it appears that non-science major courses are valuable for expanding students' knowledge as well and helping them acquire scientific literacy. They accomplished course goals by analyzing the assigned readings and participating in classroom discussions, activities and demonstrations. Students learned to appreciate and understand more about how science works (Nature of Science) and realized how the topics discussed and studied in these courses were relevant to their lives.

**Table 1. KCC BOAT TRIP**

| Questions | Superior (%) | Excellent (%) | Satisfactory (%) |
|---|---|---|---|
| 1.How easy was to understand the instructor? | 56 | 33 | 6 |
| 2. How well did you feel that you understood the science behind the activities? | 56 | 33 | 6 |
| 3. How much did this experience increase your interest in Marine Biology? | 44 | 39 | 6 |
| 4. How do you rate this field trip overall? | 50 | 39 | 6 |

**Table 2. Trip to NY Aquarium**

| Questions | Superior (%) | Excellent (%) | Satisfactory (%) |
|---|---|---|---|
| 1. How well did you feel that you understood the science behind the animal displays ? | 39 | 39 | 17 |
| 2. How much did this experience increase your interest in Marine Biology? | 39 | 39 | 22 |
| 3. How do you rate this field trip overall? | 44 | 39 | 17 |

**Table 3:- Walk on KCC Beach**

| Questions | Superior (%) | Excellent (%) | Satisfactory (%) |
|---|---|---|---|
| 1. How well did you feel that you understood the science behind the activities? | 39 | 39 | 22 |
| 2. How much did this experience increase you interest in Marine Biology? | 0 | 28 | 56 |
| 3. How do you rate this field trip overall? | 33 | 50 | 17 |

**Table 4 - Bio 28 (Biology of Women)**

| Questions | Superior (%) | Excellent (%) | Satisfactory (%) |
|---|---|---|---|
| 1.How easy was it to understand the instructor's explanations using PowerPoint presentations? | 45 | 42 | 12 |
| 2.How easy was it to understand the PowerPoint presentations? | 46 | 38 | 14 |
| 3. How useful did you find the copies of the PowerPoint slides? | 68 | 25 | 6 |
| 4. How well did you feel you understood the science taught in this course? | 31 | 54 | 14 |
| 5. How did this course increase your Interest in Biology? | 40 | 31 | 22 |
| 6.How much did the information you learned influence the way you will take care of your own health? | 72 | 17 | 9 |
| 7.Did this course increase your interest in human reproductive Biology? | 48 | 35 | 11 |
| 8.How do you rate this course overall? | 51 | 34 | 12 |

**Table 5. Marine Biology Course Activities (Bio 25)**

| Activity | Description |
|---|---|
| 1 | Trip to KCC beach: Collections and measurements |
| 2 | Trip to NY Aquarium : Choosing organisms to study in detail. |
| 3 | KCC boat trip: Dredging and identifications of organisms, etc. |
| 4 | Dissections: Jellyfish, sea star, shark |
| 5 | Sexual Reproduction: Study of sea urchin and sea star embryology. |
| 6 | Chick Egg Embryology |
| 7 | Prey-Predator Activity: a simulation. |
| 8 | Analysis of natural behavior of organisms seen in "Finding Memo" film. |
| 9 | Sharks and Sea Lions: Predators and prey (Video). |
| 10 | Biodiversity: a taxonomy demonstration |
| 11 | Students' presentation of their notes taken during the semester. |
| 12 | Students' presentations of their collections of organisms. |

**Table 6. Biology of Women Course Activities (Bio 28)**

| Activity | Description |
|---|---|
| 1 | Choice of movie (subject pertinent to women's issues). |
| 2 | Submission of paper with responses to questions specific to each movie. |
| 3 | Class discussion on movies questions and answer. |
| 4 | Class discussion on a topic chosen by the majority of the students. |

**Table 7. Selected Students' Comments from Bio 25 (Marine Biology)**

"Everything I learned was interesting. The ocean is like a whole other world. I think since the ocean is 80% {sic} of the world we should be very knowledgeable about it. Also, I learned that we should be aware of the ocean's health. Humans impact the ocean a great deal and it is important to pay attention to the negative effect."

"The most interesting information in the course is the evolution of animals and their habitats."

"We are ruining the ocean a lot more than I thought."

**Table 8. Selected Students' Comments from Bio 28 (Biology of Women)**

"The most useful information from this course was how to take care of my body, as a female, as a whole; such as how to aid in preventing breast cancer (I have a family history) and taking care so that I don't contract infections. ….learning about STDs."

"I believe it was very informative. It should be recommended to all students, males and females alike. This may encourage smarter decisions to be made by students."

"My favorite aspect was the time when we were learning about pregnancy…. Before taking this class I never knew that breastfeeding was so important in a developing baby."

## References


[1]Goldsmith, T. H. *et al*., 1990. Fulfilling the Promise: Biology Education in the Nation's School. *National Academy Press* Washington, D.C. 152 pp.

[2]Lewes, D., 2010. The Unteacheables. *The NEA* Higher Education Advocate A publication of the National Education Association. **28(1):** 1-5.

[3]Glynn, S.M., 2007. Non-Science Majors Learning Science: A theoretical Model of Motivation. Journal of Research in Science Teaching **44 (8):** 1088-1107.

[4]Krajcik, J.S., 2010. Supporting Students in Developing Literacy in Science. Science **328 (5977):** 456-459.

[5]Georgia State University, 2001. Proposed Standards for a Non-Science Major Biology Course. Quality in Undergraduate Education.

[6]Pauling, L., 1951. The Significance of Chemistry to Man in the Modern World. Engineering and Science **14:** 10-12.

[7]Bowling, B.V., 2008. Genetic Literacy of Undergraduate Non-Science Majors and the Impact of Introductory Biology and Genetics Courses. BioScience **58(7):** 654-660.

[8]Pain, E., 2010. Teaching Science to Non-Science Majors. Available on line at: http:// sciencecareers.sciencemag.org

[9]Tuberty, B., P. Das and M. Windelspecht, 2011. Student Understanding of Scientific Hypotheses, Theories & Laws: Exploring the Influence of a Non-Majors College Introductory Biology Course. International Journal of Biology Education **1(1):** 23-44.

[10]Butts, D., 1983. The Survey - A Research Strategy Rediscovered. Journal of Research in Science Teaching **20(3):** 187-193.

[11]Frye, V., 1981. *Lab Manual to Accompany Exploring Biology*. Saunders/College Cook, 1981.

# Mislocalization of Glutamate Transporter Protein GLT-1 in a Cell Model of Alexander Disease

**Rujin Tian**

**Department of Biology and MLT, Bronx Community College, CUNY, NY**

## Abstract

Heterozygous mutations in GFAP, which encodes the major intermediate filament protein of astrocytes, result in Alexander Disease (AxD), a fatal neurological disorder. The mutation causes protein aggregates in astrocytes, but paradoxically a loss of oligodendrocytes and neurons. Uptake of glutamate (Glu) via the major glutamate transporter (GLT-1) of astrocytes is important for limiting Glu-mediated toxicity to other cell types. The hippocampi of AxD patients, AxDWT/R236H mice, and AxD astrocytes in slice culture show reduced GLT-1 level and function, but the underlying mechanisms remain unclear. To help address this issue, we generated a human astrocyte line that reproduced the aggregations of GFAP+ proteins in AxD. Moreover, confocal fluorescence microscopy revealed mislocation of GLT-1 proteins from cell membrane to multiple intracellular patches or aggregates that are partially colocalized with lysosome in astrocytes expressing AxD mutant. These results are consistent with the idea that defective Glu uptake in AxD is caused by aberrant trafficking and membrane targeting of GLT-1. Our study illuminates links between GFAP mutations, altered Glu homeostasis and toxic interactions between astrocytes and neurons/oligodendrocytes, thus revealing novel pathogenic mechanisms underlying the neuronal loss and demyelination in AxD.

## Introduction

Alexander disease (AxD), a degenerative neurological disorder mainly of infants and children, causes progressive macrocephaly, psychomotor regression, intractable seizures and eventual death. More than 20 heterozygous missense mutations in the gene for glialfibrillary acidic protein (GFAP) have been identified in AxD patients[1]. The pathological hallmark of AxD is the Rosenthal fiber (RF), prominent protein aggregates within astrocytes. Another striking feature of AxD pathology is a profound loss of myelin and oligodendro-cytes, and a variable loss of neurons. Among the known components of RFs are the intermediate filament proteins GFAP, small heat shock proteins including αB-crystallin and hsp27, and plectin, a filament binding protein[2,3]. The fact that a mutation in GFAP, a structural protein found almost exclusively in the CNS astrocytes, results in a combination of local tissue destruction and death of neurons and oligodendrocytes, implies that the cellular degeneration in the AxD brain is secondary to compromised communica-tion links between astrocytes and other cell populations.

GLT-1 is the major glutamic acid transporter of the CNS, mediating a sodium-dependent uptake of glutamate into astrocytes to control extracellular levels of this excitatory neurotransmitter and prevent excitotoxic cell death[4]. Neurons, axons and oligodendrocytes are all susceptible to high levels of glutamate[5, 6]. In vivo studies that suggest aberrant astro-cytes in AxD decrease their ability to take up glutamate include (1) Hippocampal sections of infantile AxD patients displays dramatic losses of GLT-1 immunoreactivity; (2) The expression of GLT-1 protein is significantly reduced in the hippocampus of a knock-in mouse model of AxD; (3) Patch-clamp recordings in acute hippocampal slice of these mice exhibits a strikingly decreased glutamate transporter current[7].

Despite these observations, the cellular machinery underlying GLT-1 dys-regulation in AxD remains unknown. Part of the challenge stems from the difficulty in establishing a cell model that can adequately mimic RFs. Previous work suggests that achieving consistently high level of GFAP is central to the pathogenesis of AxD. Astrocytes from neonatal knock-in mice, however, show relatively few GFAP inclusions in vitro (≤5% with inclusions)[8]. To overcome this limitation and address the molecular link between GFAP mutation and GLT-1 dysfunction, we generated a human astrocyte cell line stably overexpressing R239C mutation, the most common GFAP mutations found in AxD. We observed R239C astrocytes formed cytoplasmic inclusions resembling RFs accompanied by inappropriate

targeting of GLT-1 proteins from cell membrane to lysosomal compartments. Our findings support a direct link between GFAP mutation and altered GLT-1 function, and provide new insights into the pathogenesis of neuronal/oligodendrocyte loss in AxD.

## Material and Methods

### Fluorescent DNA Constructs and Viral Transduction

To construct N-terminally GFP-tagged versions of wild-type (WT) and R239C (RC) GFAP, we used the modified pcDNA3 plasmid containing a peGFP-N1 (Clontech, Palo Alto, CA). The full GFAP coding region was amplified and subcloned into the modified eGFP-N1 plasmids between the EcoRI and the XhoI sites (New England Biolabs, Beverly, MA). U251 human astrocytoma cells were infected with retroviral construct pQCXIX-IRES-eGFP (modified from Clontech's pQCXIX) containing cDNA encoding wildtype or arg239cys (R239C) GFAP inserted between the BamHI and EcoRI sites. Retroviral expression vectors (WT-GFAP-IRES-eGFP and RC-GFAP-IRES-eGFP) were generated and propagated in gp293 cells with the help of VSVG. RFP-tagged GLT-1 was a gift from Dr. Michael Robinson at the children's hospital of Philadelphia. All plasmid inserts were fully sequenced.

### Flow Cytometry

After 48 hr retroviral infection, U251 cells were trypsinized and resuspended in 20% FBS in 1x PBS according to the instructions and protocols of FACS facility at Herbert Irving Comprehensive Cancer Center of Columbia Medical Center.

### Cell Culture and Transfection

U251 Cells were cultured in 10% fetal calf serum in DMEM medium (Sigma, St. Louis, MO) supplemented with 50 μg/ml penicillin. Transient transfections were performed in serum-free medium using Lipofectamine 2000 reagents (Invitrogen, Carlsbad, CA).

### Confocal Microscopy

Cells were grown on 22-mm glass coverslips. Twenty-four hours after transfection, cells were fixed in 4% paraformaldehyde for 10 to 15 minutes and subjected to indirect fluorescence/confocal laser-scanning microscopy (LSM 510 META, 100X, 1.4 N.A; Zeiss, Thornwood, NY). 50nM LysoTracker Blue DND-22 (Molecular Probes, Invitrogen) was applied for the last 30 min of incubation before examined by confocal microscopy. Dual or triple color images were acquired by consecutive scanning with only one laser line active per scan to avoid cross-excitation. All images are projections generated from confocal serial sections of fluorescently labeled cells. For nuclear staining, cells were treated with 10 μg/mL Hoechst 33258 (Sigma) in PBS for 15 min followed by incubating with primary and secondary antibodies and mounted in Gel/Mount medium (Biomedia).

## Results

### Generation of a human astrocyte cell line stably expressing WT and R239C GFAP using retroviral vector

A human astrocytoma cell line (U251) was infected with retroviral vector expressing Green Fluorescent Protein (GFP)-fused to the cDNA coding wildtype (WT) GFAP and R239C GFAP. Figure 1 depicts these cells at day 20 post-viral transduction. A total of 30 lines were established through FACS selection of cells expressing equivalent levels of GFP tagged WT and R239C-GFAP, a reporter and marker gene in our retroviral construct stably integrated into the astrocyte genome. The cell lines with the modest levels of GFAP expression were chosen for further analysis. Note WT lines exhibited filamentous pattern of GFAP while R239C astrocytes possess large number of mutant GFAP aggregates and form RF-like inclusions. 86%±8% of cell population containing stably integrated GFP-tagged GFAP and exhibited a process-bearing morphology after being passaged more than 35 times, with more than 6 months in continual culture. These selected cell lines were further characterized by western blot analysis[3]. Compared to WT cell lines, R239C mutant cells showed decreased proteasome proteolytic activity, increased stress-related kinase activity, such as p38 and JNK, and increased autophagy, all of which we see in human tissues and in the AxD mice[9,10] .

**Cell membrane localization of GLT-1 is disrupted in R239C astrocytes**

To determine whether loss of GLT-1 protein in AxD could be reproduced in our in vitro cell model, we performed immunofluorescence staining for GLT-1. Confocal analysis revealed a strikingly different distribution of endogenous GLT-1 between the cells expressing WT GFAP and those expressing the R239C mutant. GLT-1 showed a dispersed punctate pattern in WT line (Fig. 2, upper panel); consistent with the observations that endogenous GLT1 is primarily localized to perisynaptic membranes of astrocytes in primary astrocytes and astrocytes in slice culture[11, 12].

In R239C astrocytes, however, GLT-1 formed large concentrated "patches" or aggre-gates, which are primarily localized in the cell body (Fig. 2, lower panel). To further determine the subcellular distribution of GLT-1 and visualize the movement of GLT-1 clusters in live astrocytes expressing mutant GFAP, we transiently transfected our astrocytes with cDNA encoding a Red Fluorescence Protein (RFP) fused GLT-1. As expected, most RFP-GLT-1 was essentially confined to the cell surface with almost no intracellular staining in WT astrocytes (Fig.3, left). In contrast, these clusters are restricted to distinct intracellular compartments of R239C astrocytes (Fig. 3, right panel), a similar pattern as seen in Figure 2 (lower panel).

**Defective intracellular trafficking of GLT-1 in R239C astrocytes**

To determine if the intracellular GLT-1-positive puncta are associated with specific subcellular compartments, we labeled our astrocytes with an ionic dye, Lysotracker blue (50 nM; Molecular Probes), which is selectively sequestered in acidic organelles. In WT astrocytes we observed a fine punctate pattern of labeling within the cytoplasm (Fig. 4c), consistent with lysosomal staining. No appreciable colocalization was found between RFP-GLT-1 (cell surface, Fig. 4b) and Lysotracker (intracellular, Fig. 4d) in d (overlay of a, b and c). R239C astrocytes (Fig. 4e) showed intense labeling of large cytosolic aggregates of RFP-GLT -1 (Fig. 4f) and Lysotracker blue (Fig. 4g), which resembled colocalized vacuoles/inclusions (Fig. 4h). A similar phenotype was also seen with immunostaining for another lysosome marker (LAMP1) in R239C astrocytes (data not shown).

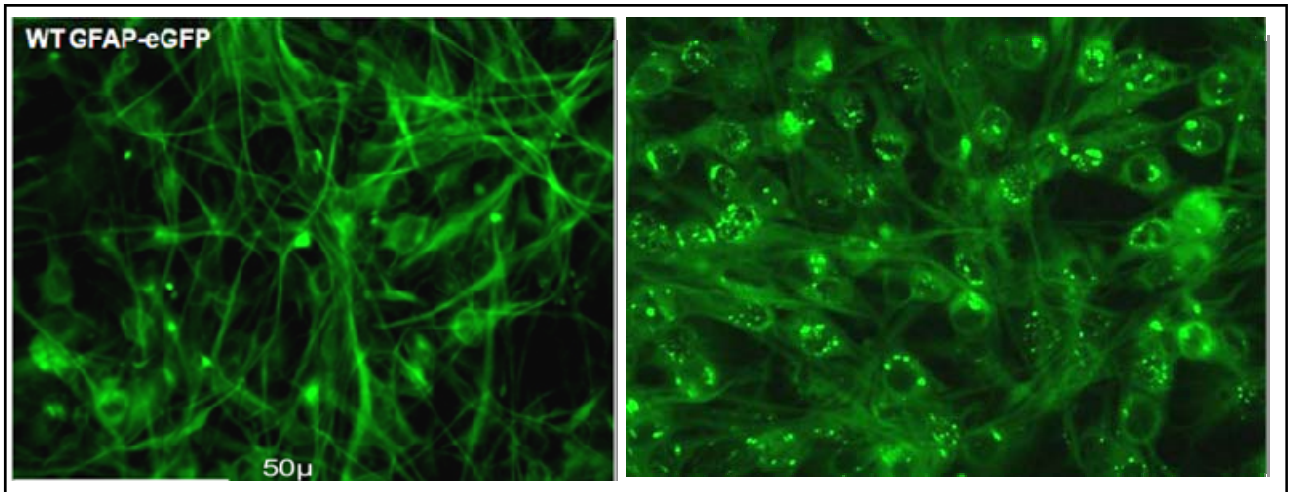


**Fig. 1. Expression of R239C GFAP resulted in a complete disruption of the endogenous GFAP network in a human astrocyte model of AxD.** A human astrocyte cell line stably overexpressing WT GFP-tagged GFAP (left panel) showed filamentous bundles of green GFAP (left panel). In contrast, R239C mutation exhibits the pathological hallmark of AxD astrocytes: intracellular inclusions or RFs (right panel).

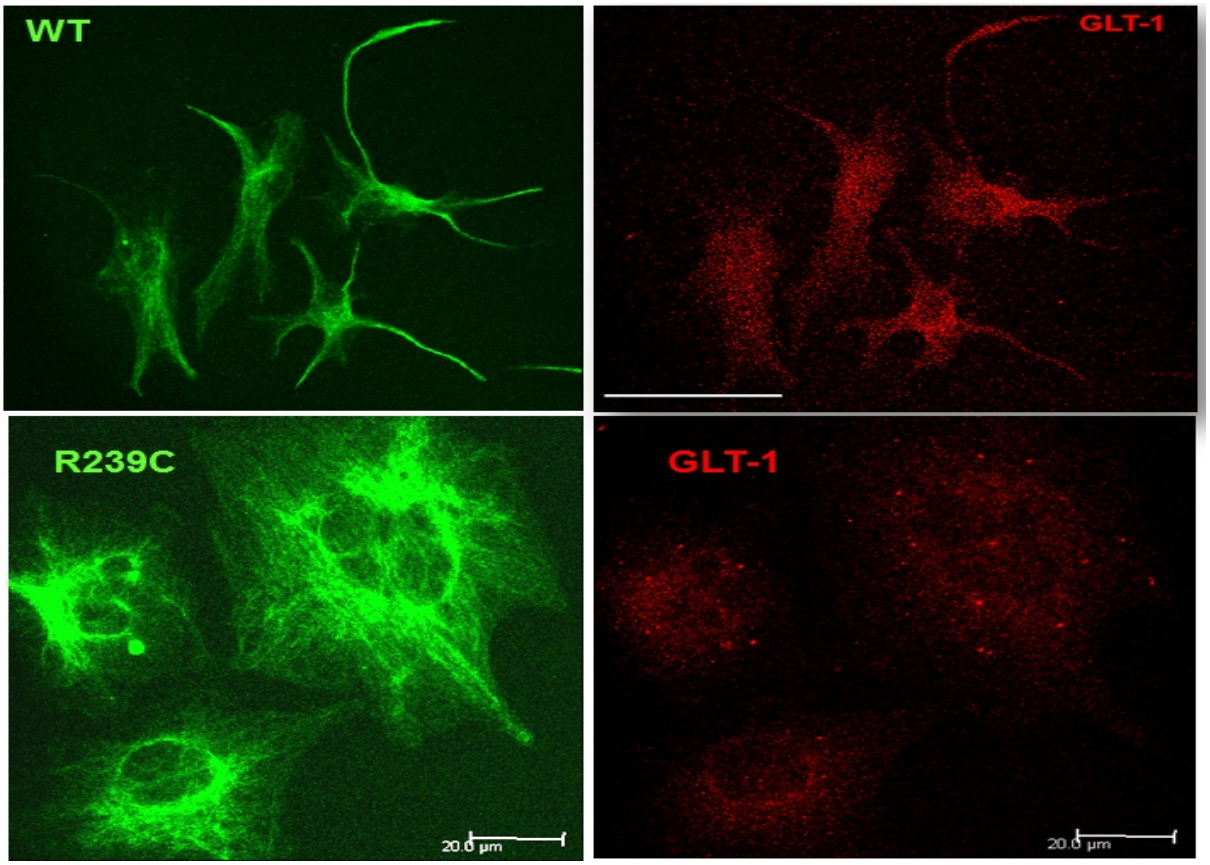


**Fig. 2. Endogenous GLT-1 proteins formed intracellular clusters in R239C astrocytes.** WT astrocytes (Green) showed a diffuse and punctate pattern of GLT-1 immunoreactivity (Red) outlining cell bodies and branches (upper panels). In contrast, GLT-1 in R239C astrocytes is primarily organized into intracellular clusters (lower panels).

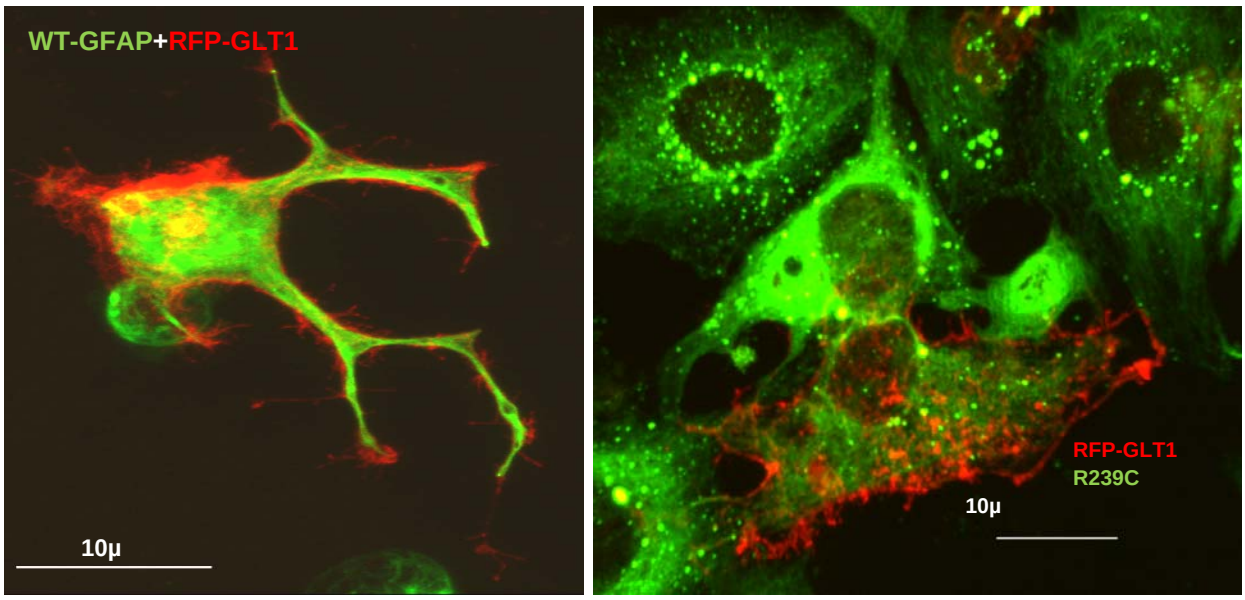


**Fig. 3. R239C GFAP caused distinct intracellular puncta of RFP-GLT-1.** RFP-GLT-1 clusters are predominantly present in the plasma membrane, circumscribing cell bodies and processes of WT astrocytes (left panel), whereas they formed abnormally large intracellular puncta with a scarce distribution along the plasma membrane in the R239C astrocytes (right panel).

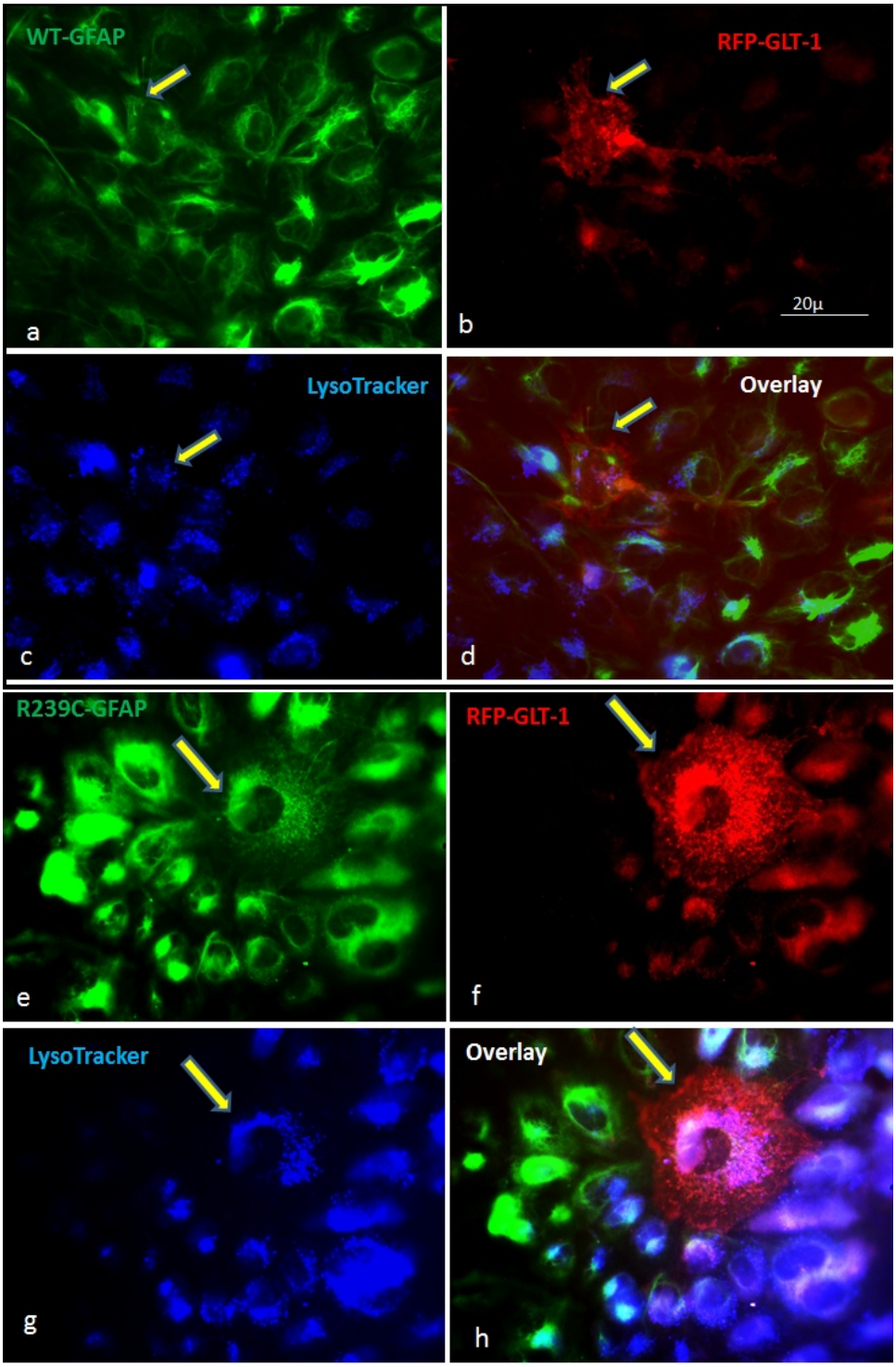


**Fig. 4. RFP-GLT-1 are partially colocalized with lysosomes in R239C astrocytes.** WT (a) and R239C (e) astrocytes transiently transfected with RFP-GLT-1(red, b and f) were immunostained for LysoTracker(blue, c and g).Images are confocal optical cross sections taken near the middle of the cell. RFP-tagged GLT-1 is expressed at or near the plasma membrane and does not appreciably overlap with staining for the lysosome (d). In contrast, RFP-GLT-1 partially overlapped with lysoTracker in R239C astrocytes (h), indicating that this protein is retained in the lysosome. Arrows indicate RFP-GLT-1 positive cells.

## Discussion

A body of evidence supports the idea that achieving high amount of GFAP -a protein threshold is critical to mimic the pathology of AxD[13, 14, 15]. To facilitate mechanistic studies of GLT-1 dysfunction in AxD, we used a retroviral vector to generate a human astro-cyte cell line stably overexpressing GFP tagged wild type (WT) or the R239C mutant GFAP. Note the distinct advantage of using retrovirus to generate stable cell lines over other vector systems (e.g. clone screening) is that retroviral constructs are stably integrated into the astrocyte genome. In combination with FACS selection of GFP+ cells, there is no need to screen and isolate antibiotics resistant clones. Although it is possible that insertion of a GFP tag renders the GFAP molecule unable to polymerize properly, expression of the GFP-GFAP constructs in Cos7 cells showed staining patterns indistinguishable from those obtained with untagged versions of GFAP (data not shown). Cytoplasmic inclusions resembling RFs, the histopathological signature of AxD; decreased proteasome activity and increased stress-related kinase activity and autophagy were observed in our R239C GFAP astrocytes, indicating that our astrocyte model has successfully recapitulated some key pathological features of AxD.

Decreased expression and function of the astrocyte glutamate transporters GLT-1 has been observed in the hippocampi of AxD patients, AxD WT/R236H mice, and AxD astrocytes in slice culture. GLT-1 is abundantly expressed in the perisynaptic membranes of astrocytes *in vivo*. GLT-1 activity is related to the relative amount of GLT-1 protein translocating to the cell surface[16]. Inappropriate targeting of GLT-1 from cell membrane to intracellular compartments would be consistent with reduced GLT-1 function, defective glutamate clearance, high vulnerability to excitotoxic insults and seizures seen in AxD patients and R236H knock-in mouse model of AxD[14]. Therefore, we are interested in determining whether loss of GLT-1 function in AxD results from trafficking defects of GLT-1. We carried out transient transfections of RFP-tagged GLT-1 in our cell model followed by colocalization analysis of multicolor confocal microscopy to determine the subcellular localization of GLT-1. GLT-1 in R239C mutant astrocytes was found to partially colocalize with lysosomal marker proteins, in striking contrast to the cell surface distribution seen in WT astrocytes. We reasoned that GLT-1 undergoes rapid dynamic trafficking between the cell surface and intracellular storage compartments[17, 18] and levels of endocytosed GLT-1 could be rapidly down-regulated in AxD via lysosomal degradation[19, 20].

Future studies with pharmacological inhibitors, along with genetic knockdown of clathrin, small GTPase and dynamin will provide further confirmation for the aberrant clathrin-mediated endocytosis of GLT-1. Nonetheless, our study provides the first direct evidence linking GFAP mutations to altered Glutamate homeostasis in AxD. The in vitro cell model we created can also be used for rapid screening of disease pathogenesis and drug candidates. A b-lactam antibiotic, ceftriaxone, has recently been identified as a potential treatment for Amyotrophic Lateral Sclerosis (ALS) by enhancing the surface expression of GLT-1 in astrocytes both in vitro and *in vivo*[21]. To Further elucidate the molecular events underlying the perturbed transcriptional and post-transcriptional regulation network of GLT -1, the development of selective pharmacological reagents along with genetic approaches interfering with the expression of the individual glutamate transporters in AxD are crucial.

## Acknowledgments

This research was a continuation of NIH Grant PO1-NS042803 and supported by PSC-CUNY 41 Research Award. The author is most grateful to Drs. James Goldman, Guomei Tang and Michael Robinson for their gifts of constructs and antibodies, to Dr. Martin Fein for discussions and comments on the manuscript.

## References

[1]Messing, A., C.M. Daniels and T.L. Hagemann, 2010. Strategies for treatment in Alexander disease. Neurotherapeutics **7(4):** 507-515.

[2]Brenner, M., A.B. Johnson, O. Boespflug-Tanguy, D. Rodriguez, J.E. Goldman and A. Messing, 2001. Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. Nat Genet **27(1):** 117-120.

[3]Tian, R., M. Gregor, G. Wiche and J.E. Goldman, 2006. Plectin regulates the organi-zation of glial fibrillary acidic protein in Alexander disease. Am J Pathol **168(3):** 888-897.

[4]Maragakis, N.J., M. Dykes-Hoberg and J.D. Rothstein, 2004. Altered expression of the glutamate transporter EAAT2b in neurological disease. Ann Neurol **55(4):** 469-477.

[5]Deng, W., Q. Yue, P.A. Rosenberg, J.J. Volpe and F.E. Jensen, 2006. Oligodendrocyte excitotoxicity determined by local glutamate accumulation and mitochondrial function. J Neurochem **98(1):** 213-222.

[6]Stys, P.K., 2005. General mechanisms of axonal damage and its prevention. J NeurolSci **233(1-2):** 3-13.

[7]Tian, R., X. Wu, T.L. Hagemann, A.A. Sosunov, A. Messing, G.M. McKhann and J.E. Goldman, 2010. Alexander disease mutant glialfibrillary acidic protein compromises glutamate transport in astrocytes. J Neuropathol Exp Neurol **69(4):** 335-345.

[8]Cho, W. and A. Messing, 2009. Properties of astrocytes cultured from GFAP over-expressing and GFAP mutant mice. Exp Cell Res. **315(7):** 1260-1272.

[9]Tang, G., Z. Yue, Z. Talloczy and J.E. Goldman, 2008a. Adaptive autophagy in Alexander disease-affected astrocytes. Autophagy **4(5):** 701-703.

[10]Tang, G., Z. Yue, Z. Talloczy, T. Hagemann, W. Cho, A. Messing, D.L. Sulze and J.E., Goldman, 2008b. Autophagy induced by Alexander disease-mutant GFAP accumulation is regulated by p38/MAPK and mTOR signaling pathways. Hum Mol Genet **17(11):** 1540-1555.

[11]Chaudhry, F., K. Lehre, M. van Lookeren Campagne, O.P. Ottersen, N.C. Danbolt and J.Storm-Mathisen, 1995. Glutamate transporters in glial plasma membranes: highly differentiated localizations revealed by quantitative ultrastructural immunocytochemistry. Neuron **15:** 711–720.

[12]Benediktsson, A.M., G.S. Marrs, J.C. Tu, P.F Worley, J.D. Rothstein, D.E. Bergles and M.E. Dailey, 2012. Neuronal activity regulates glutamate transporter dynamics in developing astrocytes.Glia **60(2):** 175-188.

[13]Tang, G., M.D. Perng, R. Quinlan and J.E. Goldman, 2010. Oligomers of mutant glialfibrillary acidic protein (GFAP) Inhibit the proteasome system in Alexander disease astrocytes, and the small heat shock protein alphaB-crystallin reverses the inhibition. J BiolChem **285(14):** 10527-10537.

[14]Hagemann, T.L., J.X. Connor and A. Messing, 2006. Alexander disease-associated glialfibrillary acidic protein mutations in mice induce Rosenthal fiber formation and a white matter stress response. J Neurosci **26(43):** 11162-11173.

[15]Tang, G., Z. Xu and J.E. Goldman, 2006. Synergistic effects of the SAPK/JNK and the proteasome pathway on glialfibrillary acidic protein (GFAP) accumulation in Alexander disease. J BiolChem **281(50):** 38634-38643.

[16]Robinson, M.B., 2006. Acute regulation of sodium-dependent glutamate trans-porters: A focus on constitutive and regulated trafficking. Handb Exp Pharmacol **175:** 251-275.

[17]Beart, P.M. and R.D. O'Shea, 2007. Transporters for L-glutamate: an update on their molecular pharmacology and pathological involvement. Br J Pharmacol **150(1):** 5-17.

[18]Dabir, D.V., M.B. Robinson, E. Swanson, B. Zhang and M.S. Forman, 2006. Impaired glutamate transport in a mouse model of tau pathology in astrocytes. J Neurosci **26(2):** 644-654.

[19]Nakagawa, T., Y. Otsubo, Y. Yatani, H. Shirakawa and S. Kaneko, 2008. Mechanisms of substrate transport-induced clustering of a glial glutamate transporter GLT-1 in astroglial-neuronal cultures. Eur J Neurosci **28(9):** 1719-1730.

[20]Susarla, B.T. and M.B. Robinson, 2008. Internalization and degradation of the glutamate transporter GLT-1 in response to phorbol ester. NeurochemInt **52(4-5):** 709-722.

[21]Rothstein, J.D., S. Patel, M.R. Regan, C. Haenggeli, Y.H. Huang, D.E. Bergles, L. Jin, M.D. Hoberg, S. Vidensky, D.S. Chung, S.V. Toan, L.I. Bruijn, Z. Su, P. Gupta and P.B. Fisher, 2005. Betalactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature **433:** 73-77.

**The Metropolitan Association of College and University Biologists thanks the following Affiliate Members for their support:**

**AD Instruments**
**Anatomy in Clay Systems**
**BioPac Systems**
**Cengage Learning**
**Heyden McNeil Publishing**
**I. Miller Microscopes**
**John Wiley & Sons**
**McGraw Hill Publishing**
**Micro-Optics Precision Instruments**
**Pasco Scientific**
**Pearson Education**
**W. H. Freeman and Company**

**Please make every effort to support these affiliate members. Their participation help us to keep the registration fees at a reasonable price.**


**Dr. Edward J. Catapane**
**Department of Biology**
**Medgar Evers College**
**1150 Carroll Street**
**Brooklyn, New York 11225**