

MICRONUCLEI PROFILE: AN INDEX OF CHROMOSOMAL ABERRATIONS IN FRESHWATER FISHES (*SYNODONTIS CLARIAS* AND *TILAPIA NILOTICA*)

J.C. OKONKWO^{1*}, M.O. OBIAKOR² and P.C. NNABUDE³

¹Department of Agricultural and Bio-resources Engineering, Nnamdi Azikiwe University, PMB 5025 Awka, Nigeria

²Department of Environmental Management, Nnamdi Azikiwe University, PMB 5025 Awka, Anambra State, Nigeria

³Department of Botany, Nnamdi Azikiwe University, PMB 5025 Awka, Anambra State, Nigeria

*Email: drjckonkwo@yahoo.com

ABSTRACT: Incidence of chromosomal aberrations in *Synodontis clarias* and *Tilapia nilotica* (Linnaeus 1757) were measured using the conventional micronucleus assay in fish erythrocytes. The species showed varying degree of micronuclei frequencies in their respective genomes of sampled gill and kidney blood. Cytological examinations showed bi-nucleated cells, deformed nuclei including the main aberrations, micronucleus formations in various genomes of the fish from different locations considered in this study. Comparison of the micronucleus rates in peripheral and kidney blood of the two species revealed no statistical difference ($P > 0.05$). On species occurrence of the measured chromosomal aberrations, averages of micronucleus frequencies recorded in *Synodontis clarias* showed visible variation and to be 2.2 folds higher than the values obtained in the corresponding *Tilapia* sp. but there was no statistical difference ($P > 0.01$) among the two breeds. The work recommends that micronuclei tests in fish erythrocytes be carried out at various times, thus making it possible to follow-up the changing micronuclei frequencies and concludes that gills and kidney erythrocytes can be used in studies concerning chromosomal aberrations since the sampling of the peripheral blood is appropriate as it allows collecting several samples from the same individuals, without having to sacrifice it.

Keywords: Micronucleus assay, chromosomal aberrations, *Synodontis clarias*, *Tilapia nilotica*, Anambra River

INTRODUCTION

Chromosomal and cytogenetic studies on fish have received considerable attention in recent years (Okonkwo and Obiakor 2010; Galetti et al 2000; Ozouf-Costaz and Foresti 1992). Fish chromosome data have great importance in studies concerning evolution, systematics, aquaculture and mutagenesis (Amemiya 1986; Al-Sabti 1991).

The erythrocyte micronucleus bioassay has been used with different fish species to monitor aquatic pollutants displaying mutagenic features (De Flora et al 1993). Kligerman (1982) demonstrated that fishes inhabiting polluted waters have greater frequencies of micronuclei compared to those raised in clean pond. The micronuclei frequencies may vary according to the season, the kind of pollution involved, and the species of fish. These structures are easy to visualize in erythrocytes and are therefore often used as a measure of chromosomal aberrations (Rabello-Gay 1991; Hartwell et al 2000).

Odo et al (2009) reported that *Tilapia nilotica* and *Synodontis clarias* are the most preponderant species of fish found in the Anambra River, and constitute the main diet for over one million rural dwellers living along the river bank.

Micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. Because genetic damage that results in chromosome breaks or spindle abnormalities leads to micronucleus formation, the incidence of micronuclei serves as an index of these types of damage (Fagr et al 2008). Because counting of micronuclei

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is much faster and less technically demanding, the micronucleus assay has been widely used to screen for chemicals that cause these types of damage (Fagr et al 2008) and the damage resulting from it. The failure of two sister chromatids to separate during mitotic anaphase generates reciprocal trisomic and monosomic daughter cells. Mistakes such as a lagging chromatid not pulled to either spindle pole at mitotic anaphase, result in a chromosome loss that produces one monosomic daughter cell. These result in classic deviation from the normal chromosomal diploidy (Hartwell et al 2000).

Changes in chromosome number evidenced by micronucleus formations may affect gene activity or gene transmission by altering the position, order, or number of certain genes in a cell. Such changes often, but not always, lead to a genetic imbalance that is harmful to the organism or its progeny (Hartwell et al 2000). Going by the same author, if the chromosomal inversion (half-circle rotation of a chromosomal region) is paracentric and a crossover occurs within the inversion loop, the recombinant chromatids will be unbalanced not only in gene dosage, but also in centromere number. One crossover products will be the main chromosomal aberration, an acentric fragment lacking a centromere; while the reciprocal crossover product will be a dicentric chromatid with two centromeres. Because the acentric fragment without a centromere cannot attach to the spindle apparatus during the first meiotic division in reproduction, the cell cannot package it into either of the daughter nuclei; as a result, this chromosome is lost and will not be included in a gamete.

In fish, the kidney is responsible for erythropoiesis as well as filtration. Upon fish exposure to toxins, defective erythrocytes undergo passage from the kidney into the peripheral blood, from where they are removed by the hemocathesis organs (Palhares and Grisolia 2002). One of the hypotheses of this study was that the examination of kidney erythrocytes would provide more sensitive detection of micronuclei frequencies than peripheral blood erythrocytes under natural conditions. Chromosomal aberration studies with preponderant native fish species represent an important effort in delineating the extent of a particular chromosome damage and change such as micronuclei formations and likely agents inducing the visible aberration in the fish genome. The study was carried out to evaluate chromosomal aberration of micronucleus formation in genotypes of the two preponderant fish species in Anambra River under natural conditions using micronucleus test.

MATERIAL AND METHODS

Study Area

Anambra State in Nigeria lies between latitude 5° 40'N and 6° 45'N and longitude 6° 35'E and 7° 21'E. The climate is tropical with average annual rainfall of 200mm and mean temperature of 27°C (Anyanwu 2006). The study area, which is Anambra River spatially lies between latitude 6° 00'N and 6° 30'N and longitude 6° 45'E and 7° 15'E. The river on the other hand is located in the South Central region of Nigeria, just close to the East of the Niger River into which it empties (Awachie and Hare 1977). Anambra River is approximately 207.4 km to 210 km in area (Odo 2004; Shahin 2002), rising from the Ankpa hills (ca. 305-610m above sea level) and discharging into River Niger at Onitsha (Odo 2004). The entire river basin drains an area of approximately 14010km² (Awachie and Hare 1977).

Sampling Stations

The sampling stations were established to cover possibly the whole area along the river course based on an earlier field reconnaissance tour. The locations (L_x) of the various sampling stations are;

L_A = Enugu Otu (Station A): Rice production and farming site.

L_B = Ezi Aguleri (Station B): Farming, Fishing and effluent discharge.

L_C = Otuocha (Station C): Rice production, marketing activities and waste disposal.

L_D = Otu Nsugbe (Station D): Wastewater effluent discharge, farming and marketing activities.

L_E = Onono (Station E): Sand mining and excavation, agricultural activities, fishing and sewage disposal. The location is close to Onitsha metropolis and mouth of Oyi River, the repository of industrial/domestic effluents.

Sample Collection

Live *Synodontis clarias* and *Tilapia nilotica* (Linnaeus 1757) of fairly similar live weight were collected from Anambra River at the five locations/stations using set nets, long-lines and traps. The relative distance between each station was approximately 12 km and all the sample collections were made during the morning hours at the peak of rainy season in the month of July, 2009.

Micronucleus Test

Blood samples were collected from caught fish. The peripheral blood smears were obtained through the gills and kidney blood by means of a medial-kidney imprint following dissection as described by Fagr et al (2008) and Palhares and Grisolia (2002). The slides were then, air-dried for 24h, fixed in methanol for 10min, followed by 10% Giemsa (v/v) staining. 2000 erythrocytes of each fish were examined, from both peripheral blood and the kidney. To determine micronuclei in erythrocytes, the slides were examined using oil-immersion (x 1000). For the scoring of micronuclei, the following criteria were adopted from Fenech et al (2003); the diameter of the micronucleus (MN) should be less than one-

third of the main nucleus; MN should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary; and MN should have similar staining as the main nucleus.

Data Analysis

Statistical analysis was performed using Student's *t* test.

RESULTS

Results reveal that the two fish species represent various degrees of sensitivity in monitoring genetic damage (especially clastogenic effect). This is indicated by variations in averages of the micronucleated cells among species at various locations/ stations. The obtained results are summarized in Table 1 and Figure 1 - 3. The chromosomal aberrations represented by the formation of micronucleus showed marked increase in the following level of occurrences; A<B<D<C<E. Location/station E was observed to possess fish with higher level of micronucleus frequencies. Generally, the spontaneous micronuclei frequencies observed in kidney and in peripheral blood erythrocytes were statistically not different ($P>0.05$, Table 1), indicating no difference between kidney and gill in both *Synodontis clarias* and *Tilapia nilotica* ($P=1.988$, Table 1). Micronuclei formation in *Synodontis clarias* was visibly found to be higher than the levels detected in *Tilapia nilotica* as shown in Table 1, the averages of micronucleated erythrocytes formed in kidneys and gills of different genomes of fish at different locations. The results revealed that the micronucleus percentages were proven to be 2.2 folds higher in *Synodontis clarias* than in *Tilapia nilotica*, although the observed differences were not statistically significant ($P=1.861$) at 1% level of significance. Figure 1 shows micronucleated cells in different fish species caught from the five locations (stations) studied. Cytological evaluation (Baker et al 1998) revealed binucleated and deformed nucleus including the main type of chromosomal aberrations (micronucleus) observed. These are shown in Figure 2 and 3.

Table 1 - Mean (\pm SE) of micronucleus frequencies (MN/1000 erythrocytes) examined in gill and kidney blood of fish sourced from different locations in Anambra River.

Species (Erythrocytes)				
Station	<i>T. nilotica</i> (Gill)	<i>T. nilotica</i> (Kidney)	<i>S. clarias</i> (Gill)	<i>S. clarias</i> (Kidney)
A	0.4 \pm 0.3 ^{ab}	0.2 \pm 0.3 ^{ab}	1.4 \pm 0.8 ^{ab}	0.3 \pm 0.4 ^{ab}
B	0.3 \pm 0.4 ^{ab}	0.2 \pm 0.3 ^{ab}	2.7 \pm 1.1 ^{ab}	1.8 \pm 1.8 ^{ab}
C	3.0 \pm 2.2 ^{ab}	1.6 \pm 0.8 ^{ab}	5.3 \pm 1.9 ^{ab}	2.1 \pm 0.4 ^{ab}
D	2.3 \pm 1.5 ^{ab}	2.1 \pm 1.0 ^{ab}	4.9 \pm 2.9 ^{ab}	1.6 \pm 0.8 ^{ab}
E	4.8 \pm 2.1 ^{ab}	2.1 \pm 1.2 ^{ab}	6.4 \pm 3.1 ^{ab}	4.6 \pm 1.7 ^{ab}

^a significantly not higher ($P= 1.861$) at 1% level of significance, comparing the two specie.
^b significantly not higher ($P= 1.988$) at 5% level of significance, comparing gill and kidney.

DISCUSSION

Micronucleus bioassay offers several types of unique information as a bioindicator for chromosomal aberrations not available from other methods: (1) the integrated effect of a variety of environmental stresses on the health of an organism and the population, community, and ecosystem; (2) early warning of potential harm to human health based on the responses of wildlife to pollution; and (3) the effectiveness of remediation efforts in decontaminating waterways (Villela et al 2006). In fish, the micronucleus test is usually based on erythrocytes, but liver and gill tissues have been used (Al-Sabti and Metcalfe 1995).

Palhares and Grisolia (2002) compared between the micronucleus frequencies of kidney and gill erythrocytes in tilapia fish, following mitomycin C treatment detecting no significant difference between the frequencies of the micronuclei. Similarly, Manna and Sadhukan (1986) maintained that there was no statistically significant difference between the frequency of micronuclei in gill and kidney cells after irradiation in the two tissues. While they included various types of cells, our study was focused on the erythrocytes. A hypothesis to explain the fact that we did not detect any difference between kidney and peripheral blood micronuclei counts may be that circulating peripheral erythrocytes also undergo mitosis (Palhares and Grisolia 2002). However, if the kidney is the main hemopoietic tissue in fish, and if micronuclei are formed during cell proliferation (Palhares and Grisolia 2002; Hartwell et al 2000), more micronucleated erythrocytes should be expected in the kidney than in the gill. Alternatively, as reported by Palhares and Grisolia (2002), we may have sampled peripheral blood during kidney imprinting and practically, the cephalic kidney is a frequently chosen organ for cytogenetic aberration studies in fish. There was no significant difference ($P> 0.05$) between the frequencies of micronuclei obtained in *Synodontis clarias* and *Tilapia nilotica*. Fagr et al (2008) observed the African walking catfish, *Clarias gariepinus* from freshwaters of Egypt to have higher incidence of the chromosomal aberrations of micronuclei in its genome than the three tilapia species employed in the study, maintaining the species to be highly

tolerant of that particular genetic damage without triggering the genetically programmed event that allows cells to commit suicide (Fagr et al 2008). Hence, the statistical results of no difference between the species of catfish and tilapia in our own study might be as a result of seasonal effect, physiological variations and responses to the local agents inducing the chromosomal damage, which as advocated by Kligerman (1982), the micronuclei frequencies may vary according to the season, the kind of pollution involved, and the species of fish. Sampling was carried out at peak of the tropical rainfall in July.

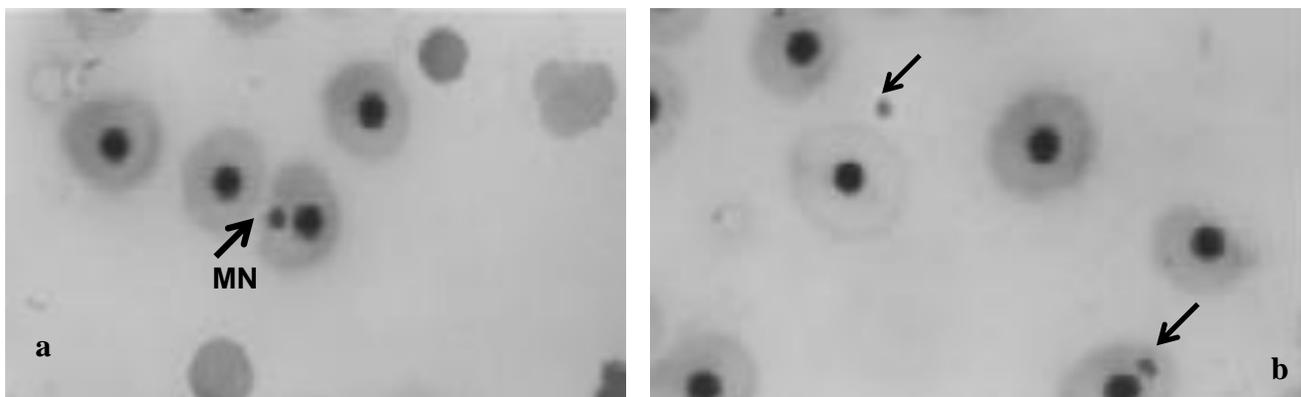


Fig. 1- Photomicrographs showing micronucleated erythrocyte (MN) from *Synodontis clarias* and *Tilapia nilotica* caught from location or station C (a) and E (b), respectively.

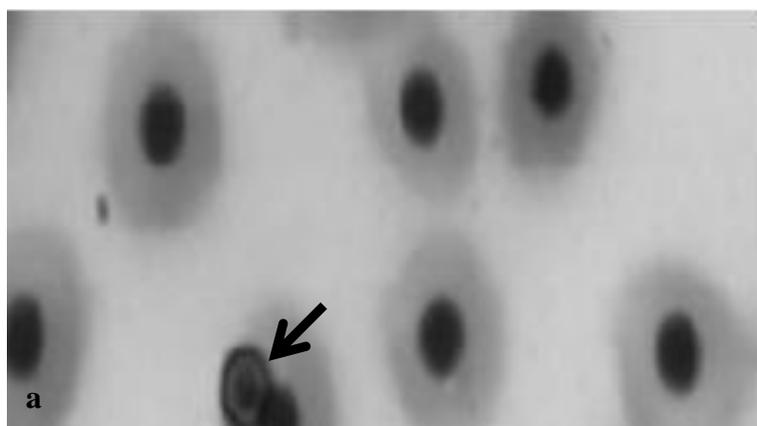


Fig. 2 - Photomicrograph showing binucleated erythrocyte (a) in *Tilapia nilotica* from location or station E.

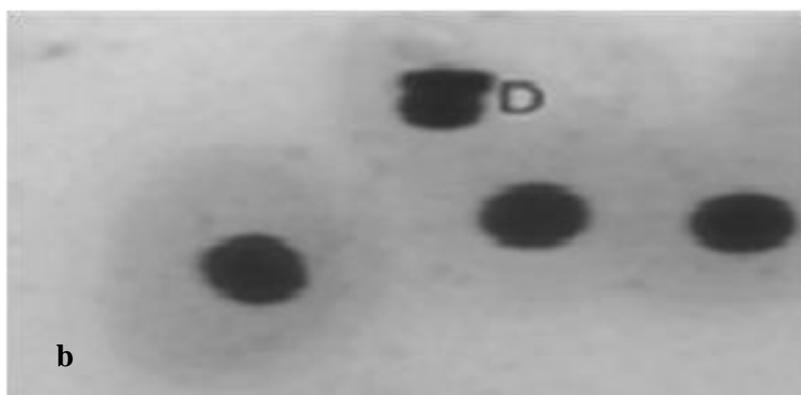


Fig. 3 - Photomicrograph showing Deformed Nucleus (D, b) in kidney blood of *Tilapia nilotica* sourced from location or station C.

At anaphase of meiosis 1, opposing spindle forces pull the dicentric chromatid toward both spindle poles at the same time with such strength that the dicentric chromatid breaks at random positions along the chromosome. These broken chromosome fragments (micronuclei) are deleted for many of their genes. This loss of the acentric fragments, together with breakage of the dicentric chromatid results in genetically unbalanced gametes, which at fertilization are lethal to the zygote's development. Consequently, no recombinant progeny resulting from such aberration survive except non recombinant progeny (Hartwell et al 2000). This observation lends substance to the abstract exposition of the foregoing drastic decline in fish diversity reported at Nsugbe, Ogurugu and Otuocha axes of the Anambra River by Odo et al (2009), which had earlier been mentioned by Ndakide (1988) in the entire river, attributing it to recent anthropogenic influences.

Karyotypes generally remain constant within a species, not because changes in chromosome number occur infrequently (they are, in fact, quite common), but because the genetic instabilities and imbalances produced by such chromosome changes usually place individual cells or organisms and their progeny at a selective disadvantage (Hartwell et al 2000). *Synodontis clarias* from the current work shows relatively high chromosomal anomalies than *Tilapia nilotica* and might hypothetically possess stable karyotype, contrasting observations made in a similar species, *Clarias gariepinus* (Burchell 1822) by Okonkwo and Obiakor (2010). The authors documented karyotypic polymorphism in *Clarias gariepinus* (Burchell 1822) from the Anambra River. The observed polymorphism in that species might be that the species genome well tolerates such type of cytogenetic damage (micronucleus) without apoptosis and as such fewer aberrations will be expected on the chromosomes of *Clarias gariepinus* than the former. It is suggested that karyological analysis of *Synodontis clarias* be carried out in Anambra River to establish its karyotypic forms and stability, including the chromosomal aberration status of *Clarias gariepinus* to portray the relationships with their karyotypes.

CONCLUSION

The results demonstrated that different fish species can respond in completely different ways to a given genotoxic agent. Depending on the toxic agent and on the species, the behavior of micronuclei rates may exhibit significant variations, probably related to the chemical kinetics of the toxins and to the speed of the hemopoietic cycle (Kligerman 1982). It is recommended that micronuclei tests in fish erythrocytes be carried out at various times, thus making it possible to follow-up the changing micronuclei frequencies. It is concluded from the study that gills and kidney erythrocytes can be used in studies concerning chromosomal aberrations. The sampling of the peripheral blood is appropriate since it allows collecting several samples from the same individuals, without having to sacrifice it (Lyne et al 1992).

REFERENCES

- Al-Sabti, K (1991). Handbook of Genotoxic Effects and Fish Chromosomes, J Stephan Institute, P.O Box 100, Jamova 39, Ljubjiana Yugoslavia
- Al-Sabti K and Metcalfe C (1995). Fish Micronuclei for Assessing Genotoxicity in Water, Mutation Resources 343: 121-135
- Amemiya C T (1986). Cytogenetic and Cytosystematic Studies on the Nucleolus Organizer Regions of North American Cyprinid Fishes, Texas A&M University, Ph.D. Thesis
- Anyanwu JC (2006). Study of Entrophication of Nkisi and Anambra Rivers and Anthropogenic Activities in Anambra State, M.SC Thesis, Nnamdi Azikiwe University, Awka, Anambra, Nigeria
- Awachie JB E and Hare L (1977). The Fisheries of the Anambra, Ogun and Oshun River Systems in Southern Nigeria, CIFA Technical paper 5: 170-184.
- De Flora S, Vigario L, D'Agostini F, Camoirano A, Bagnasco M, Bennecelli C, Melodia F and Arillo A (1993). Multiple Biomarkers in Fish Exposed *In situ* to Polluted River Water. Mutat. Resour., 319: 167-177
- Fagr A, El-shehawi AM and Seehy MA (2008). Micronucleus Test in Fish Genome: A sensitive Monitor for Aquatic Pollution, African J. Biotechnol., 7: 606-612.
- Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S and Zeiger E (2003). Human Micronucleus Project, HUMN Project: Detailed Description of the Scoring Criteria for the Cytokinesis-block Micronucleus Assay Using Isolated Human Lymphocyte Cultures. Mutat. Resour., 534: 65-75.
- Galetti PM, Aguliar CT and Molina WF (2000). An Overview of Marine Fish Cytogenetics. Hydrobiologia, 420: 55-62.
- Hartwell LH, Hood L, Goldberg ML, Reynolds AE, Silver LM and Veres RC (2000). Genetics: from Genes to Genomes. McGraw Hill Higher Education, ISBN 0-07-540923-2.
- Kligerman D (1982). Fishes as Biological Detectors of the Effects of Genotoxic Agents. In: Mutagenicity: New Horizons in Genetic Toxicology, Heddle J (Ed) Academic Press, New York, 435-456.

- Lyne TB, Bickham JW, Lamb T and Gibbons JW (1992). The Application of Bioassays in Risk Assessment of Environmental Pollution. *Risk Anal.*, 12: 361-365.
- Manna G and Sadhukhan A (1986). Use of Cells of Gill and Kidney of Tilapia Fish in Micronucleus Test. *Current Sci.*, 55: 498-501.
- Ndakide (1988). Personal Communication In: Odo, G.E; Didigwu, N.C. and Eyo, J.E. (2009). The Fish Fauna of Anambra River Basin, Nigeria: Species Abundance and Morphometry. *Rev. Biol. Trop.*, 57: 177-186.
- Odo GE (2004). Studies on the Ecology of Macro Invertebrate Fauna as Fish Food in Anambra River Basin, Nigeria, Ph.D Thesis, University of Nigeria, Nsukka, Nigeria
- Odo GE, Didigwu NC and Eyo JE (2009). The Fish Fauna of Anambra River Basin, Nigeria: Species Abundance and Morphometry. *Rev. Biol. Trop.*, 57: 177-186.
- Okonkwo JC and Obiakor MO (2010). Karyological and Chromosomal Study of Catfish (Clariidae, *Clarias gariepinus*, Buchell 1822) from Anambra River, Anambra State, Nigeria. *Pakistan J. Nurt.*, 9: 112-115.
- Ozouf-Costaz C and Foresti F (1992). Fish Cytogenetic Research: Advances, Applications and Perspectives. *Netherl. J. Zool.*, 42: 277-290.
- Palhares D and Grisolia CK (2002). Comparison between the Micronucleus Frequencies of Kidney and Gill Erythrocytes in Tilapia fish, Following Mitomycin C Treatment. *Genet. Molec. Biol.*, 25: 281-284.
- Rabello-Gay MN (1991). Teste do micronúcleo em medula óssea. In: *Mutagênese, Teratogênese e Carcinogênese: Métodos e Critérios de Avaliação*, Sociedade Brasileira de Genética (Ed), 83-90.
- Shahin M (2002). *Hydrology and Water Resources of Africa*, Springer, ISBN-1-402-00866-x, 307-309
- Villela IV, De Oliveira IM, Da Silva J and Henriques JA (2006). DNA Damage and Repair in Haemolymph Cells of Golden Mussel Exposed to Environmental Contaminants. *Mutat. Resour.*, 605:78-86.