Acta Universitatis Lodziensis
——— 2010 ———
Folia Biologica et Oecologica 6: 5–32
(Acta Univ. Lodz, Folia Biol. Oecol.)

JOLANTA DOMINIKA ŻOŁNIERCZYK & ZOFIA MARIA KILIAŃSKA

Department of Cytobiochemistry, University of Łódź, Pomorska 141/143, 90-236 Łódź, Poland, jolantazolnierczyk@gmail.com

MAIN PRO-APOPTOTIC MEMBER OF BCL-2 FAMILY PROTEINS – BAX

Abstract: Programmed cell death (apoptosis) plays a vital role in the regulation of cellular homeostasis. Because of apoptosis fundamental importance, this process is highly regulated. One important set of factors involved in apoptosis regulation is the Bcl-2 family proteins. Bcl-2 family members form a complex regulatory network that controls cell survival and death in response to different physiological and pathological signals. This family includes both pro- and anti-apoptotic members, and Bax protein (Mol wt 21 kDa) is a major pro-apoptotic factor with multifunctional activity. This review summarizes new data about the main representative of Bcl-2 family – Bax, its structure and mechanism(s) by which this protein modulates apoptosis.

Key words: apoptosis, Bcl-2 family, Bax, apoptosis mitochondrial pathway

1. INTRODUCTION

Apoptosis is a precisely controlled mode of eliminating injured, mutated or unwanted cells from the body or an organ of multicellular organism that, in contrast to necrosis, does not trigger the inflammation (HENGARTNER 2000; WYLLIE 2010). It is widely accepted that apoptosis may go through two major pathways (extrinsic and intrinsic), one of which involves mitochondria with proteins localized inside the intermembrane compartment of these organelles (KILIAŃSKA 2002). The mitochondrial (intrinsic) pathway of apoptosis is activated in response to several proapoptotic stimuli such as DNA damages, alterations in cytoskeleton stability

DOI: 10.2478/v10107-009-0004-3

(RODOLFO, PIACENTINI 2002; NDOZANGUE-TOURIGUINE *et al.* 2008), reactive oxygen species (ROS), endoplasmic reticulum (ER) stress (MEIR *et al.* 2010), hypoxia (GREIJER, VAN DER WALL 2004), cytokine deprivation (SAVARAJ *et al.* 2010; WYLLIE 2010), oncogene overdrive (WYLLIE 2010) as well as a large number of anticancer drugs (ŻOŁNIERCZYK *et al.* 2009; ROGALIŃSKA *et al.* 2010). All these death signals lead to permeabilization of outer mitochondrial membrane (OMM) and to the leakage of proapoptotic factors (procaspases, including procaspase-9, cytochrome c, Smac/DIABLO (Second mitochondria-derived activator of caspases/Direct IAP-binding protein with low pI), apoptosis inducing factor – AIF, endonuclease G, Omi/HtrA2 (High temperature requirement protein A2) protease as well as some heat shock proteins – Hsp10 or Hsp 60) into the cytosol where they initiate caspase activation and DNA fragmentation (KRAJEWSKI *et al.* 1999; SHAN *et al.* 2003; LOWE *et al.* 2004; BEDNAREK, KILIAŃSKA 2005). It is thought that protein efflux out of mitochondria is irreversible step of apoptosis. Therefore, it must be accurately controlled (CHIPUK *et al.* 2006; CHIPUK, GREEN 2008). A key role in this regulation is attributed to Bcl-2 (B-cell leukemia/lymphoma 2) family proteins many of which have become the objects of targeted therapy in oncology (BORNER 2003; WARR, SHORE 2008; WYLLIE 2010). This review focuses on the current data concerning the structure and biological activity of the main pro-apoptotic member of Bcl-2 family proteins, Bax, and especially the mechanisms by which it evokes OMM permeabilization and apoptosis induction.

2. BCL-2 FAMILY PROTEINS

Up to now, over 30 proteins have been identified in higher eukaryotic cells that belong to Bcl-2 family (SKOMMER *et al.* 2007). They are all generally characterized by the presence of at least one highly conserved motif referred as Bcl-2 homology domain (BH) in their molecules. These domains, in number 1-4, take part in the mutual interactions between Bcl-2 family proteins as well as in the communication with other factors endowed with similar BH domain i.e. HAX-1 (Hematopoietic-specific protein 1-associated protein X-1) (YEDAVALLI *et al.* 2005;

HOSSINI, EBERLE 2008). The Bcl-2 family comprises three subfamilies of proteins (Fig. 1):

- **Bcl-2-like survival factors** – multi-domain anti-apoptotic proteins i.e. Bcl-2, Bcl-X_L (Bcl-2 related gene, long isoform), Bcl-w (Bcl-2 widely expressed), Mcl-1 (Myeloid cell leukemia-1), Bcl-B (Bcl-2 family protein resembling Boo) and A1/Bfl-1 (KE *et al.* 2001; BORNER 2003; CORY *et al.* 2003; DROIN, GREEN 2004; REED 2008; SCHRÖDER 2008). These subfamily members typically possess 4 BH domains in their structure and they are usually anchored to intracellular membranes of the cell.
- **Bax-like death factors** – multi-domain pro-apoptotic proteins e.g. Bax (Bcl-2 associated x protein), Bak (Bcl-2 antagonist/killer 1), Bok/Mtd (Bcl-2-related ovarian killer/Spanish: Matador – the killer), Bcl-X_S. They have three (or 4 in case of Bok/Mtd) BH domains and play a role as effector molecules capable of directly inducing mitochondrial permeabilization and apoptosis (BORNER 2003).
- **BH3-only death factors** – single-domain pro-apoptotic proteins e.g. Bad (Bcl-2 antagonist of cell death is equivalent to Bcl-2 associated death promoter), Bid (BH3-interacting domain death agonist), Bik (Bcl-2 interacting killer), Bim (Bcl-2 interacting mediator of cell death), Bmf (Bcl-2 modifying factor), BNIP3 (Bcl-2 and adenovirus E1B 19-kDa protein interacting protein 3), Bnip3L (Bnip3-like), Hrk (Harakiri), Noxa/APR, Puma (p53-upregulated modulator of apoptosis) and Spike (Small protein with inherent killing effect). The members of this subfamily contain exclusively one domain – BH3; some of BH3-only proteins contain transmembrane domain, TM. They interact with Bax-like as well as Bcl-2-like proteins determining the fate of a cell (GUO *et al.* 2001; MOLDOVEANU *et al.* 2006; SKOMMER *et al.* 2007; HOSSINI, EBERLE 2008; REED 2008).

Recently, several other pro-apoptotic proteins have been discovered, however their classification turned out to be very difficult due to the presence of only two BH domains in their structure. For example, Bfk (Bcl-2 family kin) and Bcl-G proteins contain BH2 and BH3 while Bcl-X_S (Bcl-2 related gene, short isoform) and Bcl-X_{AK} (Bcl-2 related gene, alternative killer) are equipped with BH3

and BH4 or BH2 and BH4 domains, respectively; the latter is usually connected with anti-apoptotic potential of proteins (BORNER 2003; KARST, LI 2007; HOSSINI, EBERLE 2008). Similarly, Bcl-rambo shares the structural homology with anti-apoptotic factors of Bcl-2 family although its overexpression induces apoptosis (KATAOKA *et al.* 2001; HOSSINI, EBERLE 2008). Boo/Diva (Death inducer binding to vBcl-2 and Apaf-1) protein that comprises 4 BH domains may in turn act as pro-apoptotic or pro-survival molecule depending on the cellular context (LEE *et al.* 2001).

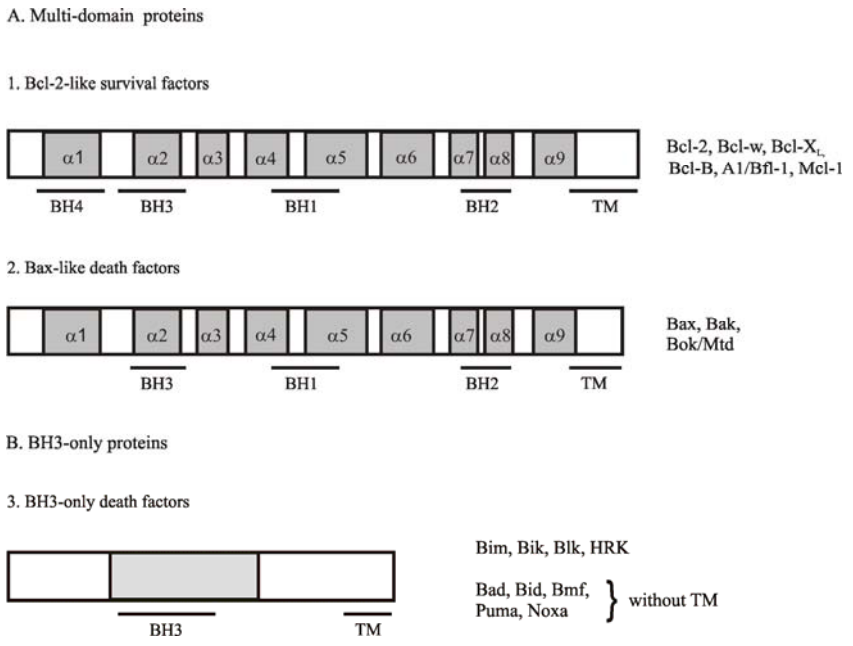

Fig. 1. Domain structure of Bcl-2 family proteins (based on: CHIPUK *et al.* 2006; CHIPUK *et al.* 2008; BORNER 2003). The Bcl-2 family of proteins (multi-domain – A; BH3-only – B) is divided into 3 functional subfamilies (1, 2, and 3) based on their composition of BH (1-4) domains. For more details see text. α1-9 – α-helices; BH – Bcl-2 homology domain; TM – transmembrane domain. The BH3 domain in the pro-apoptotic proteins is a ligand for the hydrophobic pocket formed by the BH1-BH3 domains of the anti-apoptotic members of Bcl-2 family.

The classification of Bcl-2 family proteins based on their role in apoptosis seems to be rather conventional since the phenotype of many pro- or anti-apoptotic

proteins can be changed into the opposite one under some special conditions (LIN *et al*. 2004; HANSON *et al.* 2008). For example, Bcl-X_L protein, known as anti-apoptotic factor, may interact with mitochondria and induce OMM permeabilization after the binding phosphorylated K-Ras (phosphorylation on Ser^{181}). Likewise, interaction of Bcl-2 protein with orphan receptor – Nur 77 (Nuclear receptor 77) converts the anti-apoptotic potential of Bcl-2 to pro-apoptotic (KOLLURI *et al.* 2008). It was demonstrated that Nur 77 also affected other generally anti-apoptotic proteins, such as Bcl-B, A1/Bfl-1, leading to reversion of their phenotype (LUCIANO *et al.* 2007).

2.1. Structure of Bcl-2 family proteins

Examination of the solution structure of Bcl-2 family proteins revealed some structural resemblances between pro- and anti-apoptotic molecules. These proteins adopt donut-shaped structure with two central, hydrophobic α-helices surrounded with 6-7 amphipathic α-helical regions. Additionally, they contain a long (60 aa), unstructured loop between the first and the second helical region of proteins (PETROS *et al.* 2004; SKOMMER *et al.* 2007; BORNER 2003).

BH1, BH2, and BH3 domains play an important role in the interactions of anti-apoptotic proteins with their pro-apoptotic opponents (PETROS *et al.* 2004; HOSSINI, EBERLE 2008)]. In pro-survival as well as in inactive pro-apoptotic proteins above domains form together a hydrophobic pocket which binds BH3 motif of pro-apoptotic molecule by electric and hydrophobic interactions (BORNER 2003; PETROS *et al.* 2004; HOSSINI, EBERLE 2008). Evolutionary conserved amino acid residues – Gly^{145} and Arg^{146} localized in BH1 as well as Trp^{188} in BH2 domain of anti-apoptotic proteins play a key role in the communication between the receptor pocket and BH3 domain of pro-death factor. It was indicated that the substitution of Gly145Ala/Glu and Trp188Ala prevented the heterodimerization of Bcl-2 family proteins, suggesting that these residues take part in the binding of hydrophobic clef to BH3 domain (GURUDUTTA *et al.* 2005).

The *in vitro* tests revealed that both Bax and Bcl-2 proteins were able to form channels permeable for chloride or potassium ions, respectively in the membranes

(BORNER 2003; PETROS *et al.* 2004). The process of pore forming required hydrophobic α-5 and α-6 helices overlapping the BH1 domain and the stretch between BH1 and BH2 regions. Both of them bear a resemblance to toxins produced by some bacterial strains (i.e. *Escherichia coli* or *Corynebacterium diphtheriae*) against others. It is thought that the selectivity of Bcl-2 family protein-forming channel is determined by amino acid sequence and electric charge of molecule(s) engaged in its assembly. Moreover, the chemical composition of the membrane surrounding the protein is not insignificant for its poration activity (CHRISTENSON *et al.* 2008). It has been shown that negatively charged phospholipid – cardiolipin supports the pore-forming activity of Bax whereas cholesterol-rich bilayers inhibit Bax integration into the membranes (CHRISTENSON *et al.* 2008).

BH3 domain is composed of 9-16 amino acid residues highly conserved among all Bcl-2 family proteins. It fulfils the role of ligand binding region which is necessary for both hetero- and homo-dimerization and apoptosis triggering (FREY *et al.* 2008). The BH3 domain of anti-apoptotic proteins is an integral part of hydrophobic cleft built with BH1, BH2 and BH3 in contrast to the BH3 domains of pro-apoptotic factors of this family which are hidden inside the molecules of inactive proteins or are exposed to the outside of the molecules when they are active (BORNER 2003; PETROS *et al.* 2004). Unbound BH3 domain of free pro-apoptotic proteins indicates unorganized structure while after their dimerization with pro-survival partners it forms amphiphatic helix penetrating the hydrophobic pocket (BORNER 2003). It is worth mentioning that the substitution of Asp^{160} in BH3 domain of Bad with Arg that facilitates the adoption of α-helical structure widely increased the affinity of BH3 peptide to Bcl-X_L protein (PETROS *et al.* 2004).
In addition, the amino acid sequence of Bcl-2 family proteins plays an important role for this interaction. It has been reported that conserved residues – Leu and Asp occupy the position 1 and 6 of BH3 domain in all Bcl-2 family members, whereas Ala or any hydrophobic amino acid (usually Ile) is situated at position 4 of BH3 domain in anti- or pro-apoptotic proteins, respectively.

BH4 domain is localized in amino terminal region of anti-apoptotic Bcl-2 family members as well as some pro-apoptotic proteins (Bcl-x_s, Bcl-rambo,

Bok/Mtd, Boo/Diva) (HOSSINI, EBERLE 2008). For a long time, it was thought that BH4 domain was responsible for apoptosis inhibiting activity of pro-survival molecules (HOSSINI, EBERLE 2008). However, some anti-apoptotic proteins, e.g. Mcl-1, do not possess the above conserved region, but they all include a helical region − α1. In most of anti-apoptotic proteins this motif is enclosed in the BH4 domain. Currently, it seems that just this helical structure controls pro-survival function of Bcl-2 inhibitors regardless of its amino acid sequence. Similar α-helical region was found in Bax molecule in which it negatively regulates pro-apoptotic function of this protein (CARTRON *et al.* 2004).

Some anti-apoptotic Bcl-2 family members contain a 60-aa regulatory loop between α-1 and α-2 helices that seems to work as a region of autoinhibition (Lin 2004, PETROS *et al.* 2004). Chemical modifications (such as phosphorylation) within a given region result in abolishing the pro-survival potential of anti-apoptotic proteins and conversion of their phenotype.

Most of Bcl-2 family representatives contain a C-terminal 15-20-aa hydrophobic sequence – transmembrane (TM) domain – neighboring positively-charged amino acid residues of Lys or Arg, that enables them to attach to the membranes of mitochondria, endoplasmic reticulum or cell nuclei (PETROS *et al.* 2004; SCHINZEL *et al.* 2004a). Some of the proteins anchor to membranes immediately after their synthesis while others possess a transmembrane domain hidden inside their molecule and require to pass some conformational changes to expose TM on the surface (BORNER 2003; SCHINZEL *et al.* 2004a).

3. BAX PROTEIN – A KEY EFFECTOR IN MITOCHONDRIAL PATHWAY OF APOPTOSIS

Human *Bax* gene is located on long arm of chromosome 19 within the region q13.3-q13.4 (APTE *et al.* 1995). This gene is about 4500 bp long and contains six exons. Alternative splicing of its primary transcript produces several isoforms of Bax (α, β, γ, δ, ω, σ and Ψ) (CARTRON *et al.* 2002; DROIN, GREEN 2004). In ischemic rat brain a novel splice variant of Bax, Bax κ, was identified (JIN *et al.* 2001). Main variant, firstly isolated – Baxα is encoded by all exons and has a molecular weight

of 21 kDa (192 aa). Its name – Bax (Bcl-2 associated x protein) is derived from its ability to neutralize and block the function of the main anti-apoptotic protein − Bcl-2 (BORNER 2003). Function of the rest of Bax variants still remains unknown (DROIN, GREEN 2004). The promoter of *Bax* has two canonical E-box motifs that bind c-Myc/Max (*Myc associated factor X*) heterodimers. Moreover, *Bax* is under the control of P53 transcription factor and is involved in P53-mediated apoptosis (CARTRON *et al.* 2002).

Bax protein – an effectory promoter of apoptosis is constitutively expressed in the cells. In vital cells the described polypeptide is identified only as an inactive monomer or as the molecule bound to anti-apoptotic partner. Apoptosis-inhibitory proteins have a potential to neutralize the function of pro-death factors (CHIPUK *et al.* 2006; CHIPUK, GREEN 2008). It is accepted that in vital cells Bax is mainly localized in the cytosol, although it can be loosely attached to intracellular membranes (SCHINZEL *et al.* 2004b; SCHLESINGER, SAITO 2006). After activation in response to pro-death signals, Bax undergoes some conformational changes thereby incorporating the mitochondrial membranes persistently, forming pores and inducing OMM permeabilization (CHIPUK *et al.* 2006; CHIPUK, GREEN 2008). In active state this protein indicates diminished sensitivity to alkaline treatment, trypsin digestion as well as decreased susceptibility to proteolysis of α5 and α6 helices (BORNER 2003). In this state a significant increase of N-terminal region of Bax immunoreactivity (6A7 epitope between 13 and 19 amino acids) was demonstrated. However, this feature is thought to be reversible and independent of pro-survival Bcl-2 proteins (BORNER 2003; SHARPE *et al.* 2004). It would seem that the 6A7 epitope exposition on the surface of Bax appears at very early stage of its conformational changes and it precedes protein oligomerization. Therefore, this immunoreactivity elevation can not be strictly identified with Bax activation and apoptosis induction (SHARPE *et al.* 2004). In addition, it is believed that Bax translocation and oligomerization are not enough to induce permeabilization of OMM, although they both are required (SKOMMER *et al.* 2007).

Bax protein shares a strong similarity of its tertiary structure with anti-apoptotic members of Bcl-2 family, such as Bcl-2 and Bcl-X_L (SUZUKI *et al.* 2000;

BORNER 2003; PETROS *et al.* 2004). Above polypeptide contains 9 α-helices. The seven of them surround two centrally-localized hydrophobic α-helical structures. The helix α5 is positioned the most internally in the molecule. Between helices α-1 and α-2 a long flexible loop region can be observed. BH1, BH2 and BH3 domains of the described protein, embraced with α-2, α-3 and α-4 helices, lay in the same orientation as the domains of Bcl-X_L. In living cells they form hydrophobic cleft in analogous manner as anti-apoptotic proteins. It prevents from Bax dimerization under normal conditions (DROIN, GREEN 2004). In contradiction with Bcl-X_L or Bcl-2 proteins, BH3 domain of Bax does not go so deep into the hydrophobic pocket of a protein. Additionally Bax, similarly as anti-apoptotic factors, possesses in C-terminus a transmembrane region responsible for directing of the molecule to the mitochondrial membrane (Fig. 1) (SUZUKI *et al.* 2000; SCHINZEL *et al.* 2004b). However, TM region of Bax, unlike of Bcl-2 or Bcl-X_L, is not exposed to the cytosol. Instead of that, it moves back towards the pocket and binds to it as Bak or Bad proteins bind the hydrophobic pocket of Bcl-X_L (SUZUKI *et al.* 2000; BORNER 2003; PETROS *et al.* 2004). It has been revealed that a hydrogen bound formed by hydroxyl group of Ser^{184} side chain and carboxyl group of Asp^{98} is directly involved in the interaction between C-terminus of Bax and α4 region of its hydrophobic pocket (SUZUKI *et al.* 2000). It is also possible that C-terminus of Bax protein takes part in pocket stabilization and plays a role in the regulation of its translocation and oligomerization (SUZUKI *et al.* 2000; CARTRON *et al.* 2004). If the C-terminus of Bax is folded the pocket remains in a stable conformation. While the region is removed out of the hydrophobic cleft, the pocket collapses and the protein structure changes leading to protein dimerization and to its interaction with mitochondrial membrane. It is suggested that a removal of α-9 helical region (TM) is initiated by posttranslational modification of Bax (i.e. phosphorylation of Ser and Thr residues present in that region in large numbers) or some currently unknown factor, from behind of BH3-only subfamily, that competes with Bax for binding of its hydrophobic cleft. Interestingly, Pro^{168} can be a direct or indirect linker between N- and C-termini of Bax protein displaying a function in the stabilization of its structure (SCHINZEL *et al.* 2004b). Destruction of this interaction under apoptotic signal(s)

results in the exposition of TM domain and translocation of pro-apoptotic protein to the mitochondria. It is possible that Pro^{168} is a target for Pin 1 isomerase activity (peptidylprolyl cis/trans isomerase, NIMA-interacting 1) or a substrate for hydroxylation reactions.

BORNER suggested sequential events that may occur in Bax-like proteins during the apoptosis induction (2003). According to this model Bax-like proteins are present in living cells in the cytosol in the form of monomers or remain loosely attached to OMM. This kind of contact does not lead to remodeling of Bax hydrophobic cleft which is stabilized by interactions with membrane phospholipids or some unidentified inhibitory factors. In the case of Bax the C-terminus of the protein needs to be released from the binding with its hydrophobic pocket. In contrast, Bak doesn't require any extra signals because of its anchoring region that is always exposed outside the molecule. An alternative scenario assumes that the pushing aside α9 helix of the molecule evokes immediate changes in the protein structure: disintegration of hydrophobic cleft and exposition of BH3 domain. However, even then the incorporation of Bax to the membranes cannot be achieved because of the Bcl-2 anti-apoptotic proteins inhibiting BH3 domain of this pro-death molecule. Only in the presence of activated BH3-only proteins there occurs the release of Bax from the interaction with Bcl-2 or Bcl-X_L. As a result of the liberation, Bax α5/α6 helices can stably incorporate into the mitochondrial membranes. It is also possible that Bax may be auto-activated or cross-activated with already active Bax-like molecules without the participation of BH3-only proteins. The next step of OMM permeabilization is the oligomerization of effector proteins (Bax, Bak, Bok) and pore forming. It is conceivable that this stage can also be stopped by Bcl-2-like proteins if they are overexpressed in the cells (BORNER 2003). Therefore, apoptosis-mediating signals must generate multidirectional response to abrogate the multistage inhibition of anti-apoptotic proteins.

It was thought for a long time that α9 helix of Bax was only one region directing the molecule to the mitochondria. However, in 2004 CARTRON *et al.* suggested the presence of additional mitochondrial targeting sequence in α1 helix of that protein and proposed an alternative to Borner's manner of Bax activation and

translocation. In viable cells, α1 helix of inactive Bax protein is situated close to α2 (BH3 domain), α5 and α6 regions and guards against their mitochondrial insertion (GRIFFITHS *et al.* 2001, MOLDOVEANU *et al.* 2006). According to the cited authors (CARTRON *et al.* 2004) the negative regulation of above helix is abrogated by its interaction with BH3 domain of the so-called direct activators from BH3-only proteins, e.g. Bid, Bim or Puma. This interaction leads to the opening of Bax molecule and to the exposure of α1 on the surface of pro-apoptotic protein. It has been established that Asp^{33} of Bax and Gly^{94}, Met^{97}, Arg^{84} of BH3-only protein play a key role in this binding. The hypothesis of CARTRON *et al*. (2004) arouses many controversies (SCHINZEL *et al.* 2004b, MING *et al.* 2006). It has been indicated that mitochondrial localization sequence is preceded by ART (apoptosis-regulating targeting domain) inhibitory domain (SCHINZEL *et al.* 2004b). Only if it was removed from the protein, the sequence would be uncovered. Thus, N-terminus of protein performs a role presumably only in inhibition of Bax activity. Indeed, its elimination from the molecule results in producing more potent pro-apoptotic protein − p18 Bax (CAO *et al.* 2003). Interestly, during apoptosis N-terminus of Bax may be cleaved by calpains (GAO, DOU 2000). Furthermore, transfection of Bax/p18 fragment into cancer cells targeted this cleaved protein segment to mitochondria, which was accompanied by release of cytochrome c.

It has been demonstrated very recently (GEORGE *et al.* 2010) that Bax contains two functional mitochondrial targeting sequence that may act independently in mitochondrial translocation of Bax. They are located in helices α6 and α9 and uncovering of one of them after conformational changes of Bax, directs the molecule to mitochondrial membrane. It has been revealed that only combined mutations in both of these regions preclude its mitochondrial translocation in HeLa cell line.

4. MECHANISMS OF BAX-INDUCED MITOCHONDRIAL OUTER MEMBRANE PERMEABILIZATION

Bcl-2 family proteins regulate apoptosis via their influence on mitochondrial membrane permeability and efflux of pro-apoptotic factors from intermembrane

space of mitochondria, releasing Ca^{2+} ions from endoplasmic reticulum as well as on redox state in cell resulting from a translocation of cytochrome c to the cytosol, disruption of respiratory chain in mitochondria and production of reactive oxygen species (ROS) (BRECKENRIDGE *et al.* 2003; REED 2008; HANSON *et al.* 2008; HETZ, GLIMCHER 2008).

At present, it is accepted that Bax-mediated permeabilization may run through one of the following models. The most common mode of Bax action is that after homo- or heterodimerization with other pro-apoptotic Bcl-2 family molecules, Bax-like proteins form in outer mitochondrial membrane channels big enough to let pass cytochrome c and/or other pro-apoptotic factors, including procaspase-9 which together with cytochrome c forms apoptosome and initiates apoptosis (KRAJEWSKI *et al.* 1999; SCHLESINGER, SAITO 2006; KUMARSWAMY, CHANDNA 2009).

According to the next hypothesis, supported by experiments with liposome, Bcl-2 family proteins interact with channels that already exist in the membrane, e.g. with voltage-dependent anion channel (VDAC), and create structure permeable for molecules of the size of cytochrome c and even bigger (KUMARSWAMY, CHANDNA 2009).

Importantly, Bax may cooperate with proteolytically truncated BH3-only member Bid (tBid) and Ca^{2+} in permeabilizing the outer mitochondrial membrane and releasing mitochondrial apoptogenic factors (LUO *et al.* 1998; WEI *et al.* 2000; DESAGHER *et al.* 1999; BEDNAREK, KILIAŃSKA 2005; BRUSTOVETSKY *et al.* 2010). Recent data have indicated that Bax and tBid together may induce the formation of a large lipid pore in mitochondrial membrane (TERRONES *et al*. 2004). It has been revealed that both of these proteins destabilize the structure of lipid bilayer and provoke pore formation. tBid protein facilitates lipid pore forming through its CBD domain (cardiolipin-binding domain) that directs pro-apoptotic proteins to cardiolipin-rich membranes (TERRONES *et al*. 2004).

Both, Bcl-2 and Bax proteins seem to be also involved in the regulation of Ca^{2+} release from endoplasmic reticulum (ER). During ER stress followed by accumulation of improperly folded proteins in the ER, overexpression of Bik protein induces Bax/Bak heterodimerization and leads to Ca^{2+} efflux from ER. Increase of

the level of Ca^{2+} in the cytosol may stabilize open conformation mitochondrial megachannel – a large structure embracing both of mitochondrial membranes which, among others, can participate in cytochrome c releasing (CHIPUK *et al.* 2006).

5. REGULATORS OF BAX ACTIVITY

5.1. Activators of Bax protein

The main regulators of pro-apoptotic activity of Bax-like proteins are BH3-only members of Bcl-2 family. They may be divided into two subgroups depending on the way how they reveal pro-apoptotic potential of the so-called effectors (CHIPUK *et al.* 2006; LEBER *et al*. 2007; SKOMMER *et al.* 2007):

- Direct activators (Bid-like proteins) e.g. Bid or Bim – proteins that transiently bind to Bax-like proteins and trigger their activation according to 'hit and run' model. They can also neutralize anti-apoptotic members of Bcl-2 family molecules.
- Derepressors or sensitizers (Bad-like proteins) e.g. Bad, Bik, Bmf, Noxa, Puma – proteins that do not exhibit a potential to activate Bax-like proteins directly but are able to release effectors from interaction with anti-apoptotic molecules.

The best known and the first described example of Bcl-2 family effector activation was tBid-dependent rearrangement of Bax molecule (ZAMZAMI *et al.* 2000). It has been established that the interaction of Bax with tBid induces conformational change in pro-apoptotic effectory molecule (exposure of 6A7 epitope on the molecule) resulting in protein translocation, incorporation to mitochondrial membranes and eventually their permeabilization.

Importantly, Bax can be also directly activated by factors that do not belong to Bcl-2 family. It has been demonstrated that cytoplasmic form of tumor suppressor protein – P53 induces Bax oligomerization and permeabilization of lipid vesicles at the level comparable to that obtainable with tBid (CHIPUK, GREEN 2004). It appears that a proline-rich region of P53 located between 62. and 91. amino acid residues is required for direct activating of Bax molecule. The deletion of this segment fails to permeabilize the membrane and arrests cytochrome c and other pro-apoptotic molecules inside the mitochondria. Additionally, P53 may also act as a derepressor –

a molecule that binds to anti-apoptotic protein (Bcl-X_L) and releases of Bax from the interaction with its inhibitor.

Recently, several reports have revealed that calpain-activated protein product of *Atg5* (autophagy-related gen 5) gene exhibits potential to activate the members of Bax-like family (CODOGNO, MEIJER 2006, YOUSEFI *et al.* 2006). Its 24 kDa N-terminal region, freed after the calpain-mediated cleavage, translocates from the cytosol into the mitochondrial membrane, thereby inducing apoptosis. Thus, Atg5 plays a role of autophagy-apoptosis switch. However, the mechanism of Atg-mediated Bax activation remains unclear.

Furthermore, CUDDEBACK *et al*. (2001) have reported the Bax-activating action of Bif-1/endophilin B1 (Bax-interacting factor 1) in murine pre-B hematopoietic FL5.12 cell line following IL-3 withdrawal. It has been also revealed that loss of Bif-1 protein inhibits Bax/Bak-mediated apoptosis in HeLa cells treated with siRNA, as well as in Bif-1-knockout mouse embryonic fibroblasts (MEFs), obtained from mouse ES cells through homologous recombination (TAKAHASHI *et al.* 2005). It seems that after apoptosis induction Bif-1 undergoes some posttranslational modification(s) enabling its binding to Bax protein. This association triggers conformational rearrangement in Bcl-2 family molecule and this incident is accompanied by its activation and incorporation into the mitochondrial membrane. Once Bax is activated, complex branches out (CUDDEBACK *et al*. 2001, TAKAHASHI *et al.* 2005). Moreover, it is thought that a failure of Bif-1 may contribute to the development of tumors under *in vitro* as well as under *in vivo* conditions.

In addition, Bax can also interact with P53-dependent protein – ASC (Apoptosis associated speck-like protein containing a caspase recruitment domain) which moves to mitochondria after apoptosis induction and, in this way, facilitates Bax relocation (LEBER *et al.* 2007). Similarly, it has been indicated that histone H1.2 trafficking is a part of events leading to Bcl-2 family pro-apoptotic proteins activation, mitochondrial membrane permeabilization and apoptosis induction upon cell death stimulation (YAN, SHI 2003). It is unclear, however, how exactly H1.2 histone acts to provoke Bax/Bak oligomerization after its release to the cytosol.

It is worth mentioning that under special environmental circumstances, Bax and Bak molecules, can be activated *in vitro* without contribution of any additional proteins e.g. in response to nonionic detergents, pH increase or growth of temperature (BORNER 2003). Nevertheless, it is not known if this kind of Bax activation occurs under physiological conditions.

5.2. Inhibitors of Bax protein

Among Bax regulators, with the exception of Bcl-2-like proteins that neutralize pro-apoptotic function of Bax, Ku70 protein – a component of DNA-PK (DNA-dependent protein kinase), 14-3-3 protein, and some Hsp proteins (Heat shock proteins) (αA and αB-crystalins, Hsp70/dj1 and Hsp70/dj2 complexes) are found (LUCKEN-ARDJOMANDE, MARTINOU 2005; GOTOH *et al.* 2004; SHARPE *et al.* 2004; SCHINZEL *et al.* 2004a). Anti-apoptotic activities of the above factors are probably associated with a sequestering of inactive Bax molecule in the cytosol. It has been revealed that the proteins abrogate apoptosis induced by several agents e.g., UV irradiation or staurosporine treatment, while their removal from the cells enhances cell susceptibility towards apoptosis.

Additionally, ASO (antisense oligonucleotides) strategy showed anti-apoptotic potential of ARC protein (Activity-regulated cytoskeleton-associated protein), a factor, that is highly expressed in cardiac and skeletal muscle cells (LUCKEN-ARDJOMANDE, MARTINOU 2005). It was shown that the silencing of ARC expression contributed to conformational changes in the pro-apoptotic molecule Bax and mediated apoptosis. Bax activation and translocation can be brought to a halt by a new endogenous peptide – humanin, too (ZHAI *et al.* 2005). It was indicated that this 24-aa peptide is able to bind both with Bax and its direct activator – Bid (and tBid) and to inhibit tBid-induced Bax oligomerization.

It has been also suggested that Bax mediated apoptosis can be abolished by hexokinase-mitochondria interaction in a process regulated by Akt/PKB kinase activity (MAJEWSKI *et al.* 2004).

In 1998, XU and REED reported about the new anti-apoptotic protein, BI-1 (Bax inhibitor 1), discovered by functional screening in yeast. Above inhibitor is

highly conserved in animals and plants (XU, REED 1998; HÜCKELHOVEN 2004). It has been well documented that BI-1 protein abrogates apoptosis followed by a large number of apoptotic stimuli, e.g. Bax overexpression, growth factor and cytokine deprivation, drug treatment (etoposide, staurosporine), bacterial pathogens (e.g. *Chlamydia psittacci*) in various cell lines (HÜCKELHOVEN 2004). This protein neutralizes pro-apoptotic function of Bax. However, the mechanism of BI-1 action does not seem to be related to its physical interaction with Bax molecule. These two proteins do not appear to associate with themselves.

The main negative regulators of pro-apoptotic function of Bax are Bcl-2 family proteins. Bax can heterodimerize with Bcl-2, Bcl-X_L, Bcl-w and, perhaps, with Mcl-1 proteins and these interactions prevent apoptosis in live cells (DROIN, GREEN 2004; EWINGS *et al.* 2007). After apoptosis induction, the release of Bax from the complexes with proteins that counteract its function takes place due to the fact that BH3-only proteins indicate approximately 100 times stronger affinity for anti-apoptotic Bcl-2-like members than their affinity for Bax (BORNER 2003). Free Bax molecules multimerize and/or interact with the components which already exist in the mitochondrial membranes forming a large channel permeable for cytochrome c and other pro-death factors. Thus, the cell fate depends essentially on the equilibrium between pro-survival and pro-apoptotic proteins. Indeed, it is well known that disruption of the balance between Bcl-2 family partners in the cells entails serious consequences manifested in the disorder of life-or-death decision and on the one hand, cell accumulation, or on the other hand, excessive cell elimination. Defective apoptosis causes a large number of diseases, including cancers, autoimmune syndromes, neurodegenerative disorders, diabetes, immunodeficiency syndromes, infertility (KILIAŃSKA 2002; PEREZ *et al.* 1997; BORNER 2003; HAYASHI, FAUSTMAN 2003; MOLDOVEANU *et al.* 2006; FREY *et al.* 2008).

6. BCL-2 FAMILY PROTEINS – NEW TARGET FOR THE THERAPY OF DISORDERS WITH DEFECTIVE APOPTOSIS

In viable cells pro-apoptotic proteins co-exist in dynamic balance with anti-apoptotic counterparts from Bcl-2 family (DAY *et al.* 2008). It means that decreased

expression of one of them leads to the reduction in the level of the other one. For example, in viable cells of HEK-293 cell line pro-apoptotic Bak is kept in check by pro-survival Mcl-1 protein, whereas a proper number of B lymphocytes in the body is strictly regulated by Noxa/Mcl-1 ratio in the cells. Admittedly, Bax requires some additional signals for its activation, but after apoptotic stimulation cell fate depends to a high degree on the equilibrium between Bax and its main inhibitor – Bcl-2 (OLTVAI, KORSMEYER 1993). The overexpression of anti-apoptotic protein results in cell accumulation and may conduce to survival of transformed cells (CORY *et al.* 2003). Bcl-2 over-expression has been indicated in leukemias, lymphomas and a large number of human solid tumors and its high level is often associated with unfavorable prognosis (CASTLE *et al.* 1993; CORNBLAU *et al*. 1999; DROIN, GREEN 2004). Correspondingly, *Bax*α gene is mutated in many cancers (e.g. derived from colorectum, stomach, endometrium cells) and its expression level influences on the effectiveness of apoptosis induction (BARGOU *et al.* 1995, WAGENER *et al.* 1996; RAMPINO *et al.* 1997; OUYANG *et al.* 1998; REED 1999; FERREIRA *et al.* 2002). Therefore, Bax/Bcl-2 ratio may reflect susceptibility of neoplastic cells to spontaneous as well as drug-induced apoptosis. For instance, the cellular level of Bax and Bcl-2 proteins in prostate cancer cells is a predictive factor forecasting the result of the therapy based on androgen deprivation and radiation (KHOR *et al.* 2007). It has been also shown that Bax/Bcl-2 rate in chronic lymphocytic leukemia (CLL) inversely correlates with resistance of leukemic cells to cytotoxic drug – chlorambucil *in vitro* as well as with clinical responsiveness (PEPPER *et al.* 1997). Above parameter mirrors the potential of the examined cells to apoptosis induction and its analysis may serve *in vitro* as potential tool for evaluating whether neoplastic cells are drug resistant or not (KOBYLINSKA *et al.* 2006).

Owing to the key role of Bcl-2 family proteins in apoptosis regulation they are an attractive target for modern anti-cancer therapy. There are many therapeutical approaches leading to modification of cellular balance between pro- and anti-apoptotic Bcl-2 family molecules. Most of them are targeted to pro-survival proteins and are based on antisense oligonucleotides (JANSEN *et al.* 1998; KONOPLEVA *et al.* 2000; FERREIRA *et al.* 2002). However, the number of trials aiming at inducing *Bax*

gene in tumor cells is growing all the time (WAGENER *et al.* 1996; LI *et al.* 2001; PIROCANAC *et al.* 2002; FALKE *et al.* 2003). As the introduction of a vector expressing *Bax* gene into cells involves a lot of difficulties associated with putative toxic effect of its protein product on normal cells, it is a great challenge for scientists to cause a cancer cell-directed expression of this gene (SEO *et al.* 2009).

A new engineered artificial transcription factor, 5ZFAV, seems to be a solution to this problem (FALKE *et al.* 2003). It has been revealed that 5ZFAV, containing zinc fingers from mouse Zif268 and transactivation domain from herpes simplex virus protein, VP16, selectively induces the expression of endogenous *Bax* and efficiently turns apoptosis on in P53-deficient sarcoma osteogenic Saos-2 cells. Interestingly, the impact of the novel molecule on P53 positive osteosarcoma cell line – U-2 OS appears to be much less potent giving the chance for selective pro-apoptotic activity against P53-mutated tumor cells.

The Tet-On inducible system, containing reverse tetracycline transactivator (rtTA) and inducing transcription after the binding with tetracycline and its derivatives, seems to be very helpful in controlling Bax gene expression (WAGENER *et al*. 1996, SEO *et al.* 2009). It has been reported that the stable transfectants obtained from DLD-1 colon cancer cells transfected with above system (*pTET-On* and *p-TRE Bax* plasmids) and selected in the medium with geneticin, occur to be more sensitive to various drug-induced apoptosis than the cells without introduced *Bax* gene (KOBAYASHI *et al.* 2000).

On the other hand, it has been discovered that *bax* inactivation in oocytes of mice undergoing treatment with doxorubicine reduced their sensitivity to apoptosis and increased their viability (PEREZ *et al.* 1997). Since it is well known that development of infertility in anticancer drugs-treated women is one of the most common side effect of chemotherapy, the inhibition of *Bax* gene in germinal cells during the chemotherapy of cancer could be interesting strategy of infertility preservation (PEREZ *et al.* 1997).

In conclusion, it seems that overexpression of Bax alone in cancer cells does not induce apoptosis but enhances its induction following anticancer treatment. A combination of conventional chemotherapy with gene therapy may be a new, very

potent strategy in cancer treatment. It is conceivable that the better understanding of apoptosis regulation may help to overcome apoptosis deficiency (typical in many cancers) and therapy resistance.

Acknowledgements

Preparation of this article was supported by grant 505/0375 from the University of Łódź.

7. REFERENCES

APTE, S.S., MATTEI, M.G., OLSEN, B.R. 1995. Mapping of the human *bax* gene to chromosome 19q13.3-13.4 and isolation of a novel alternatively spliced transcript Bax delta. Genomics 26: 592–594.

BARGOU, R.C., BOMMERT, K., WEINMANN, P., DANIEL, P.T., WAGENER, C., MAPARA, M.Y., DÖRKEN, B. 1995. Expression of the *bcl-2* gene family in normal and malignant breast tissue: low bax-alpha expression in tumor cells correlates with resistance towards apoptosis. Eur. J. Immunol. 25: 770–775.

BEDNAREK, J., KILIAŃSKA, Z.M. 2005. Białka przestrzeni międzybłonowej mitochondriów uczestniczące w procesie apoptozy. Post. Biochem. 51: 447–458.

BORNER C. 2003. The Bcl-2 protein family: sensors and checkpoints for life-or-death decisions. Mol. Immunol. 39: 615–647.

BRECKENRIDGE, D.G., GERMAIN, M., MATHAI, J.P., NGUYEN, M., SHORE, G.C. 2003. Regulation of apoptosis by endoplasmic reticulum pathway. Oncogene 22: 8608–8618.

BRUSTOVETSKY, T., LI, T., YANG, Y., ZHANG, J.T., ANTONSSON, B., BRUSTOVETSKY, N. 2010. Bax insertion, oligomerization, and outer membrane permeabilization in brain mitochondria: Role of permeability transition and SH-redox regulation. Biochim. Biphys. Acta 1797: 1795–1806.

CAO, X., DENG, X., MAY, W.S. 2003. Cleavage of Bax to p18 Bax accelerates stress-induced apoptosis, and a cathepsin-like protease may rapidly degrade p18 Bax. Blood. 102: 2605–2614.

CARTRON, P.F., GALLENNE, T., BUGRAS, G., GAUTIER., MANERO, F., VUSO, P., MEFLAH, K., VALLETTE, F.M., JUIN, P. 2004. The first α-helix of Bax plays a necessary role in its ligand-induced activation by the BH3-only proteins Bid and Puma. Mol. Cell. 16: 807–818.

CARTRON, P.F., OLIVER, L., MARTIN, S., MOREAU, C., LECABELLEC, M.T., JEZEQUEL, P., MEFLAH, K., VALLETTE, F.M. 2002. The expression of a new variant of the pro-apoptotic molecule Bax, Bax psi, is correlated with an increased survival of glioblastoma multiforme patients. Hum. Mol. Genet. 11: 675–687.

CASTLE, V.P., HEIDELBERGER, K.P., BROMBERG, J., OU, X., DOLE, M., NUÑEZ, G. 1993. Expression of the apoptosis-suppressing protein Bcl-2 in neuroblastoma is associated with unfavorable histology and N-myc amplification. Am. J. Pathol. 143: 1543–1550.

CHIPUK, J.E., BOUCHIER-HAYES, L., GREEN, D.R. 2006. Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. Cell. Death Differ. 13: 1396–1402.

CHIPUK, J.E., GREEN, D. 2004. Cytoplasmic p53: bax and forward. Cell Cycle. 3: 429–431.

CHIPUK, J.E., GREEN, D. 2008. How do Bcl-2 proteins induce mitochondria outer membrane permeabilization? Trends Cell. Biol. 18: 157–164

CHRISTENSON, E., MERLIN, S., SAITO, M., SCHLESINGER, P. 2008. Cholesterol effects on Bax pore activation. J. Mol. Biol. 381: 1168–1183.

CODOGNO, P., MEIJER, A.J. 2006. Atg: more than autophagy factor. Nat. Cell. Biol. 8: 1045–1047.

CORNBLAU, S.M., THALL, P.F., ESTROV, Z., WALTERSCHEID, M., PATEL, S., THERIAULT, A., KEATING, M.J., KANTARJIAN, H., ESTEY, E., ANDREEFF, M. 1999. The prognostic impact of Bcl2 protein expression in acute myelogenous leukemia varies with cytogenetics. Clin. Cancer. Res. 5: 1758–1766.

CORY, S., HUANG, D.C., ADAMS, J.M. 2003. The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene 22: 8590–8607.

CUDDEBACK, S.M., YAMAGUCHI, H., KOMATSU, K., MIYASHITA, T., YAMADA, M., WU, C., SINGH, S., WANG, H.G. 2001. Molecular cloning and characterization of Bif-1. A novel Src homology 3 domain-containing protein that associates with Bax. J. Biol. Chem. 276: 20559–20565.

DAY, C.L., SMITS, C., FAN, F.C., LEE, E.F., FAIRLIE, W.D., HINDS, M.G. 2008. Structure of the BH3 domains from the p53-inducible BH3-only proteins Noxa and Puma in complex with Mcl-1. J. Mol. Biol. 380: 958–971.

DESAGHER, S., OSEN-SAND, A., NICHOLS, A., ESKES, R., MONTESSIUT, S., LAUPER, S., MAUNDRELL, K., ANTONSSON, B., MARTINOU, J.C. 1999. Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. J. Cell Biol. 144: 891–901.

DROIN, N. M., GREEN, D. R. 2004. Role of Bcl-2 family members in immunity and disease. Biochim. Biophys. Acta. 1644: 179–188.

EWINGS, K.E., HADFIELD-MOORHOUSE, K., WIGGINS, C.M., WICKENDEN, J.A., BALMANNO, K., GILLEY, R., DEGENHARDT, K., WHITE, E., COOK, S.J. 2007. ERK1/2-dependent phosphorylation of Bim_{EL} promotes its rapid dissociation from Mcl-1 and Bcl-$X_{L.}$ EMBO J. 26: 2856–2867.

FALKE, D., FISHER, M., YE, D., JULIANO, R.L. 2003. Design of artificial transcription factors to selectively regulate the pro-apoptotic bax gene. Nucleic Acids Res. 31(3): e10.

FERREIRA, C.G., EPPING, M., KRUYT, F.A.E., GIACCONE, G. 2002. Apoptosis: target of cancer therapy. Clin. Cancer Res. 8: 2024–2034.

FREY, V., VIAUD, J., SUBRA, G., CAUQUIL, N., GUICHOU, J.F., CASARA, P., GRASSY, G., CHAVANIEU, A. 2008. Structure-activity relationships of Bak derived peptides: Affinity and specificity modulations by amino acid replacement. Eur. J. Med. Chem. 43: 966–972.

GAO, G., DOU, Q.P. 2000. N-terminal cleavage of Bax by calpain generates a potent proapoptotic 18 kDa fragment that promotes bcl-2-independent cytochrome c release and apoptotic cell death. J. Cell. Biochem. 80: 53–72.

GEORGE, N.M., TARGY, N., EVANS, J.J., ZHANG, L., LUO, X. 2010. Bax contains two functional mitochondrial targeting sequences and translocates to mitochondria in

a conformational change- and homo-oligomerization-driven process. J. Biol. Chem. 285: 1384–1392.

GOTOH, T., TERADA, K., OYADOMARI, S., MORI, M. 2004. Hsp70-DnaJ chaperone pair prevents nitric oxide- and CHOP-induced apoptosis by inhibiting translocation of Bax to mitochondria. Cell. Death. Differ. 11: 390–402.

GREIJER, A.E., VAN DER WALL, E. 2004. The role of hypoxia inducible factor 1 (HIF-1) in hypoxia induced apoptosis. J. Clin. Pathol. 57: 1009–1014.

GRIFFITHS, G. J., CORFE, B.M., SAVORY, P., LEECH, S., ESPOSTI, M.D., HICKMAN, J.A., DIVE, C. 2001. Cellular damage signals promote sequential changes at the N-terminus and BH-1 domain of the pro-apoptotic protein Bak. Oncogene 20: 7668–7676.

GUO, B., GODZIK, A., REED, J.C. 2001. Bcl-G, a novel pro-apoptotic member of the Bcl-2 family. J. Biol. Chem. 276: 2780–2785.

GURUDUTTA, G.U., VERMA, Y.K., SINGH, V.K., GUPTA, P., RAJ, H.G., SHARMA, R.K., CHANDRA, R. 2005. Structural conservation of residues in BH1 and BH2 domains of Bcl-2 family proteins. FEBS Lett. 579: 3503–3507.

HANSON, C.J., BOOTMAN, M.D., DISTELHORST, C.W., MARALDI, T., RODERICK, H.L. 2008. The cellular concentration of Bcl-2 determinates its pro- or anti-apoptotic effect. Cell. Calcium 44: 243–258.

HAYASHI, T., FAUSTMAN, D.L. 2003. Role of defective apoptosis in type 1 diabetes and other autoimmune diseases. Rec. Prog. Horm. Res. 58: 131–153.

HENGARTNER, M.O. 2000. The biochemistry of apoptosis. Nature 407: 770–776.

HETZ, C., GLIMCHER, L. 2008. The daily job of night killers: alternative roles of the Bcl-2 family in organelle physiology. Trends. Cell. Biol. 18: 38–44.

HOSSINI, A. M., EBERLE, J. 2008. Apoptosis induction by Bcl-2 proteins independent of the BH3 domain. Biochem. Pharmacol. 76: 1612–1619.

HÜCKELHOVEN, R. 2004. Bax inhibitor-1, an ancient cell death suppressor in animals and plants with prokaryotic relatives. Apoptosis 9: 299–307.

JANSEN, B., SCHLAGBAUER-WADL, H., BROWN, B.D., BRYAN, B.D., VAN ELSAS, A., MÜLLER, M., WOLFF, K., EICHLER, H.G., PEHAMBERGER, H. 1998. Bcl-2 antisense therapy chemosensitizes human melanoma in SCID mice. Nat. Med. 4: 232–234.

JIN, K.L., GRAHAM S.H., MAO, X.O., HE, X., NAGAYAMA, T., SIMON, R.P., GREENBERG, D.A. 2001. Bax kappa, a novel Bax splice variant from ischemic rat brain lacking an ART domain, promotes neuronal cell death. J. Neurochem. 77: 1508–1519.

KARST, A.M., LI, G. 2007. BH3-only proteins in tumorigenesis and malignant melanoma. Cell. Mol. Life Sci. 64: 318–330.

KATAOKA, T., HOLLER, N., MICHEAU, O., MARTINON, F., TINEL, A., HOFMANN, K., TSCHOPP, J. 2001. Bcl-rambo, a novel Bcl-2 homologue that induces apoptosis via its unique C-terminal extension. J. Biol. Chem. 276: 19548–19554.

KE, N., GODZIK, A., REED, J.C. 2001. Bcl-B, a novel Bcl-2 family member that differentially binds and regulates Bax and Bak. J. Biol. Chem. 276: 12481–12484.

KHOR, L.Y., MOUGHAN, J., AL-SALEEM, T., HAMMOND, E.H., VENKATESAN, V., ROSENTHAL, S.A., RITTER, MA., SANDLER, H.M., HANKS, G.E., SHIPLEY, W.U., POLLACK, A. 2007. Bcl-2 and Bax expression predict prostate cancer outcome in men treated with androgen deprivation and radiotherapy on radiation therapy oncology group protocol 92-02. Clin. Cancer Res. 13: 3585–3590.

KILIAŃSKA, Z.M. 2002. Apoptoza organizmów zwierzęcych. In: L. KŁYSZEJKO-STEFANOWICZ (ed.) Cytobiochemia. Biochemia niektórych struktur komórkowych. Wydawnictwo Naukowe PWN, Warszawa, pp. 772–814.

KOBAYASHI, T., SAWA, H., MORIKAWA, J., ZHANG, W., SHIKU, H. 2000. *Bax* induction activates apoptotic cascade via mitochondrial cytochrome *c* release and *Bax* overexpression enhances apoptosis induced by chemotherapeutic agents in DLD-1 colon cancer cells. Jpn. J. Cancer Res. 91: 1264–1268.

KOBYLINSKA, A., BEDNAREK, J., BLONSKI, J.Z., HANAUSEK, M., WALASZEK, Z., ROBAK, T., KILIANSKA, Z.M., 2006. In vitro sensitivity of B-cell chronic lymphocytic leukemia to cladribine and its combinations with mafosfamide and/or mitoxantrone. Oncol. Rep. 16: 1389–1395.

KOLLURI, S.K., ZHU, X., ZHOU, X., LIN, B., CHEN, Y., SUN, K., TIAN, X., TOWN, J., CAO, X., LIN, F., ZHAI, D., KITADA, S., LUCIANO, F., DONNELL, E., CAO, Y., HE, F., LIN, J., REED, J.C., SATTERTHWAIT, A.C., ZHANG, X.K. 2008. A short Nur77-

derived peptide converts Bcl-2 from a protector to a killer. Cancer Cell.14: 285–298.

KONOPLEVA, M., TARI, A.M., ESTROV, Z., HARRIS, D., XIE, Z., ZHAO, S., LÓPEZ-BERESTEIN, G., ANDREEFF, M. 2000. Liposomal Bcl-2 antisense oligonucleotides enhance proliferation, sensitize acute myeloid leukemia to cytosine-arabinoside, and induce apoptosis independent of other antiapoptotic proteins. Blood 95: 3929–3938.

KRAJEWSKI, S., KRAJEWSKA, M., ELLERBY, L.M., WELSH, K., XIE, Z., DEVERAUX, Q.L., SALVESEN, G.S., BREDESEN, D.E., ROSENTHAL, R.E., FISKUM, G., REED, J.C. 1999. Release of caspase-9 from mitochondria during neuronal apoptosis and cerebral ischemia. Proc. Natl. Acad. Sci. U.S.A. 96: 5752–5757.

KUMARSWAMY, R., CHANDNA, S. 2009. Putative partners in Bax mediated cytochrome-c release: ANT, CypD, VDAC or none of them? Mitochondrion 9: 1–8.

LEBER, B., LIN, J., ANDREWS, D.W. 2007. Embedded together: the life and death consequences of interaction of the Bcl-2 family with membranes. Apoptosis. 12: 897–911.

LEE, R.M., CHEN, J., MATTHEWS, C.P., MCDOUGALL, J.K., NEIMAN, P.E. 2001. Characterization of NR13-related human cell death regulator, Boo/Diva, in normal and cancer tissues. Biochim. Biophys. Acta 1520: 187-194.

LI, X., MARANI, M., YU, J., NAN, B., ROTH, J.A., KAGAWA, S., FANG, B., DENNER, L., MARCELLI, M. 2001. Adenovirus-mediated Bax overexpression for the induction of therapeutic apoptosis in prostate cancer. Cancer Res. 61: 186–191.

LIN, B., KOLLURI, S.K., LIN, F., LIU, W., HAN, Y.H., CAO, X., DAWSON, M.I., REED, J.C., ZHANG, X.K. 2004. Conversion of Bcl-2 from protector to killer by interaction with nuclear orphan receptor Nur77/TR3. Cell 116: 527–540.

LOWE, S.W., CEPERO, E., EVAN, G. 2004. Intrinsic tumour suppression. Nature 432: 307–315.

LUCIANO, F., KRAJEWSKA, M., ORTIZ-RUBIO, P., KRAJEWSKI, S., ZHAI, D., FAUSTIN, B., BRUEY, J.M., BAILLY-MAITRE, B., LICHTENSTEIN, A., KOLLURI, S.K., SATTERTHWAIT, A.C., ZHANG, X.K., REED, J.C. 2007. Nur77 converts phenotype

of Bcl-B, an antiapoptotic protein expressed in plasma cells and myeloma. Blood. 109: 3849–3855.

LUCKEN-ARDJOMANDE, S., MARTINOU, J.C. 2005. Regulation of Bcl-2 proteins and of the permeability of the outer mitochondrial membrane, Regulation of Bcl-2 proteins and of the permeability of the outer mitochondrial membrane. C. R. Biol. 328: 616–631.

LUO, X., BUDIHARDJO, J., ZOU, H., SLAUGHTER, C., WANG, X. 1998. Bid, an Bcl-2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 94: 481–490.

MAJEWSKI, N., NOGUEIRA, V., BHASKAR, P., COY, P.E., SKEEN, J.E., GOTTLOB, K., CHANDEL, N.S., THOMPSON, C.B., ROBEY, R.B., HAY. N. 2004. Hexokinase-mitochondria interaction mediated by Akt is required to inhibit apoptosis in the presence or absence of Bax and Bak. Mol. Cell. 16: 819–830.

MEIR, O., DVASH, E., WERMAN, A., RUBINSTEIN, M. 2010. C/EBP-beta regulates endoplasmic reticulum stress-triggered cell death in mouse human models. PLOS One 5: e9516.

MING, L., WANG, P., BANK, A., ZHANG, L. 2006. PUMA dissociates Bax and Bcl-$_{XL}$ to induce apoptosis in colon cancer cells. J. Biol. Chem. 281: 16034–16042.

MOLDOVEANU, T., LIU, Q., TOCILJ, A., WATSON, M., SHORE, G., GEHRING, K. 2006. The X-ray structure of Bak homodimer reveals an inhibitory zinc binding site. Mol. Cell. 24: 677–688.

NDOZANGUE-TOURIGUINE, O., HAMELIN, J., BRÉARD, J. 2008. Cytoskeleton and apoptosis. Biochem. Pharmacol. 76: 11–18.

OLTVAI, Z., KORSMEYER, S. 1993. Bcl-2 heterodimrizes in vivo with conserved homolog, Bax, that accelerates programmed cell death. Cell 74: 609–619.

OUYANG, H., FURUKAWA, T., ABE, T., KATO, Y., HORII, A. 1998. The *BAX* gene, the promoter of apoptosis, is mutated in genetically unstable cancers of the colorectum, stomach, and endometrium. Clin. Cancer. Res. 4: 1071–1074.

PEPPER, C., HOY, T., BENTLEY, D.P. 1997. Bcl-2/Bax ratios in chronic lymphocytic leukemia and their correlation with in vitro apoptosis and clinical resistance. Br. J. Cancer 76: 935–958.

PEREZ, G.I., KNUDSON, C.M., LEYKIN, L., KORSMEYER, S.J., TILLY, J.L. 1997. Apoptosis-associated signaling pathways are required for chemotherapy-mediated female germ cell destruction. Nat. Med. 3: 1228–1332.

PETROS, A.M., OLEJNICZAK, E.T., FESIK, S.W. 2004. Structural biology of the Bcl-2 family of proteins. Biochim. Biophys. Acta 1644: 83–94.

PIROCANAC, E.C., NASSIRPOUR, R., YANG, M., WANG, J., NARDIN, S.R., GU, J., FANG, B., MOOSSA, A.R., HOFFMAN, R.M., BOUVET, M. 2002. Bax-induction gene therapy of pancreatic cancer. J. Surg. Res. 106: 346–351.

RAMPINO, N., YAMAMOTO, H., IONOV, Y., LI., Y., SAWAI., H., REED, J.C., PERUCHO, M. 1997. Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science 275: 967–969.

REED, J.C. 1999. Dysregulation of apoptosis in cancer. J. Clin. Oncol. 17: 2941–2953.

REED, J.C. 2008. Bcl-2 family proteins and hematologic malignancies: history and future prospects. Blood. 111: 3322–3330.

RODOLFO C., PIACENTINI M. 2002. Does cytoskeleton `Akt' in apoptosis? Cell. Death Differ. 9: 477–478.

ROGALIŃSKA, M., BŁOŃSKI, J.Z., KOMINA, O., GÓRALSKI, P., ŻOŁNIERCZYK, J.D., PIEKARSKI, H., ROBAK, T., KILIAŃSKA, Z.M., WESIERSKA-GADEK, J. 2010. R-roscovitine (Seliciclib) affects CLL cells more strongly than combinations of fludarabine or cladribine with cyclophosphamide: Inhibition of CDK7 sensitizes leukemic cells to caspase-dependent apoptosis. J. Cell. Biochem. 76: 11–18.

SAVARAJ, N., YOU, M., WU, C., WANGPAICHITR, M., KUO, M.T., FEUN, L.G. 2010. Arginine deprivation, autophagy, apoptosis (AAA) for the treatment of melanoma. Curr. Mol. Med. 10: 405–412.

SCHINZEL, A., KAUFMANN, T., BORNER, C. 2004a. Bcl-2 family members: integrators of survival and death signals in physiology and pathology [corrected]. Biochim. Biophys. Acta. 1644: 95–105.

SCHINZEL, A., KAUFMANN ,T., SCHULER, M., MARTINALBO, J., GRUBB, D., BORNER, C. 2004b. Conformational control of Bax localization and apoptotic activity by Pro168. J. Cell. Biol. 164: 1021–1032.

SCHLESINGER, P.H., SAITO, M. 2006. The Bax pore in liposomes. Biophysics. Cell. Death Differ.13: 1403–1408.

SCHRÖDER, M. 2008. Endoplasmic reticulum stress responses. Cell. Mol. Life Sci. 65: 862–894.

SEO, E., KIM, S., JHO, E.H. 2009. Induction of cancer cell-specific death via MMP2 promoter-dependent Bax expression. B. M. B. Rep. 42: 217–222.

SHAN, Y.X., LIU, T.J., SU, H.F., SAMSAMSHARIAT, A., MESTRIL, R., WANG, P.H. 2003. Hsp10 and Hsp60 modulate Bcl-2 family and mitochondria apoptosis signaling induced by doxorubicin in cardiac muscle cells. J. Mol. Cell. Cardiol. 35: 1135–1143.

SHARPE, J.C., ARNOULT, D., YOULE, R.J. 2004. Control of mitochondrial permeability by Bcl-2 family members. Biochem. Biophys. Acta 1644: 107–113.

SKOMMER, J., WLODKOWIC, D., DEPTALA, A. 2007. Larger than life: mitochondria and the Bcl-2 family. Leuk. Res. 31: 277–286.

SUZUKI, M., YOULE, R.J., TJANDRA, N. 2000. Structure of Bax: coregulation of dimer formation and intracellular localization. Cell 103: 645–654.

TAKAHASHI, Y., KARBOWSKI, M., YAMAGUCHI, H., KAZI, A., WU, J., SEBTI, S.M., YOULE, R.J., WANG, H.G. 2005. Loss of Bif-1 suppresses Bax/Bak conformational change and mitochondria apoptosis. Mol. Cell. Biol. 25: 9369–9382.

TERRONES, O., ANTONSSON, B., YAMAGUCHI, H., WANG, H.G., LIU, J., LEE, R.M., HERRMANN, A., BASAÑEZ, G. 2004. Lipidic pore formation by the concerted action of proapoptotic Bax and tBid. J. Biol. Chem. 279: 30081–30091.

WAGENER, C., BARGOU, C., DANIEL, P.T., BOMMERT, K., MAPARA, M.Y., ROYER, H.D., DÖRKEN, B. 1996. Induction of the death-promoting gene bax-α sensitizes cultured breast-cancer cells to drug-induced apoptosis. Int. J. Cancer 67: 138–141.

WARR, M.R., SHORE, G.C. 2008. Small-molecule Bcl-2 antagonists as targeted therapy in oncology. Curr. Oncol. 15: 256–261.

WEI, M.C., LINDSTEN, T., MOOTHA V.K, WEILER, S., GROSS, A., ASHIYA, M., THOMPSON, C.B., KORSMEYER, S.J., 2000. tBid, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. Genes Dev. 14: 2060–2071.

WYLLIE, A.H. 2010. "Where, o death, is thy sting?" A brief review of apoptosis biology. Mol. Neurobiol. 42: 4–10.

XU, Q., REED, J.C., 1998, Bax inhibitor-1, a mammalian apoptosis suppressor identified by functional screening in yeast. Mol. Cell. 1: 337–346.

YAN, N., SHI, Y. 2003. Histone H1.2 as a trigger for apoptosis. Nat. Struct. Biol. 10: 983–985.

YEDAVALLI, V.S., SHIH, H.M., CHIANG, Y.P., LU, C.Y., CHANG, L.Y., CHEN, M.Y., CHUANG, C.Y., DAYTON, A.I., JEANG, K.T., HUANG, L.M. 2005. Human immunodeficiency virus type 1 Vpr interacts with antiapoptotic mitochondrial protein HAX1, J. Virol. 79: 13735–13746.

YOUSEFI, S., PEROZZO R., SCHMID, I., ZIEMIECKI, A., SCHAFFNER, T., SCAPOZZA, L., BRUNNER, T., SIMON, H.U. 2006. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nat. Cell. Biol. 8: 1124–1132.

ZAMZAMI, N., HAMEL, E., MAISSE, C., BRENNER, C., MUÑOZ-PINEDO, C., BALZACQ, A.S., COSTANTINI, P., VIEIRA, H., LOEFFLER, M., MOLLE, G., KROEMER, G. 2000. Bid acts on the permeability transition pore complex to induce apoptosis. Oncogene 19: 6342–6350.

ZHAI, D., LUCIANO, F., ZHU, X., GUO, B., SATTERTHWAIT, A.C., REED, J.C. 2005. Humanin binds and nullifies Bid activity by blocking its activation of Bax and Bak. J. Biol. Chem. 280: 15815–15824.

ŻOŁNIERCZYK, J.D., BŁOŃSKI, J.Z., ROBAK, T., KILIAŃSKA, Z.M., WESIERSKA-GADEK, J. 2009. Roscovitine triggers apoptosis in B-cell chronic lymphocytic leukemia cells with similar efficiency as combinations of conventional purine analogs with cyclophosphamide. Ann. N.Y. Acad. Sci. 1171: 124–131.

Acta Universitatis Lodziensis
——— 2010 ———
Folia Biologica et Oecologica 6: 33–47
(Acta Univ. Lodz, Folia Biol. Oecol.)

ADAM I. CYGANKIEWICZ

Department of Cytobiochemistry, University of Łódź, Pomorska 141/143, 90-236 Łódź, Poland, adam.c@biol.uni.lodz.pl

PHYSIOPATHOLOGICAL IMPLICATIONS OF 7TM RECEPTORS

Abstract: Seven-transmembrane (7TM) receptors are one of the most important proteins involved in perception of extracellular stimuli and regulation of variety of intracellular signaling pathways. Divergence of receptor types, their ligands and signaling pathways makes 7TM receptors important factors in pathology of many diseases. This review focused on the main diseases in which involvement of 7TM receptors was established e.g., retinitis pigmentosa, severe obesity, and dwarfism. Recent findings of aberrant expression of 7TM receptors in development of cancer were also summarized.

Key words: Seven-transmembrane receptors, G protein coupled receptors, pathology, cancer

1. 7TM RECEPTORS AS UNIVERSAL SIGNAL MEDIATORS

During evolution an extremely diverse family of heptahelical receptor proteins emerged. These proteins are called seven-transmembrane receptors (7TMRs) or G-protein coupled receptors (GPCRs) due to the fact of signal transmission via heterotrimeric G-proteins. The superfamily of 7TM receptors is the largest class of cell membrane receptor found in metazoans. The completion of the human genome project revealed over 800 genes encoding 7TM receptors (LAGERSTROM, SCHIOTH 2008). In view of existence of alternative splice variants and editing isoforms of 7TM receptors (CADET *et al.* 2003; HIRASAWA *et al.* 1995; NELSON, CHALLISS 2007) it is anticipated that true number of functional receptors is

DOI: 10.2478/v10107-009-0005-2

much higher. Sequence of these receptors is highly variable and the only homology between all 7TM receptors is the presence of seven α-helical transmembrane segments joined by intra- and extracellular loops with N-terminal chain located in the extracellular space and C-terminal chain located in cytoplasm. 7TM receptors react in response to a varied range of stimuli e.g., light, ions, peptides, lipids, and odorants. Upon ligand binding 7TM receptors constrain conformational change in α subunit of trimeric G-protein complex. This leads to exchange of GDP molecule for GTP and dissociation of G-protein and activation of subsequent intracellular responses (BOURNE *et al.* 1991).

Biological significance of 7TM receptors was consolidated after reports showing that heterotrimeric G-proteins are common, although not exclusive, mediators of cellular response after activation of these molecules (PIERCE *et al.* 2001; PIERCE *et al.* 2002). Unusual diversity among 7TM receptors allow them to play an important role in wide spectrum of biological processes ranging from neurotransmission and hormonal control to perception of taste, smell, light and pain.

Divergence of stimuli and rich pattern of intracellular signaling pathways governed by 7TM receptors makes this group of proteins undoubtedly prone to be involved in many pathological processes. In most cases mutation will exhibit as gain/loss of function phenotype of 7TM receptor. As a result of inherited mutations aberrant receptor will be present in every cell expressing particular gene while in somatic mutations even in the case of ubiquitous gene the expression of mutated gene will be limited to the cells derived from the progenitor of mutation (SPIEGEL 1997).

Mutations in nearly every part of the receptor may cause improper protein expression, folding, endoplasmic reticulum retention, or inability to interact with other proteins of the signalosome. Such abnormalities will exhibit loss of function phenotype and its presence will lead to ligand resistance. Mutations which lead to loss of function mutations are usually recessive and unveil their presence in homozygotes. Gain of function mutations will in most cases cause constitutive activation of the receptors and in case of hormonal signaling cause endocrine

hyperfunction. Since such mutations are dominant, heterozygotes are developing symptoms of the disease (SPIEGEL 2000).

At present several separate disease entities caused by mutations in 7TM receptors have been described. However, this number is likely to grow fast given to the fact, that over 160 7TM receptors are targeted in mice for homology with human diseases (SCHONEBERG *et al.* 2004).

2. DISEASES CAUSED BY INACTIVATION MUTATIONS IN 7TM RECEPTORS

The chances of mutational inactivation of the receptor are high. Amino acid substitution, deletion or insertion may cause loss or prematurely terminated transcription, improper protein folding or its inability to reach cell surface. Moreover, disruption of ligand binding pocket or receptor inability to bind with downstream signaling molecules, all result in loss of function phenotype (SPIEGEL 2000; SPIEGEL, WEINSTEIN 2004).

Diseases linked with inactivating mutations in 7TM receptors include: retinitis pigmentosa (RP) caused by mutations in rhodopsin; hypothyroidism and resistance to thyroid-stimulating hormone (TSH) caused by mutations in TSH receptor; nephrogenic diabetes insipidus (NDI) characterised by polyuria, polydipsia and hyposthenuria as a effect of mutations in vasopressin type 2 (V2) receptor (BARAK *et al.* 2001); Blomstrand chondrodysplasia in which mutations in parathyroid hormone/parathyroid hormone – related peptide (PTH/PTH-related peptide type 1) receptor; rare disease of familial hypocalciuric hypocalcaemia and potentially lethal neonatal severe hyperparathyroidism are linked with inactivating mutations in calcium-sensing receptor. Human red-hair color phenotype characterized by red hair, fair skin and poor ability to tan associated with loss of brown/black pigment eumelanin production, and susceptibility to development of skin cancers, have been linked with more than 60 variants of melanocortin type 1 (MC1R) receptor (SANCHEZ-LAORDEN *et al.* 2007; TAO 2006). Interestingly, inactivating mutations of CCR5 chemokine receptor, especially CCR5Δ32 are

associated with strong resistance to HIV infections, and slower progression of the disease (BALISTRERI *et al.* 2007).

2.1 Rhodopsin mutations and retinitis pigmentosa

A growing list of over 150 mutations, most of them missense or small in frame deletions, are primary causes of retinitis pigmentosa. Patients carrying this phenotypically and genetically diverse disease suffer from retinal dystrophy with symptoms ranging from night blindness to progressive loss of visual field (SCHONEBERG *et al.* 2004; TAO 2006).The first ever described mutation that causes this disease in humans was P23H substitution in rhodopsin. Mutated rhodopsin is retained in endoplasmic reticulum due to misfolding and aggregation. Although many single nucleotide mutations which causes retinitis pigmentosa can be found throughout the rhodopsin, cytoplasmatic part of the receptor and regions surrounding disulfide bridge connecting second extracellular loop with top of transmembrane three are more prone to hold retinitis pigmentosa mutations (STOJANOVIC, HWA 2002). The mechanism of retinal cells entering apoptotic pathway remains elusive, however, growing evidence suggest that aberrant formation of multimeric receptors complexes may contribute to this phenomena (ABDULAEV 2003).

2.2 Leptin/Melanocortin circuit; melanocortin receptor and severe obesity

Circulating leptin levels give the brain a reading of energy storage for the purposes of regulating appetite and metabolism. Leptin works by inhibiting the activity of neurons that contain neuropeptide Y (NPY) and agouti-related peptide (AgRP), and by increasing the activity of neurons expressing melanocyte-stimulating hormone (MSH).

Melanocortins are an important mediator of satiety. Leptin is produced in the adipocytes and mutations in the gene for the melanocortin receptors (MCRs) are linked to obesity in humans (TAO 2005). MC3R seems to exert its function in fat depository processes, rather than regulation of food intake. Inactivation of MC3R in mice results in elevated amount of fat mass while total body weight remains

unchanged (BUTLER *et al.* 2000; CHEN *et al.* 2000). Only recently first mutations of MC3R in obese patients were identified (LEE *et al.* 2002; RACHED *et al.* 2004; TAO, SEGALOFF 2004), confirming the importance of MC3R receptor in maintaining energy balance in the body. Heterozygous MC4 receptor mutations are found in 1–6% of severe cases of human obesity. More than 50 mutations in MC4 receptor gene, many of which were identified as heterozygous missense mutations, linked to obesity have been described in adults with morbid obesity or children with early onset obesity (GOVAERTS *et al.* 2005). The exact mechanism of observed dominant-negative effect of mutated MC4R is still far being clear, however recent reports suggest that altered sequence of MC4R may contribute to aberrant formation of multimeric complexes (BIEBERMANN *et al.* 2003).

2.3 7TM receptors mutations and reproductive physiology

Gonadothropin releasing hormone (GnRH) plays pivotal role in neuroendocrine regulation of reproduction. Hypogonadotropic hypogonadism occurs in patients with lack of GnRH receptor function. Characteristic for this disease is absence or decreased function of male testes or the female ovaries. Follitropin (FSH) receptor inefficiency is responsible for female infertility due to ovarian dysgenesis. At least 22 mutations in lutropin (LH) receptor responsible for production of testosterone have been reported to cause pseudohermaphroditis in males and hypergonadotropic hypogonadism, and primary amenorrhea in females (TAO 2006).

2.4 Growth hormone releasing hormone receptors and dwarfism

Growth hormone releasing hormone (GHRH) is synthesized and secreted by the arcuate nucleus of the hypothalamus. GHRH stimulates synthesis and secretion of growth hormone. Defective signaling in GHRH axis results in somatotroph hypoplasia and growth deficiency. GHRH receptor mutation was first identified in *little* mouse strain. Mutated murine GHRH receptor is unable to bind its ligand properly. In humans mutant receptors show aberrant signaling (TAO 2006). Inactivating mutations of growth hormone releasing hormone receptor lead to isolated growth hormone (GH) deficiency (LIN-SU, WAJNRAJCH 2002). At the same

time overexpression of GHRH was found to be an important factor in development of pituitary adenomas in mice (MAYO *et al.* 1988).

3. DISEASES CAUSED BY ACTIVATING MUTATIONS IN 7TM RECEPTORS

Gain of function mutations of 7TM receptors are in most cases missense mutations, however due to the fact that most of them are lethal during embryogenesis and thus undetectable, only 13% of diseases caused by mutated 7TM receptors are characterized by induction of agonist independent signaling. Constitutive activation of luteinizing hormone and thyrotropin receptors are cause of familial male precocious puberty characterized by accelerated sexual development at the age of 2 – 5 years. Familial hypocalcaemia is caused by calcium receptor hypersensivity to circulating Ca^{2+} and thus excessive hypercalcuria (SCHONEBERG *et al.* 2004; SPIEGEL, WEINSTEIN 2004).

Since many agonists for 7TM receptors exhibit mitogenic activity, many somatic gain of function mutations in 7TMRs are linked to development of adenomas and malignant tumors. In approximately 80% of thyroid adenomas activating mutations in thyrotropin receptors are reported. Smoothened, a member of frizzled family of 7TMRs that signals through hedgehog pathway is supposed cause of basal cell carcinoma (SCHONEBERG *et al.* 2004). Tumorigenic activity has been demonstrated for constitutively active receptors encoded by Kaposi's sarcoma associated virus and human cytomegalovirus (SODHI *et al.* 2004; VISCHER *et al.* 2006)

4. ABERRANT 7TM RECEPTORS EXPRESSION IN NEOPLASMS

Alterations in expression of different 7TM receptors have been reported in numerous human both benign and malignant neoplasms. It has been showed that aberrant expression of gastric inhibitory polypeptide and luteinizing hormone receptors is a sufficient event to trigger hyperplastic growth of adrenogortical cells (MAZZUCO *et al.* 2007). Systematic study of data from microarray analysis of primary lung, breast, prostate, gastric and melanoma cancers has revealed that

expression of multiple e.g., chemokine, PAR, neuropeptide, adenosine, purine and calcium receptors are significantly upregulated in human neoplasms (LI *et al.* 2005). Summary of latest findings on changes in 7TM receptors expression changes in various human neoplasms is presented in Tables 1a and 1b.

Table 1a. Abberations in 7TM receptors expression in neoplastic cells

Receptor	Ligand	Expression	Neoplasm		Reference
AXOR12	KiSS-1 peptide	↓	high grade epithelial ovarian cancer		HATA *et al.* 2007
Cb2	cannabinoids	↑	acute myelogenous leukemia	overexpressed in human myeloid leukemia cell lines	JORDA *et al.* 2004
CRH-R	corticotropin-releasing hormone	↑	corticotroph tumours		DE KEYZER *et al.* 1998
CysLT2R	leukotriene C4	↑	colorectal adenocarcinoma	subsequent downregulation causes poor prognosis	MAGNUSSON *et al.* 2007
D-GPCR	odorants	↑	malignant prostate	upregulation correlated with advancement of tumor	FUESSEL *et al.* 2006
FZD7	Wnt proteins	↑	hepatic cancers	subsequent activation of Wnt/beta catenin pathway	MERLE *et al.* 2005
Ghrelin 1a	ghrelin	↓	adenoid cystic carcinoma	parallel overexpression of 1b isoform	BARZON *et al.* 2005
GnRH	gonadotropin-releasing hormone	↑	multiple		EAVERI *et al.* 2004
GPR30	estrogen	↑	breast cancer	upregulation correlated with tumor size, invasiveness, Her2/neu expression	FILARDO *et al.* 2006
		↓	infiltrating ductal carcinoma	correlation in ER+ cells	KUO *et al.* 2007

Table 1b. Abberations in 7TM receptors expression in neoplastic cells (continued)

Receptor	Ligand	Expression	Neoplasm		Reference
GPR48	orphan	↑	multiple	downregulation of p27(Kip1) is associated with increased tumor malignancy and poor prognosis	GAO *et al.* 2006
GPR49	orphan	↑	colon, primary ovarian		MCCLANAHAN *et al.* 2006
GPR54	Kisspeptin	↑	bladder, thyroid		NICOLLE et al. 2007
GPR56	orphan	↓	pancreas	in vitro study	HUANG *et al.* 2007
		↑	gliomas		SHASHIDHAR *et al.* 2005
GPR87	lysophosphatidic acid	↑	lung squamous cell carcinoma		GUGGER *et al.* 2008
LPA2/3	lysophosphatidic acid	↑	colon	in vitro study; LPA induced proliferation mediated by beta catenin pathway	YANG *et al.* 2005
LPA2	lysophosphatidic acid	↑	invasive ductal carcinoma		KITAYAMA *et al.* 2004
Metastin	KiSS-1 peptide	↑	thyroid papillary carcinoma		RINGEL *et al.* 2002
Orphan BTR	orphan	↑	prostate		PARMIGIANI *et al.* 2004
PAR1	thrombin	↑	colon, prostate, aggressive melanoma, invasive breast		ARORA *et al.* 2007
			high grade endometrial cancer	no expression in benign tumors	GRANOVSKY-GRISARU *et al.* 2006
SCTR	secretin	↓	pancreas	dominant negative effect of truncated secretin receptor	KORNER *et al.* 2005
V3	vasopressin	↑	corticotroph tumors		DE KEYZER *et al.* 1998

5. COMMERCIAL POTENTIAL OF 7TM RECEPTORS

7TM receptors are one of the most studied targets for present and future therapy targets. More than 30% of all drugs on the market are believed to exert their

clinical action through one of the 7TM receptors family members (HOPKINS, GROOM 2002). About half of the commercially exploited 7TMRs are activated by polypeptide/protein ligands, further over 25% with biogenic amine ligands. But still less than 30% of 7TMRs which are identified in human genome are currently targeted by pharmacological therapies (LAGERSTROM, SCHIOTH 2008).

7TMRs make a very good drug targets, however pharmacological profiling of still existing, orphan receptors and searching for new drug candidates for receptors with established natural ligands have forced scientists and pharmacological industry to come up with highly efficient systems able to efficiently screen 7TMRs of interest against vast compound libraries. The general strategy that enables resourceful characterization of orphan is termed "reverse pharmacology" approach. It employs studied receptor as a bait to fish out its ligand at first step, usually out of biologically active tissue or organ extracts (WILSON *et al.* 1998).

Out of top 20 drug best sellers in the U.S. in 2003, 35% were 7TMR related, and have brought over 16 billion $ of income (SCHLYER, HORUK 2006).

6. REFERENCES

ABDULAEV, N.G. 2003. Building a stage for interhelical play in rhodopsin. Trends Biochem. Sci. 28: 399–402.

ARORA, P., RICKS, T.K., TREJO, J. 2007. Protease-activated receptor signalling, endocytic sorting and dysregulation in cancer. J. Cell Sci. 120: 921–928.

BALISTRERI, C.R., CARUSO, C., GRIMALDI, M.P., LISTI, F., VASTO, S., ORLANDO, V., CAMPAGNA, A.M., LIO, D., CANDORE, G. 2007. CCR5 receptor: biologic and genetic implications in age-related diseases. Ann. N. Y. Acad. Sci. 1100: 162–172.

BARAK, L.S., OAKLEY, R.H., LAPORTE, S.A., CARON, M.G. 2001. Constitutive arrestin-mediated desensitization of a human vasopressin receptor mutant associated with nephrogenic diabetes insipidus. Proc. Natl. Acad. Sci. U. S. A 98: 93–98.

BARZON, L., PACENTI, M., MASI, G., STEFANI, A.L., FINCATI, K., PALU, G. 2005. Loss of growth hormone secretagogue receptor 1a and overexpression of type 1b receptor transcripts in human adrenocortical tumors. Oncology 68: 414–421.

BIEBERMANN, H., KRUDE, H., ELSNER, A., CHUBANOV, V., GUDERMANN, T., GRUTERS, A. 2003. Autosomal-dominant mode of inheritance of a melanocortin-4 receptor mutation in a patient with severe early-onset obesity is due to a dominant-negative effect caused by receptor dimerization. Diabetes 52: 2984–2988.

BOURNE, H.R., SANDERS, D.A., MCCORMICK, F. 1991. The GTPase superfamily: conserved structure and molecular mechanism. Nature 349: 117–127.

BUTLER, A.A., KESTERSON, R.A., KHONG, K., CULLEN, M.J., PELLEYMOUNTER, M.A., DEKONING, J., BAETSCHER, M., CONE, R.D. 2000. A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. Endocrinology 141: 3518–3521.

CADET, P., MANTIONE, K.J., STEFANO, G.B. 2003. Molecular identification and functional expression of mu 3, a novel alternatively spliced variant of the human mu opiate receptor gene. J. Immunol. 170: 5118–5123.

CHEN, A.S., MARSH, D.J., TRUMBAUER, M.E., FRAZIER, E.G., GUAN, X.M., YU, H., ROSENBLUM, C.I., VONGS, A., FENG, Y., CAO, L., METZGER, J.M., STRACK, A.M., CAMACHO, R.E., MELLIN, T.N., NUNES, C.N., MIN, W., FISHER, J., GOPAL-TRUTER, S., MACINTYRE, D.E., CHEN, H.Y., VAN DER PLOEG, L.H. 2000. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nat. Genet. 26: 97–102.

DE KEYZER, Y., RENE, P., BELDJORD, C., LENNE, F., BERTAGNA, X. 1998. Overexpression of vasopressin (V3) and corticotrophin-releasing hormone receptor genes in corticotroph tumours. Clin. Endocrinol. (Oxf) 49: 475–482.

EAVERI, R., BEN-YEHUDAH, A., LORBERBOUM-GALSKI, H. 2004. Surface antigens/receptors for targeted cancer treatment: the GnRH receptor/binding site for targeted adenocarcinoma therapy. Curr. Cancer Drug Targets. 4: 673–687.

FILARDO, E.J., GRAEBER, C.T., QUINN, J.A., RESNICK, M.B., GIRI, D., DELELLIS, R.A., STEINHOFF, M.M., SABO, E. 2006. Distribution of GPR30, a seven

membrane-spanning estrogen receptor, in primary breast cancer and its association with clinicopathologic determinants of tumor progression. Clin. Cancer Res. 12: 6359–6366.

FUESSEL, S., WEIGLE, B., SCHMIDT, U., BARETTON, G., KOCH, R., BACHMANN, M., RIEBER, E.P., WIRTH, M. P., MEYE, A. 2006. Transcript quantification of Dresden G protein-coupled receptor (D-GPCR) in primary prostate cancer tissue pairs. Cancer Lett. 236: 95–104.

GAO, Y., KITAGAWA, K., HIRAMATSU, Y., KIKUCHI, H., ISOBE, T., SHIMADA, M., UCHIDA, C., HATTORI, T., ODA, T., NAKAYAMA, K., NAKAYAMA, K. I., TANAKA, T., KONNO, H., KITAGAWA, M. 2006. Up-regulation of GPR48 induced by down-regulation of p27Kip1 enhances carcinoma cell invasiveness and metastasis. Cancer Res. 66: 11623–11631.

GOVAERTS, C., SRINIVASAN, S., SHAPIRO, A., ZHANG, S., PICARD, F., CLEMENT, K., LUBRANO-BERTHELIER, C., VAISSE, C. 2005. Obesity-associated mutations in the melanocortin 4 receptor provide novel insights into its function. Peptides 26: 909–1919.

GRANOVSKY-GRISARU, S., ZAIDOUN, S., GRISARU, D., YEKEL, Y., PRUS, D., BELLER, U., BAR-SHAVIT, R. 2006. The pattern of Protease Activated Receptor 1 (PAR1) expression in endometrial carcinoma. Gynecol. Oncol. 103: 802–806.

GUGGER, M., WHITE, R., SONG, S., WASER, B., CESCATO, R., RIVIERE, P., REUBI, J.C. 2008. GPR87 is an overexpressed G-protein coupled receptor in squamous cell carcinoma of the lung. Dis. Markers 24: 41–50.

HATA, K., DHAR, D.K., WATANABE, Y., NAKAI, H., HOSHIAI, H. 2007. Expression of metastin and a G-protein-coupled receptor (AXOR12) in epithelial ovarian cancer. Eur. J. Cancer 43 (9): 1452-1459.

HIRASAWA, A., SHIBATA, K., HORIE, K., TAKEI, Y., OBIKA, K., TANAKA, T., MURAMOTO, N., TAKAGAKI, K., YANO, J., TSUJIMOTO, G. 1995. Cloning, functional expression and tissue distribution of human alpha 1c-adrenoceptor splice variants. FEBS Lett. 363: 56–260.

HOPKINS, A.L., GROOM, C.R. 2002. The druggable genome. Nat. Rev. Drug Discov. 1: 727–730.

HUANG, Y., FAN, J., YANG, J., ZHU, G.Z. 2007. Characterization of GPR56 protein and its suppressed expression in human pancreatic cancer cells. Mol. Cell Biochem. 308: 133–139.

JORDA M.A., RAYMAN, N., TAS, M., VERBAKEL, S.E., BATTISTA, N., VAN, L.K., LOWENBERG, B., MACCARRONE, M., DELWEL, R. 2004. The peripheral cannabinoid receptor Cb2, frequently expressed on AML blasts, either induces a neutrophilic differentiation block or confers abnormal migration properties in a ligand-dependent manner. Blood 104: 526–534.

KITAYAMA, J., SHIDA, D., SAKO, A., ISHIKAWA, M., HAMA, K., AOKI, J., ARAI, H., NAGAWA, H. 2004. Over-expression of lysophosphatidic acid receptor-2 in human invasive ductal carcinoma. Breast Cancer Res. 6: R640–R646.

KORNER, M., HAYES, G.M., REHMANN, R., ZIMMERMANN, A., FRIESS, H., MILLER, L.J., REUBI, J.C. 2005. Secretin receptors in normal and diseased human pancreas: marked reduction of receptor binding in ductal neoplasia. Am. J. Pathol. 167: 959–968.

KUO, W.H., CHANG, L.Y., LIU, D.L., HWA, H.L., LIN, J.J., LEE, P.H., CHEN, C.N., LIEN, H.C., YUAN, R.H., SHUN, C.T., CHANG, K.J., HSIEH, F.J. 2007. The interactions between GPR30 and the major biomarkers in infiltrating ductal carcinoma of the breast in an Asian population. Taiwan. J. Obstet. Gynecol. 46: 135–145.

LAGERSTROM, M.C., SCHIOTH, H.B. 2008. Structural diversity of G protein-coupled receptors and significance for drug discovery. Nat. Rev. Drug Discov. 7: 339–357.

LEE, Y.S., POH, L.K., LOKE, K.Y. 2002. A novel melanocortin 3 receptor gene (MC3R) mutation associated with severe obesity. J. Clin. Endocrinol. Metab 87 (3): 1423-1426.

LI, S., HUANG, S., PENG, S.B. 2005. Overexpression of G protein-coupled receptors in cancer cells: involvement in tumor progression. Int. J. Oncol. 27: 1329–1339.

LIN-SU, K., WAJNRAJCH, M.P. 2002. Growth Hormone Releasing Hormone (GHRH) and the GHRH Receptor. Rev. Endocr. Metab Disord. 3: 313–323.

MAGNUSSON, C., EHRNSTROM, R., OLSEN, J., SJOLANDER, A. 2007. An increased expression of cysteinyl leukotriene 2 receptor in colorectal adenocarcinomas correlates with high differentiation. Cancer Res. 67: 9190–9198.

MAYO, K.E., HAMMER, R.E., SWANSON, L.W., BRINSTER, R.L., ROSENFELD, M.G., EVANS, R.M. 1988. Dramatic pituitary hyperplasia in transgenic mice expressing a human growth hormone-releasing factor gene. Mol. Endocrinol. 2: 606–612.

MAZZUCO, T.L., CHABRE, O., FEIGE, J.J., THOMAS, M. 2007. Aberrant GPCR expression is a sufficient genetic event to trigger adrenocortical tumorigenesis. Mol. Cell Endocrinol. 265-266: 23–28.

MCCLANAHAN, T., KOSEOGLU, S., SMITH, K., GREIN, J., GUSTAFSON, E., BLACK, S., KIRSCHMEIER, P., SAMATAR, A.A. 2006. Identification of overexpression of orphan G protein-coupled receptor GPR49 in human colon and ovarian primary tumors. Cancer Biol. Ther. 5: 419–426.

MERLE, P., KIM, M., HERRMANN, M., GUPTE, A., LEFRANCOIS, L., CALIFANO, S., TREPO, C., TANAKA, S., VITVITSKI, L., DE LA, MONTE S., WANDS, J.R. 2005. Oncogenic role of the frizzled-7/beta-catenin pathway in hepatocellular carcinoma. J. Hepatol. 43: 854–862.

NELSON, C.P., CHALLISS, R.A. 2007. "Phenotypic" pharmacology: the influence of cellular environment on G protein-coupled receptor antagonist and inverse agonist pharmacology. Biochem Pharmacol. 73: 737–751.

NICOLLE, G., COMPERAT, E., NICOLAIEW, N., CANCEL-TASSIN, G., CUSSENOT, O. 2007. Metastin (KISS-1) and metastin-coupled receptor (GPR54) expression in transitional cell carcinoma of the bladder. Ann. Oncol. 18: 605–607.

PARMIGIANI, R.B., MAGALHAES, G.S., GALANTE, P.A., MANZINI, C.V., CAMARGO, A.A., MALNIC, B. 2004. A novel human G protein-coupled receptor is over-expressed in prostate cancer. Genet. Mol. Res. 3: 521–531.

PIERCE, K.L., LUTTRELL, L.M., LEFKOWITZ, R.J. 2001. New mechanisms in heptahelical receptor signaling to mitogen activated protein kinase cascades. Oncogene 20: 1532–1539.

PIERCE, K.L., PREMONT, R.T., LEFKOWITZ, R.J. 2002. Seven-transmembrane receptors. Nat. Rev. Mol. Cell Biol. 3: 639–650.

RACHED, M., BURONFOSSE, A., BEGEOT, M., PENHOAT, A. 2004. Inactivation and intracellular retention of the human I183N mutated melanocortin 3 receptor associated with obesity. Biochim. Biophys. Acta 1689: 229–234.

RINGEL, M.D., HARDY, E., BERNET, V.J., BURCH, H.B., SCHUPPERT, F., BURMAN, K.D., SAJI, M. 2002. Metastin receptor is overexpressed in papillary thyroid cancer and activates MAP kinase in thyroid cancer cells. J. Clin. Endocrinol. Metab 87: 2399.

SANCHEZ-LAORDEN, B.L., JIMENEZ-CERVANTES, C., GARCIA-BORRON, J.C. 2007. Regulation of human melanocortin 1 receptor signaling and trafficking by Thr-308 and Ser-316 and its alteration in variant alleles associated with red hair and skin cancer. J. Biol. Chem. 282: 3241–3251.

SCHLYER, S., HORUK, R. 2006. I want a new drug: G-protein-coupled receptors in drug development. Drug Discov. Today 11: 481–493.

SCHONEBERG, T., SCHULZ, A., BIEBERMANN, H., HERMSDORF, T., ROMPLER, H., SANGKUHL, K. 2004. Mutant G-protein-coupled receptors as a cause of human diseases. Pharmacol. Ther. 104: 173–206.

SHASHIDHAR, S., LORENTE, G., NAGAVARAPU, U., NELSON, A., KUO, J., CUMMINS, J., NIKOLICH, K., URFER, R., FOEHR, E.D. 2005. GPR56 is a GPCR that is overexpressed in gliomas and functions in tumor cell adhesion. Oncogene 24: 1673–1682.

SODHI, A., MONTANER, S., GUTKIND, J.S. 2004. Does dysregulated expression of a deregulated viral GPCR trigger Kaposi's sarcomagenesis? FASEB J. 18: 422–427.

SPIEGEL, A.M. 1997. Inborn errors of signal transduction: mutations in G proteins and G protein-coupled receptors as a cause of disease. J. Inherit. Metab Dis. 20: 113–121.

SPIEGEL, A.M. 2000. G protein defects in signal transduction. Horm. Res. 53: 17–22.

SPIEGEL, A.M., WEINSTEIN, L.S. 2004. Inherited diseases involving g proteins and g protein-coupled receptors. Annu. Rev. Med. 55: 27–39.

STOJANOVIC, A., HWA, J. 2002. Rhodopsin and retinitis pigmentosa: shedding light on structure and function. Receptors. Channels 8: 33–50.

TAO, Y.X. 2005. Molecular mechanisms of the neural melanocortin receptor dysfunction in severe early onset obesity. Mol. Cell Endocrinol. 239: 1–14.

TAO, Y.X. 2006. Inactivating mutations of G protein-coupled receptors and diseases: structure-function insights and therapeutic implications. Pharmacol. Ther. 111: 949–973.

TAO, Y.X., SEGALOFF, D.L. 2004. Functional characterization of melanocortin-3 receptor variants identify a loss-of-function mutation involving an amino acid critical for G protein-coupled receptor activation. J. Clin. Endocrinol. Metab 89: 3936–3942.

VISCHER, H.F., LEURS, R., SMIT, M.J. 2006. HCMV-encoded G-protein-coupled receptors as constitutively active modulators of cellular signaling networks. Trends Pharmacol. Sci. 27: 56–63.

WILSON, S., BERGSMA, D.J., CHAMBERS, J.K., MUIR, A.I., FANTOM, K.G., ELLIS, C., MURDOCK, P.R., HERRITY, N.C., STADEL, J.M. 1998. Orphan G-protein-coupled receptors: the next generation of drug targets? Br. J. Pharmacol. 125: 1387–1392.

YANG, M., ZHONG, W.W., SRIVASTAVA, N., SLAVIN, A., YANG, J., HOEY, T., AN, S. 2005. G protein-coupled lysophosphatidic acid receptors stimulate proliferation of colon cancer cells through the {beta}-catenin pathway. Proc. Natl. Acad. Sci. U. S. A 102: 6027–6032.

Acta Universitatis Lodziensis
———— 2010 ————
Folia Biologica et Oecologica 6: 49–59
(Acta Univ. Lodz, Folia Biol. Oecol.)

EWA FORMA[1], MAGDALENA BERNACIAK[1], HANNA ROMANOWICZ-MAKOWSKA[2] & MAGDALENA BRYŚ[1]

[1]Department of Cytobiochemistry, University of Łódź, Pomorska 141/143, 90-236 Łódź, Poland, eforma@biol.uni.lodz.pl
[2]Department of Clinical Pathomorphology, Polish Mother's Memorial Hospital, Research Institute, Rzgowska 281/289, 93-338 Łódź, Poland

RT-PCR ANALYSIS OF TOPBP1 GENE EXPRESSION IN HEREDITARY BREAST CANCER

Abstract: Hereditary predisposition to breast cancer determined in large part by loss of function mutations in one of two genes *BRCA1* and *BRCA2*. Besides BRCA1 and BRCA2 other genes are also likely to be involved in hereditary predisposition to breast cancer. TopBP1 protein is involved in DNA replication, DNA damage checkpoint response and transcriptional regulation. Expression of *TopBP1* gene at the mRNA level was analyzed by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) in 94 samples of hereditary breast cancer. Analysis of *TopBP*1 mRNA level showed that expression of *TopBP1* is significantly downregulated in poorly differentiated breast cancer (grade III according Bloom-Richardson system ($P<0.05$).

Key words: TopBP1, gene expression, RT-PCR, hereditary breast cancer

1. INTRODUCTION

Hereditary breast and ovarian cancer syndrome is an inherited cancer susceptibility syndrome. The hallmarks of this syndrome are multiple family members with breast or/and ovarian cancer, the presence of both breast and ovarian cancer in a single individual, and early age of breast cancer onset (LU *et al.* 2009).

DOI: 10.2478/v10107-009-0006-1

Breast and ovarian cancers are among the most common malignancies of women in Western countries. About 5 – 10% of the cases are considered familial, and 40 – 50% of them can currently be explained by mutations in two main susceptibility genes, *BRCA1* and *BRCA2*. Of the remaining cases no more than 5% are caused by defects in other studied genes, such as *TP53*, *PTEN*, *ATM* and *CHK2* (KARPPINEN *et al.* 2006; EASTON 1999). TopBP1 (topoisomerase IIβ binding protein 1) displays structural as well as functional similarities with BRCA1, and both proteins have been suggested to function partly in the same cellular processes (KARPPINEN *et al.* 2006). Based on its biological significance KARPPINEN *et al.* (2006) suggested that TopBP1 is a plausible susceptibility gene for hereditary breast and/or ovarian cancer. Aberrant expression of *TopBP1* may be involved in the deregulation of processes controlled by this protein and have pathological consequences. The aim of this study was to investigate the expression of *TopBP1* at the mRNA level in hereditary breast cancer.

2. MATERIALS AND METHODS

2.1. Sample collection

Samples of 94 hereditary breast cancers were obtained from patients (age range 28 – 67 years) undergoing surgery for breast neoplasms in Polish Mather's Memorial Hospital, Poland. The inclusion criteria were: (1) at least one first-degree relative with breast cancer, regardless of age, or (2) breast cancer diagnosed below 40 years of age. None of the patient received neoadjuvant endocrine therapy, chemotherapy and radiotherapy. The pathological evaluation report was obtained for each patient (Table 1). Immediately after resection, samples of breast cancer tissue were fixed in 10% neutral buffered formalin and embedded in paraffin blocks following standard histological protocols. For our studies tissue sections were cut from the blocks with a microtome blade, extra paraffin was removed, and tissue sections was placed in a 1.5-ml microcentrifuge tube.

Table 1. Characteristics of patients and tumor samples

Characteristics	Number of patients
Age at diagnosis	
range	28 - 67
mean ± SD	55.3 ± 8.5
Type of cancer	
ductal carcinoma	79
lobular carcinoma	13
tubular carcinoma	2
Tumor grade according to Bloom-Richardson system	
I	9
II	64
III	21
Lymph node metastasis	
No	52
Yes	42
Menopausal status	
premenopausal	55
postmenopausal	39
ER status	
Negative	46
Positive	48
PR status	
Negative	42
Positive	52

2.2. Total RNA extraction and cDNA synthesis

Xylene deparaffinization: Sections were deparaffinized by two rinses in xylene for 10 min at room temperature with shaking, followed by centrifugations at

room temperature for 5 min at 12,000g. After deparaffinization, we introduced a rehydratation step (rinsing in 100% ethanol, 85%, 70% ethanol, all prepared with DEPC treated dH_2O, for 5 min). The tissue was collected by centrifugation at 12,000g for 5 min. After the final wash, alcohol was aspirated and the tissue pellets were resuspended in 500 µl of digestion buffer (10 mM NaCl, 500 mM Tris-HCl, pH 8.0, 25 mM EDTA, 1% SDS) and 1 mg/ml proteinase K was added. Sections were incubated at 45°C overnight. Prior to RNA purification, in same samples we inactivated proteinase K at 97°C for 10 min. The digested samples were extracted using TRI Reagent (Sigma Aldrich, USA) according to manufacturer's protocol. RNA was eluted in 20 µl RNase-free water, quantified by spectrophotometry at 260 nm and stored at -20°C. RNA with a 260/280 nm ratio in range 1.8 – 2.0 was considered high quality. First-strand cDNA was synthesized from each RNA pool using PCR Kit ver. 3.0 (Takara Bio Inc. Japan) according to the manufacturer's instructions. Briefly, 1 µg RNA was combined with 2.5 pmol of oligo dT-adapter primer, 4 µl of 25 mM $MgCl_2$, 2 µl 10 x RNA PCR buffer, 2 µl of 10 mM dNTP mixture, 20 units of RNase inhibitor, 5 units of AMV Reverse Transcriptase XL, and RNase-free water to total volume of 20 µl. The reaction took place at 42°C for 30 min, followed by 95°C for 5 min and 5°C for 5 min in a GeneAmp PCR System 9700 (Perkin-Elmer Co, USA). cDNA was stored at -20°C.

2.3. RT-PCR

One microliters of cDNA sample was used as a substrate for PCR reaction in a 20 µl volume with 1 µM of forward and reverse primers, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 2.0 µl 10 x PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl) and 1 units Taq polymerase (Takara Bio Inc., Japan). Specific oligonucleotide primers for *TopBP1* were as follows F: 5'GCTTCATCGCTCCTACCTTG3', R: 5'TTCCACCCACTAAATGCTCC3'. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) with primer sequences F: 5'GAAGGTGAAGGTCGGACTC3', R: 5'GAAGATGGTGATGGGATTTC 3' was used as an endogenous control for the PCR amplifications. PCR reaction was carried out as follows: initial denaturation of 4 min at 94°C) followed by 40 cycles of 1 min at 94°C, 30 s at 53°C for *TopBP1* and

60°C for *GAPDH* and 30 s at 72°C, final extension step of 10 min at 72°C. Negative control and already amplified cDNA were included in all the PCR amplifications. The size of amplified fragments was 210 and 225 bp for *TopBP1* and *GAPDH*, respectively. After amplification 10 µl of PCR products were combined with 2 µl gel loading buffer and the mixture was separated on a 8% polyacrylamide gel. The gel was silver stained. For qualitative and quantitative analysis of silver nitrate stained gels video densitometr (Biotec-Fischer, Germany) with the software program Gel-Pro® Analyzer 3.0 (Media Cybemetics, USA) was used. All RT-PCR reactions were repeated 2 times for each samples. The integrated optical density (IOD) of the bands in a digitalized picture was measured. *TopBP1* gene expression was determined as the ratio of *TopBP1* to *GAPDH*.

2.4. Statistical analysis

All data are presented as mean ± SEM. Because results obtained from RT-PCR were not normally distributed (Kolmogorov-Smirnov test), therefore nonparametric Mann-Whitney U-test (for two categories) and the Kruskal-Wallis test with post hoc multiple comparisons (for three categories) were used. Statistical significance was designated at $P<0.05$.

3. RESULTS

The expression of *TopBP1* in hereditary breast cancer was estimated at the mRNA level by semiquantitative RT-PCR analysis with *GAPDH* applied as a reference gene. Representative electropherogram of *TopBP1* mRNA expression in hereditary breast cancer with *GAPDH* mRNA as standard is presented in Fig. 1. Expression of *TopBP1* gene at the mRNA level was observed in 81 of 94 (86.2%) hereditary breast cancer samples. The comparison of transcript level of *TopBP1* gene with clinicopathological parameters of tumors is shown in Table 2, where ratio of integrated optical density *TopBP1* to *GAPDH* representing the mean level of mRNA, are used for statistical analysis. Analysis of *TopBP1* mRNA level showed significantly lower expression of *TopBP1* in the poorly differentiated hereditary breast cancer (grade III according to Bloom-Richardson scale) in comparison with

moderately and well-differentiated cancer (P<0.05). No statistically significant differences occured between *TopBP1* mRNA level in grade I and II tumors (Fig. 2).

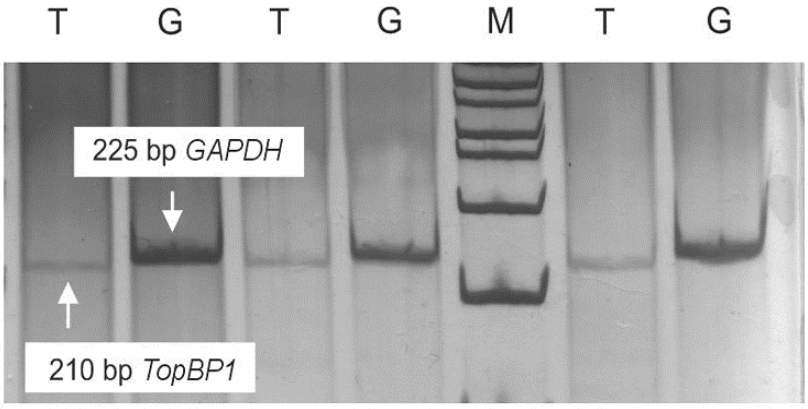

Fig. 1. Representative electropherogram of *TopBP1* mRNA expression in hereditary breast cancers with *GAPDH* mRNA as standard (M molecular weight markers). Amplification products were separated on the 8% polyacrylamide gel. T – *TopBP1*; G – *GAPDH*; M – molecular weight markers.

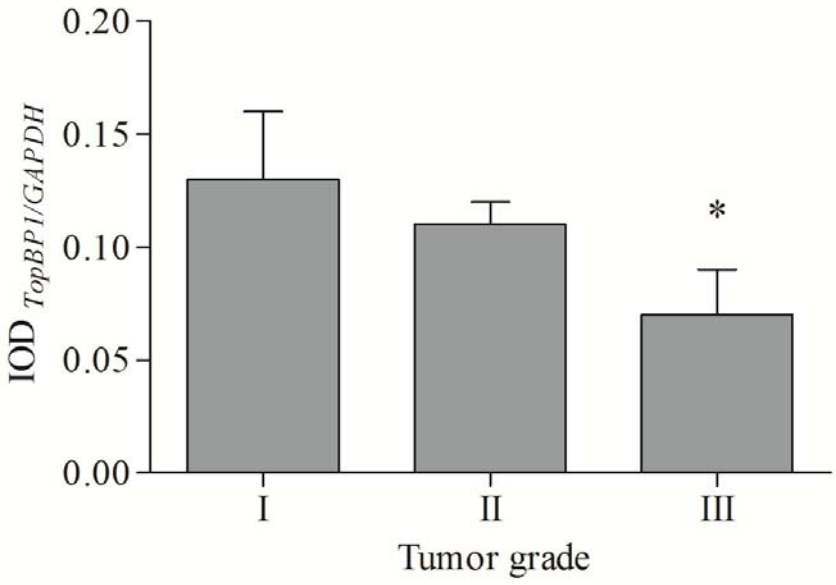

Fig. 2. Expression of *TopBP1* mRNA in hereditary breast cancers in relation to tumor grade. Asterisk indicates significant differences at P<0.05.

There were no significant associations between level of TopBP1 mRNA expression and other clinicopathological parameters, such as estrogen and progesterone receptor status, appearance of metastasis in the axillary lymph nodes and type of cancer.

Table 2. Comparison of the transcript levels of *TopBP1* gene with clinicopathological parameters of the tumors

Clinicopathological features (N)	Semiquantitative RT-PCR ($IOD_{TopBP1/GAPDH}$)	P
Type of cancer		
ductal carcinoma (79)	0.14 ± 0.01	
lobular carcinoma (13)	0.12 ± 0.02	0.45
Tumor grade		
I (9)	0.13 ± 0.03	
II (64)	0.11 ± 0.01	
III (21)	0.07 ± 0.02	0.60
Lymph node status		
No (52)	0.13 ± 0.01	
Yes (42)	0.11 ± 0.01	0.67
ER status		
Negative (46)	0.12 ± 0.01	
Positive (48)	0.10 ± 0.01	0.64
PR status		
Negative (42)	0.11 ± 0.01	
Positive (52)	0.13 ± 0.01	0.20

4. DISCUSSION

TopBP1 protein plays a key role in various aspects of DNA metabolism. It is required for the initiation of DNA replication, the maintenance of DNA replication forks when replication is stalled and for DNA damage signaling and checkpoints

(JEON *et al*. 2007; KIM *et al*., 2005; KUMAGAI *et al*. 2006; LIU *et al* 2006; MORISHIMA *et al*. 2007; SCHMIDT *et al* 2008; YAMMANE *et al.* 2003). TopBP1 is also involved in the process of mitosis in somatic cells and during meiotic recombination in germ cells (PERERA *et al.* 2004; REINI *et al.* 2004). Most of TopBP1 does not colocalize at site of ongoing DNA replication in irradiated cells but is relocalized to stalled replication forks upon DNA damage. The involvement of TopBP1 in DNA replication is supported by the demonstration that incubation of an antibody against the sixth BRCT motif of TopBP1 inhibits replicative DNA synthesis in a in vitro HeLa cell nucleus replication assay (KIM *et al.* 2004; MAKINEMI *et al*. 2001). TopBP1 can interact with human polymerase ε, checkpoint protein Rad9, Miz-1, E2F1, human papilliomavirus type 16 (HPV16) transcription/replication factor E2 (BONER *et al*. 2002; DONALDSON *et al.* 2007; DELACROIX *et al.* 2007; HEROLD *et al.* 2002; LIU *et al.* 2003, 2004, 2006; MAKINIEMI *et al.* 2001) . TopBP1 also participates in ATR activation in ATRIP-dependent manner (BURROWS, ELLEDGE 2008; CIMPRICH, CORTEZ 2008; KUMAGAI *et al.* 2006; LEE *et al.* 2007). In addition to control DNA replication, TopBP1 is also required for cell survival. Inhibition of TopBP1 expression induces apoptosis. TopBP1 is involved in several important aspects of regulation cell growth (LIU *et al.* 2003; YAMANE *et al.* 2002). The aim of this study was investigate the transcript level of *TopBP1* gene. Analysis of *TopBP1* expression shown that tumor progression is accompanied by a decrease of *TopBP1* mRNA level. Expression of TopBP1 is regulated by Rb/E2F1 and is induced when cells enter into S phase (LIU *et al.* 2004, 2009; YOSHIDA, INOUE 2004). Therefore, disruption of Rb/E2F1 pathway can lead to overexpression of TopBP1 protein in breast cancers. In the other hand, TopBP1 regulates activity of E2F1 and overexpression of TopBP1 suppressed E2F1 transcriptional activity (LIU *et al.* 2003, 2004). Thus, decreased transcriptional activity of E2F1 by TopBP1 can repress the expression of E2F1 target genes, including *TopBP1*. In the other hand, downregulation of *TopBP1* mRNA expression may be caused by aberration expression of E2F1. Many studies have found that expression of E2F1 protein in breast cancer tended to decreased as the grade

increase (HO *et al.* 2001; KWON *et al.* 2009). However, regulation of *TopBP*1 expression will need to be studied.

Acknowledgements

This work was supported by the grant of Integrated Operational Program for Regional Development, ZPORR (grant no. 10/S-UŁ/2009). The project was also co-founded by the European Union under the European Social Fund, POKL "Human – Best Investment!".

5. REFERENCES

BONER, W., TAYLOR, E.R., TSIRIMONAKI, E., YAMANE, K., CAMPO, M.S., MORGAN, I.M. 2002. A functional interaction between the human papillomavirus 16 transcription/replication factor E2 and the DNA damage response protein TopBP1. J. Biol. Chem. 277: 22297–22303.

BURROWS, A.E., ELLEDGE, S. 2008. How ATR turns on: TopBP1 goes on ATRIP with ATR. Genes Dev. 22: 1416–1421.

CIMPRICH, K.A., CORTEZ, D. 2008. ATR: an essential regulator of genome integrity. Nature Rev. Mol. Cell Biol. 9: 616–627.

DELACROIX, S., WAGNER, J.M., KOBAYASHI, M., YAMAMOTO, K.I., KARNITZ, L.M. 2007. The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. Genes Dev. 21: 1472–1477.

DONALDSON, M.M., BONER, W., MORGAN, I.M. 2007. TopBP1 regulates human papillomavirus type 16 E2 interaction with chromatin. J. Virol. 81: 4338–4342.

EASTON, D.F. 1999. How many more breast cancer predisposition genes are there? Breast Cancer Res. 1: 14–17.

HEROLD, S., WANZEL, M., BEUGER, V., FROHME, C., BEUL, D., HILLUKKALA, T., SYVAOJA, J., SALUZ, H.P., HAENEL, F., EILERS, M. 2002. Negative regulation of the mammalian UV response by Myc through association with Miz-1. Mol. Cell 10: 509–521.

HO, G.H., CALVANO, J.E., BISOGNA, M., VAN ZEE, K.J. 2001. Expression of E2F-1 and E2F-4 is reduced in primary and metastatic breast carcinomas. Breast Cancer Res. Treat. 69: 115–122.

JEON, Y., LEE, K.Y., KO, M.J., LEE, Y.S., KANG, S., HWANG. D.S. 2007. Human TopBP1 participates in cyclinE/CDK2 activation and preinitiation complex assembly during G1/S transition. J. Biol. Chem. 282: 14882–14890.

KARPPINEN, S.M., ERKKO, H., REINI, K., POSPIECH, H., HEIKKINEN, K., RAPAKKO, K., SYVAOJA, J.E., WINQVIST, R. 2006. Identification of a common polymorphism in the TopBP1 gene associated with hereditary susceptibility to breast and ovarian cancer. Eur. J. Cancer. 42: 2647–2652.

KIM, J.E., MCAVOY, S.A., SMITH, D.I., CHEN, J. 2005. Human TopBP1 ensures genome integrity during normal S phase. Mol. Cel. Biol. 25: 10907–10915.

KUMAGAI, A., LEE, J., YOO, H.Y., DUNPHY, W.G. 2006. TopBP1 activates the ATR-ATRIP complex. Cell 124: 943–955.

KWON, M.J., NAM, E.S., CHO, S.J., PARK, H.R., SHIN, H.S., PARK, J.H., PARK, C.H., LEE, W.J. 2009. E2F1 expression predicts outcome in Korean women who undergo surgery for breast cancer. Ann. Surg. Oncol. 17: 564–71.

LEE, J., KUMAGAI, A., DUNPHY, W.G. 2007. The Rad9-Hus1-Rad-1 checkpoint clamp regulates interaction of TopBP1 with ATR. J. Biol. Chem. 282: 28036–28044.

LIU, K., LIN, F.T., RUPPERT, J.M., LIN, W.C. 2003. Regulation of E2F1 by BRCT domain-containing protein TopBP1. Mol. Cell. Biol. 23: 3287–3304.

LIU, K., LUO, Y., LIN, F.T., LIN, W.C. 2004. TopBP1 recruits BRG1/BRM to repress E2F1-induced apoptosis, a novel pRb-independent and E2F1-specific control for cell survival. Genes Dev. 21: 1472–1477.

LIU, K., PAIK, J.C., WANG, B., LIN, F.T., LIN. W.C. 2006 Regulation of TopBP1 oligomerization by Akt/PKB for cell survival. EMBO J. 25: 4795–4807.

LIU, K., BELLAM, N., LIN, H.Y., WANG, B., STOCKARD, C.R., GEIZZLE, W.E., LIN, W.C. 2009. Regulation of p53 by TopBP1: a potential mechanism for p53 inactivation in cancer. Mol. Cell. Biol. 29: 2673–2693.

LU, K., KAUFF, N., POWELL, B., CHEN, L.M., CASS, I., LANCASTER, J. 2009. Hereditary breast and ovarian syndrome, ACOG Practice Bull. Gynecol. Oncol. 113: 6–11.

MAKINIEMI, M., HILLUKKALA, T., TUUSA, J., REINI, K., VAARA, M., HUANG, D., POSPIECH, H., MAJURI, I., WESTERLING, T., MÄKELÄ, T.P., SYVÄOJA, J.E. 2001. BRCT domain-containing protein TopBP1 functions in DNA replication and damage response. J. Biol. Chem. 276: 30099–30406.

MORISHIMA, K.I., SAKAMOTO, S., KOBAYASHI, J., IZUMI, H., SUDA, T., MATSUMOTO, Y., TAUCHI, H., IDE, H., KOMATSU, K., MATSUURA, S. 2007. TopBP1 associates with NBS1 and is involved in homologous recombination repair, Biochem. Biophys. Res. Commun. 362: 872–879.

PERERA, D., PEREZ-HIDALGO, L., MOENS, P.B., REINI, K., LAKIN, N., SYVÄOJA, J.E., SAN-SEGUNDO, P.A., FREIRE, R. 2004. TopBp1 and ATR colocalization at meiotic chromosomes: role of TopBP1/Cut5 in the meiotic recombination checkpoint, Mol. Biol. Cell 15: 1568–1579.

REINI, K., UITTO, L., PERERA, D., MOENS, P.B., FREIRE, R., SYVÄOJA, J.E. 2004. TopBP1 localises to centrosomes in mitosis and chromosome cores in meiosis, Chromosoma 112: 323–330.

SCHMIDT, U., WOLLMANN, Y., FRANKE, C., GROSSE, F., SALUZ, H.P., HANEL, F. 2008. Characterization of the interaction between the human DNA topoisomerase IIβ-binding protein 1 (TopBP1) and the cell division cycle protein 45 (Cdc45). Biochem. J. 409: 169–177.

YAMANE, K., CHEN, J., KINSELLA, T.J. 2003. Both DNA topoisomerase II-binding protein 1 and BRCA1 regulate the G_2-M cell cycle checkpoint. Cancer Res. 63: 3049–3053.

YAMANE, K., WU, X., CHEN, J. 2002. A DNA damage-regulated BRCT-containing protein, TopBP1, is required for cell survival. Mol. Cell. Biol. 22: 555–566.

YOSHIDA, K., INOUE, I. 2004. Expression of MCM10 and TopBP1 is regulated by cell proliferation and UV irradiation via the E2F1 transcription factor. Oncogene 23: 6250–6260.

Acta Universitatis Lodziensis
———— 2010 ————
Folia Biologica et Oecologica 6: 61–68
(Acta Univ. Lodz, Folia Biol. Oecol.)

EWA FORMA, MAGDALENA BERNACIAK & MAGDALENA BRYŚ

Department of Cytobiochemistry, University of Łódź, Pomorska 141/143, 90-236 Łódź, Poland, eforma@biol.uni.lodz.pl

BREAST CANCER IN ART PAINTING

Abstract: Breast cancer is an emotive cancer. It is a disease that affects a visible sexual organ and it is the commonest single cause of death of women between 40 and 60 years of age. Nevertheless, this type of cancer was infrequently depicted in art paintings. In this article the themes from the breast cancer in famous art paintings are discussed.

Key words: breast cancer, art paintings, famous painters

1. INTRODUCTION

Science is a systematic knowledge of nature and the physical world, derived from observation and experimentation. Art, on the other hand is the free and creative way of making or doing things that have form and beauty and is based on perceptions and intuition. Artists used their talent in order to depict with the greatest detail possible the human anatomy and later the histology of tissues, helping scientists comprehend better the world and human nature. From the other side, scientific images coming from observation or experimentation can constitute art since they can have form and beauty (BONJER, BRUINING 1999; BALJET 2000; BATISTATOU, CHARALABOPOULOS 2005; KARKABI, CASTEL 2006).

Medicine and art have long had a close connection. In fact, the practice of medicine in previous centuries was mostly considered to be art. Conversely, the study of human anatomy in the Renaissance period was an essential component of

DOI: 10.2478/v10107-009-0007-0

an artist's training, as exemplified by the likes of Leonardo Da Vinci (VAIDYA 2007a; EKMEKTZOGLOU *et al*. 2009).

Breasts had an intense symbolism for all women since ancient times. Breasts were considered the "seat for fertility", which can explain why multimammillary goddesses were created to epitomise fertility. The breasts had always been considered as a symbol of beauty in women and this is reflected in many works of painters, poets, etc (SAKORAFAS, SAFIOLEAS 2009).

Breast cancer has probably been prevalent since antiquity, but the search for historical evidence is difficult for lack of verifiable descriptions or graphic representations of the disease (DAHLGREN 2003).

From ancient Egypt until the present day, breast cancer has been a field of ongoing research. Breast cancer is an emotive cancer. It is a disease that affects a visible sexual organ and is the commonest single cause of death of women between 40 and 60 years of age. However, this type of cancer was infrequently depicted in historical art. In most of recorded history, cultural norms have dictated that the breast is unexposed to protect modesty. Therefore, only a doctor or an artist painting nude models would have had the opportunity to see any clinical signs of breast cancer. Nonetheless, in the few pieces of art in which breast cancer has inadvertently been the subject of artistic creation, interpretations have been controversial (VAIDYA 2007a; EKMEKTZOGLOU *et al*. 2009).

In our article we demonstrated the integration of art and science. We focused on how disease, specifically breast cancer, has influenced the art and vice versa.

2. BREAST ABNORMALITIES IN FAMOUS PAINTINGS

One of the most famous paintings that depict breast cancer is the oil-on-panel piece by Raphael Sanzio *La Fornarina* (Galleria Nazionale d'Arte Antica in Palazzo Barberini, Rome, Italy, oil on panel, 85x60 cm, circa 1516). The portrait is, perhaps the first, graphic evidence of breast cancer.

The woman in the portrait is thought to have been Raphael's lover, La Fornarina. Her name, according to an annotation in Giorgio Vasari's 16th century biography of Raphael, was Margherita. Her father was recorded in a Roman census

as Francesco Luti from Siena who lived at via del Governo Vecchio 48 (ESPINEL 2002; GROSS 2004).

Raphael Sanzio was the first to depict signs of breast cancer in his painting of Fornarina. Despite La Fornarina's position, turned a quarter away from the viewer, her breasts can be compared. They differ from one another in appearance. The left breast is enlarged and deformed. There is a bulge in the breast that, beginning inward from the axilla and curving horizontally to the right, slopes gently toward the nipple. This bulge seems to be a mass, oval in shape, puckering just above the tip of La Fornarina's index finger. Below this bulge, the breast has a wide retraction. The skin over the breast is discoloured. A blue hue and duskiness extends over the mass and the aureole and touches the nipple. Just inside the axilla, a slight protuberance suggests a fat pad, or perhaps a lymph node. La Fornarina's left arm appears larger than would be expected for the perspective of her position. La Fornarina presents signs that not only are diagnostic but also allow staging of the malignancy (ESPINEL 2002; CZEIZEL 2003; GROSS 2004).

La Fornarina's deformation might be a depiction of five clinical signs: a mass, a retraction, skin discoloration, a possible lymph node and arm swelling. The discoloration suggests skin invasion, perhaps into the dermatic lymphatics and the arm swelling, lymphoedema. La Fornarina's signs are compatible with the diagnosis of cancer of the left breast, at an advanced stage (ESPINEL 2002).

However, BAUM (2003) debated these claims, giving clear and plausible explanations for each of the characteristics described by ESPINEL: first, the position of the index finger is a classic pose that can easily create a dimple even in a normal breast and second, breast cancer never gives rise to a bluish tinge.

Another example of breast cancer visualization is 15th century fresco in the church of Santa Maria della Grazia in Milan, Italy, that houses Leonardo da Vinci's *Last Supper*. It presents a beautiful woman holding a child and she appears to have an ulcerating cancer in the upper outer quadrant of her right breast. This fresco is actually the depiction of The Madonna Delivers Milan From the Plague that was commissioned by the Domenicans and painted in 1631 by Il Cerano. However, the

tumour is clearly in the breast rather than the axilla so it could not have been a plague bubo (VAIDYA 2007b).

In Prado Museum in Madrid, Spain, several Ruben's paintings are shown. Among these pictures, *The Three Graces* (oil on canvas, 87x124 cm) is one of the most remarkable. The right-hand side Grace has a tumor in its external upper quadrant of the left breast which extends up to the left axila (GRAU *et al*. 2001a; GROSS 2004).

The tumor between the left breast and the left axila is exofitic, irregular, with reddness of rounding skin suggesting inflammatory component. Such visual aspect which addresses us to breast cancer was painted in the external upper quadrant of the breast where this cancer most frequently appears (GRAU *et al*. 2001a; GRAU *et al*. 2001b).

There are two other paintings in the Museum of the Prado (Madrid) that also draw our attention. One of them is *Orpheus and Euridice* and the other one is *Diana and her nymphs pursued by satyrs*, they also show abnormalities suggesting the earlier stages of the breast cancer. In *Orpheus and Euridice* the female figure who represents Euridice, also shows an abnormality in its left breast. In this case, also in the external upper quadrant there is an in-depth into the skin surface, without breast retraction. In *Diana and her nymphs pursued by satyrs* there is a woman at the front of the picture, her hands up shows like in *The three Graces* a breast which was painted obliquely from the stand point of the observer. In this model it is evident that there is a dimpling of the skin in the external upper quadrant of the left breast, together with total retraction of the breast both outwards and upwards. Observing the above-mentioned images: firstly *Orpheus and Euridice*, secondly *Diana and her nymphs pursued by satyrs*, and thirdly *The three Graces*, we can find the typical evolution of a locally advanced breast cancer (GRAU *et al*. 2001a, 2001b; GROSS 2004).

In the Royal Library of Brussels, Belgium, there is a copy of an engraving performed by Lucas Vorsterman of a Ruben's painting after Titian which shows a young woman dressed in a fur coat and a hat. Belonging to the realism style, both Ruben and Titian painted things exactly as they saw them. This allows us to

recognise many aspects of the quotidian life style in the 16th and 17th centuries but also, to recognise many clinical signs that physicians nowadays can correlate with certain specific diseases (GRAU, ESTRACH 2008).

The woman appearing in the picture has two lumps in the upper external quadrant of her right breast. These superficial tumours caused bulges in the breast contour and retraction of overlaying skin, suggesting a direct extension of a deep tumour into the skin. The nipple and the entire breast show a retraction to the right axila. The lumps are there to call the attention of the viewer and this is when the woman has partially removed her fur in order to show the lumps. The position of the model suggests that the artist wants to draw our attention to the medical problem rather than to the beauty of the breast. It is not know either, the outcome and the cause of the death of the model. Nevertheless, the clinical aspect, especially the breast lumps with retraction to the axila, supports the diagnosis of breast cancer. Of course, we have to assume that both, Rubens and Vorsterman did not change the original aspect of the model's breast and that Titian painted a real model and not a virtual composition of different body parts of more than one model. As far as we know, Titian's painting was a portrait of the beautiful Venetian courtesan he painted during the 16th century. Rubens made different versions of Titian's composition but Titian's original is lost. One of these copies was engraved by Lucas Vorsterman, which not only allows us to enjoy the beauty of the model, but also to recognise a terrible tumour in her breast (BELKIN *et al*. 2004; GRAU, ESTRACH 2008).

Rembrandt Harmenszoon van Rijn was arguably without peer as a portrait artist, and ranks among the master painters of all time. Therefore it is not surprising that there was great and justifiable interest when it was suggested that Rembrandt's famous painting of *Bathsheba at her toilet* (Louvre, Paris, oil on canvas, 142x142 cm) showed clinical signs of advanced left breast carcinoma based upon skin discolouration, distortion, axillary fullness and peau d'orange appearance (GRECO 1970; BAITHWAITE, SHUGG, 1983; GROSS 2004; HAYAKAWA *et al*. 2005).

It is generally accepted that Bathsheba was painted in 1654 modeled by Hendrickje Stoffels. In 1654, Hendrickje was 28 years old. She was de facto Rembrandt's wife from 1649, and had a pregnancy in 1652. No records on health of

Hendrickje until her premature death on 21 July 1663 at the age of 37 (GRAU *et al*. 2001b; HAYAKAWA *et al*. 2005).

An alternative diagnosis was proposed of an infective process such as tuberculous mastitis or less likely chronic lactational breast abscess. If the body of model was Hendrickje, she could hardly have lived 9 years with advanced breast cancer without any effective treatment. Possibly she had a chronic inflammatory condition, either tuberculous mastitis or, less likely, lactational breast abscess (BOURNE 2000; HAYAKAWA *et al*. 2005).

Cancer was known as a disease since prehistoric times. Management of breast cancer evolved slowly through centuries in the ancient world up to the Renaissance. This period is marked by the absence of any scientifically verifiable understanding of the true nature of cancer and its natural history and consequently by a lack of effective treatment. Breast cancer was a huge problem among common people in the 16th century. As presented in our article breast cancer was clearly visible, people could see progressing, from a small lump to a large tumor and wreaking breast skin. It was also easy to link death with disease. Women used art to help them accept their diagnosis, treatment and prognosis. Therefore it is not surprising that the presentation of this tumor by painters was so common in the past. Nowadays, clinical photography is not only used to keep a record of certain diseases, prepare lecture and lessons, share information with colleagues, it is especially used to evaluate the response to chemotherapy or to new drugs in clinical trials.

3. REFERENCES

BALJET, B. 2000. The painted Amsterdam anatomy lessons: anatomy performances in dissecting rooms? Ann. Anat. 182: 3–11.

BATISTATOU, A., CHARALABOPOULOS, K.A. 2005. The art of science: Transitional cell carcinoma of the bladder and Golconda. Med. Hypotheses 65: 970–971.

BAUM, M. 2003. La Fornarina: breast cancer or not? Lancet 361: 1129.

BELKIN, K.L., HEALY, F. MARTIN, G. 2004. Painting and sculpture. In: K.L. BELKIN, F. HEALY (eds), A house of art. Rubens as collector. Rubenshuis & Rubenianum, Antwerpen, pp 88–259.

BONJER, H.J., BRUINING, H.A. 1999. Surgery in the Netherlands. Arch. Surg. 134: 92–98.

BOURNE, R.G. 2000. Did Rembrandt's Bathsheba really have breast cancer? Aust. N. Z. J. Surg. 70: 231–232.

BRAITHWAITE, P.A., SHUGG, D. 1983. Rembrandt's Bathsheba: The dark shadow of the left breast. Ann. R. Coll. Surg. Engl. 65: 337–338.

CZEIZEL, A.E. 2003. La Fornarina: breast cancer or not? Lancet 361: 1129–1130.

DAHLGREN, A. 2003. Michelangelo and medicine. J. R. Soc. Med. 96: 256.

EKMEKTZOGLOU, K.A., XANTHOS, T., GERMAN, V., ZOGRAFOS, G.C. 2009. Breast cancer: from the earliest times through to the end of the 20th century. Eur. J. Obstet. Gynecol. Reprod. Biol.145: 3–8.

ESPINEL, C.H. 2002. The portrait of breast cancer and Raphael's La Fornarina. Lancet 360: 2061–2063.

GRAU, J.J., ESTAPE, J., DIAZ-PADRON, M. 2001a. Breast cancer in Rubens paintings. Breast Cancer Res. Treat. 68: 89–93.

GRAU, J.J., ESTRACH, T. 2008. Old masters as clinical photographers: multifocal breast cancer diagnosed 400 years ago. Breast Cancer Res. Treat. 111: 11–13.

GRAU, J.J., PRATS, M., DIAZ-PADRON, M. 2001b. Breast cancer in the Rubens and Rembrandt paints. Med. Clin. (Barc) 116: 380–384.

GRECO, T. 1970. Rembrandt and breast cancer. Osp. Ital. Chir. 22: 141–144.

GROSS, A. 2004. An epidemic of breast cancer among models of famous artists. Breast Cancer Res. Treat. 84: 293.

HAYAKAWA, S., MASUDA, H., NEMOTO, N. 2006. Rembrandt's Bathsheba, possible lactation mastitis following unsuccessful pregnancy. Med. Hypotheses 66: 1240–1242.

KARKABI, K., CASTEL, O.K. 2006. Deepening compassion through the mirror of painting. Med. Education 40: 459–489.

SAKORAFAS, G.H., SAFIOLEAS, M. 2009. Breast cancer surgery: an historical narrative. Part I. From prehistoric times to Renaissance. Eur. J. Cancer Care 18: 530–544.

VAIDYA, J.S. 2007a. Breast cancer: an artistic view. Lancet Oncology 8:583-585 http://oncology.thelancet.com

VAIDYA, J.S. 2007b. Locally advanced breast cancer in a 15th century painting in Milan. Breast 16: 102–103.

Acta Universitatis Lodziensis
———— 2010 ————
Folia Biologica et Oecologica 6: 69–80
(Acta Univ. Lodz, Folia Biol. Oecol.)

MACIEJ BARTOS

Department of Teacher Training and Biodiversity Studies, University of Łódź, Banacha 1/3, 90-237 Łódź, Poland, bartos@biol.uni.lodz.pl

PREDATORY ENCOUNTERS OF *YLLENUS ARENARIUS* (ARANEAE, SALTICIDAE) WITH FLIES (DIPTERA)

Abstract: Predatory behaviour of *Yllenus arenarius* hunting flies (Diptera) was studied. The general spider's approach and capture was typical for salticids hunting prey that has high ability to escape. Two modes of approach in close proximity of prey were observed. One was typical for the majority of predatory encounters where the spider's velocity was significantly reduced with decreasing distance to prey. Stalk and movement masking were typical for this type of approach. Second mode occurred sporadically and was characterized by a high spider's velocity that was not reduced in the vicinity of the prey.

Key words: predatory behaviour, jumping spider, *Yllenus*, Diptera

1. INTRODUCTION

Salticids are the most diverse family of spiders with over 5300 species described (PLATNICK 2011). In this group there is an amazing diversity in forms and life styles, of which only a very small fraction has been described. There is also a striking disproportion in our knowledge of different aspects of salticid biology from different regions of the world. For example, salticid fauna of the Palearctic, which belongs to the most thoroughly described by taxonomists, is still one of the least known with respect to the biology of species.

Jumping spiders (Salticidae) are typical daily hunters that do not build webs but ambush or actively pursue and capture their prey. Probably the most specific of

DOI: 10.2478/v10107-009-0008-z

these spiders are their unusual eyes, which enable precise prey identification. They have two types of eyes: one pair of frontally positioned, large principal eyes and three pairs of laterally positioned, small secondary eyes. These two groups have different visual properties and functions. Principal eyes are responsible for acute vision and perception of colours while secondary eyes are generally movement detectors (WILLIAMS, MCINTYRE 1980; PEASLEE, WILSON 1989). The eyes have an extraordinary resolving power and allow to discriminate between invertebrates of similar size (HARLAND, JACKSON 2000; HARLAND, JACKSON 2004).

Vision plays a key role in salticid behaviour, particularly in courtship and predatory strategies (RICHMAN, JACKSON 1992). Highly effective visual system enables the spiders to distinguish between sexual partners, their own predators and different prey types from the distance of about 40 body lengths on the basis of visual signals alone (HARLAND *et al.* 1999). Precise target identification plays a significant role, especially in predatory interactions, as it may not only increase the chances of hunting success but also avoid mistaking a prey and an enemy.

Jumping spiders hunt a wide variety of invertebrates and their prey may vary according to many aspects, to mention only the ability to escape or harm the predator. There are numerous examples of conditional predatory tactics characterized by four basic aspects: different direction and velocity of approach to prey, different distances from which the prey is attacked and a variety of other prey-specific behaviours observed during predatory encounters (EDWARDS, JACKSON 1993, 1994; BEAR, HASSON 1997; BARTOS 2007). Irrespective of the variety of prey-specific behavioural adaptations, most predatory encounters consist of three primary patterns: orientation, pursuit and capture (FORSTER 1977).

The predatory behaviour of jumping spiders has been well studied (RICHMAN, JACKSON 1992; JACKSON, POLLARD 1996). Although the majority of salticids are generalist predators, the bulk of our knowledge on their hunting behaviour comes from studies of species that specialize in particularly dangerous prey: ants and spiders. These studies revealed some striking behavioural adaptations to capture such prey (LI, JACKSON 1996; TARSITANO, JACKSON 1997; WILCOX, JACKSON 1998; LI, JACKSON 2003). They also shed some light on extraordinary

cognitive abilities that enable these creatures solving complex problems. One particular genus, *Portia* from subfamily Sparteinae, has become a model in the studies of invertebrate cognition (WILCOX, JACKSON 1998; HARLAND, JACKSON 2004).

There are very few salticids whose biology has been studied in more than just one aspect. In the Palearctic region an example of such species is *Yllenus arenarius* Menge 1868 – a medium-sized jumping spider with an adult body length of about 7 mm. It is a stenotopic species, which in Central Europe is mostly limited to *Spergulo-Corynephoretum* habitat, in particular to the initial stage of dune succession (MERKENS 2000; LOGUNOV, MARUSIK 2003). *Y. arenarius* is a cryptically-coloured, sit-and-wait predator feeding on a wide range of insects and spiders that inhabit open sand or are blown by the wind onto the dune surface from neighbouring habitats (BARTOS 2004). It was found that the spiders use a conditional hunting strategy manifested in prey-specific jumping distance, speed of approach, direction of approach and other prey-specific behaviours (BARTOS 2002, 2007, 2008).

The present paper presents the research on predatory encounters of *Y. arenarius* with Diptera – an insect order that constitutes a major fraction in the spider's natural diet (BARTOS 2004, 2011). The predatory interactions of *Y. arenarius* with other prey (Homoptera, Orthoptera, Thysanoptera and larvae of Lepidoptera) have been described earlier with particular attention on the spider's predatory versatility (BARTOS 2000, 2002, 2007, 2008).

2. MATERIALS AND METHODS

2.1. Prey

All prey items used in the experiments belonged to the order Diptera. They were collected in the field by sweep-netting dune grass on the day of the experiment or the day before. They were brought to the lab and kept separately. Each prey and a spider were chosen randomly for the experiments. In order to reduce the mortality of the prey, insects were stored in a refrigerator (temp. 5˚C) and taken out 15 min.

before the experiment started. Each prey item was given to a spider of approximately similar size.

2.2. Predators

Spiders were collected from a dune in Central Poland near the village of Kwilno (51°59' N, 19°30' E). In order to reduce the influence of rearing conditions on the spider's behaviour (CARDUCCI, JAKOB 2000) all experiments were carried out the same day or the next day after the spiders were collected. Before experiments, spiders were kept individually in glass containers (10x10x10 cm) with a layer of dune sand on the bottom. Each spider was used only once in the tests. The experiments in which no hunting behaviour was present (e.g., because the spider ignored the prey or the prey escaped before it was approached) were not included in the analyses.

After experiments each spider's abdomen length was measured. The measurement was used to standardize the jumping distance to correct for body size and for the condition of different spiders in the same age (see BARTOS 2002). After experiments all spiders were released back in the dune.

3.3. Experimental procedure

Experiments were carried out within a white cardboard arena (15 cm height by 20 cm diameter) with a 1 cm-thick sand layer on the bottom and were conducted between 09:00 hours and 16:00 hours (laboratory light regime, 12L:12D, lights coming on at 08:00 hours). Lighting was from a 100W PILA incandescent lamp bulb positioned 0.5 m above the arena and by fluorescent tube ceiling lights 2 m above the arena. Spiders were placed within the arena and, after one minute, a prey item was introduced about 8 cm from the spider. The prey was dropped approximately 30˚ to the left or right from the main eye's optical axis to allow the experimenter to record the moment when the predator oriented toward the prey. The prey item was left with the spider for 15 minutes. The hunting behaviour was recorded with a camera placed above the arena.

3.4. Data analysis

Movies with hunting behaviour were analyzed frame by frame. All behavioural units and hunting success were recorded. The complete sequences of hunting, namely those that started with the first dynamic behaviour (run), and that ended with subduing the prey, were used to draw flow diagram (Fig. 1). If there were multiple attacks on the same prey, only the first hunting sequence was presented in Fig. 1. The percentage of individuals that expressed certain behaviours is indicated by the width of the line that leads to the behaviour and by the number above the line. The numbers in some paths do not add up to 100%, due to rounding. The names of already reported components of salticid behaviour are taken from a classic paper by FORSTER (1977). Behaviours specific for *Y. arenarius* are defined and discussed in BARTOS (2000, 2007). Movies with selected behaviours discussed in this paper can be seen online (http://maciejbartos.pl/movies/) to enable comparison. Data are presented as mean±SD.

3. RESULTS

In the sequences of hunting flies 11 behavioural units were identified. They were depicted in the flow diagram (Fig. 1). The presence or lack of certain behaviours in the hunting sequence depended on prey's distance from the spider at the beginning of the experiment, prey's motility and direction of prey's movement. For example no approach was observed if the prey landed in the vicinity of the spider or if the prey moved towards the spider. In these situations only *alert*, *attack preparation*, *attack* and *grasping and stabbing* were present. As a result in these experiments a simplified pattern of hunting was observed. These simplified cases were not included in the flow diagram. The complete sequence of behaviours was observed in 12 out of 77 predatory encounters and only these data were used to draw Fig. 1.

In a complete hunting sequence the first observable behaviour was *alert* marked by a swivel of spider's cephalothorax. As a result the spider's main eyes were directed towards the prey. From this moment spider's eyes kept following its prey, which was noticeable by sideways movements of cephalothorax and was

defined as *observation.* Prey observation was carried out on average for 6.5±7.5 s (n=62).

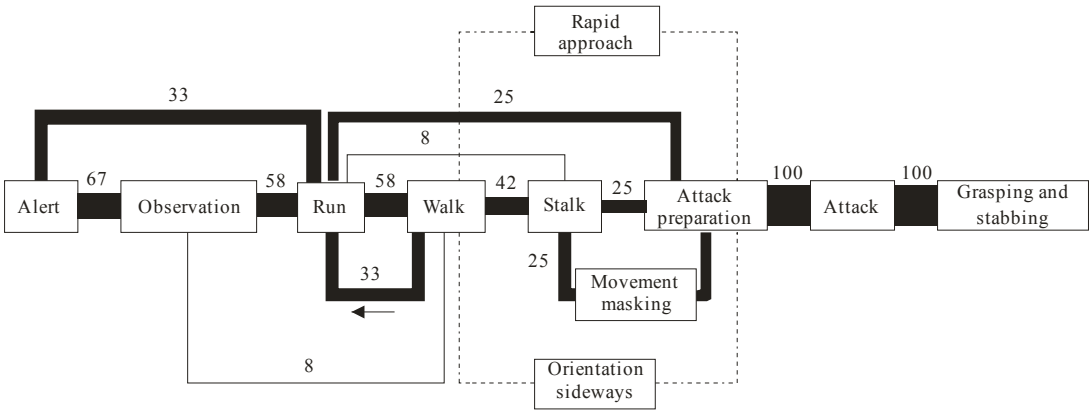

Fig. 1. The flow diagram of *Yllenus arenarius* hunting flies. Diagram is based on 12 cases of complete hunting sequences (see text for details). Transition frequencies are indicated by the per cent numbers and by an appropriate line width. Dashed line symbolizes the behaviour that was not observed in the complete hunting sequence, but was commonly recorded in incomplete sequences. The sequence should be read from left to right unless indicated by the arrow.

After the period of observation the spider started approach. The first phase was *run* towards its prey with a mean velocity of 42.3±13.0 mm/s (n=9) (Fig. 2). Run was sometimes interrupted by short pauses accompanied by the observation of the prey. Spider reduced the speed of approach with decreasing distance to prey and started to *walk* with the velocity of about 22.8±13.6 mm/s (n=9). In the vicinity of the prey *stalk* and *movement masking* were observed. Both behaviours were characterized by a robot-like gait and had the same movement velocity of 2.3±2.0 mm/s (n=9). Movement masking was, however, performed only in situations when there were alternate phases of prey's movement and stillness (while prey was moving the spider was approaching, but when the prey stopped moving the spider froze). In only one out of 77 cases of hunting flies and in none of complete hunting sequences *rapid approach* was observed (Fig. 1). The behaviour was characterized by very quick run (velocity: 122.9±10.1 mm/s, n=3) in direction to the prey followed

by a sudden stop and immediate attack. Some spiders performed sideway movements accompanied by constant observation of prey called *orientation sideways*.

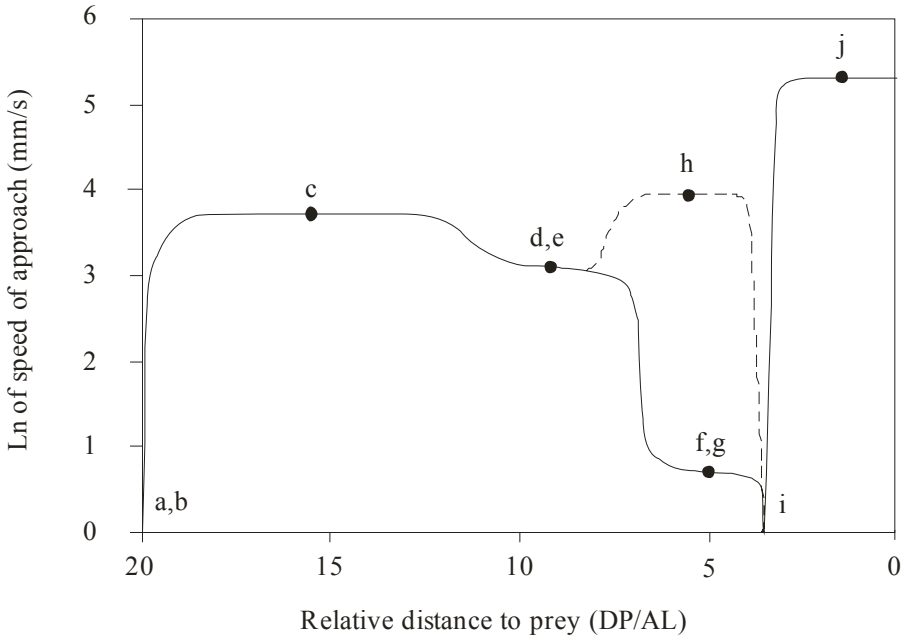

Fig. 2. The mean velocity of spiders (dots) approaching flies in relation to the spiders' relative distance to prey (DP/AL). The velocities were calculated on the basis of nine randomly selected complete hunting sequences. Lines represent tentative relationships between spider's velocity and its distance to prey. Dashed line depicts encounters in which rapid approach was observed. DP – distance to prey; AL – spider's abdomen length; a – alert; b – observation; c – run; d – walk; e – orientation sideways; f – stalk; g – movement masking; h – rapid approach; i – attack preparation; j – attack.

The last phase of predatory encounter took place in the close vicinity of the prey and was uniform. In *attack preparation* the spider lowered its body, attached the dragline to sand surface, pushed its fourth pair of legs repeatedly against sand surface (as if trying to firm sand before the jump) and finally stretched its first pair of legs towards the prey. The spider always *attacked* its prey by means of a jump

(velocity: 182.4±88.9 mm/s, n=9) and landed on the prey's dorsal side first *grasping* its wings and then *stabbing* its thorax.

The direction of approach to prey was irrespective of the preys' position in relation to the spider. Spiders always approached their prey along the shortest path. There was no difference in the direction of approach when prey was positioned frontally, sideways or backwards.

The mean relative distance of attack (distance of attack divided by spider's abdomen length) was 3.60±1.53 (n=77) (Fig. 3). In 94% of all hunting encounters (n=77) the prey was successfully captured by the spider including the attack with rapid approach (Fig. 2). Other hunting encounters were unsuccessful and the prey managed to escape after spider's attack.

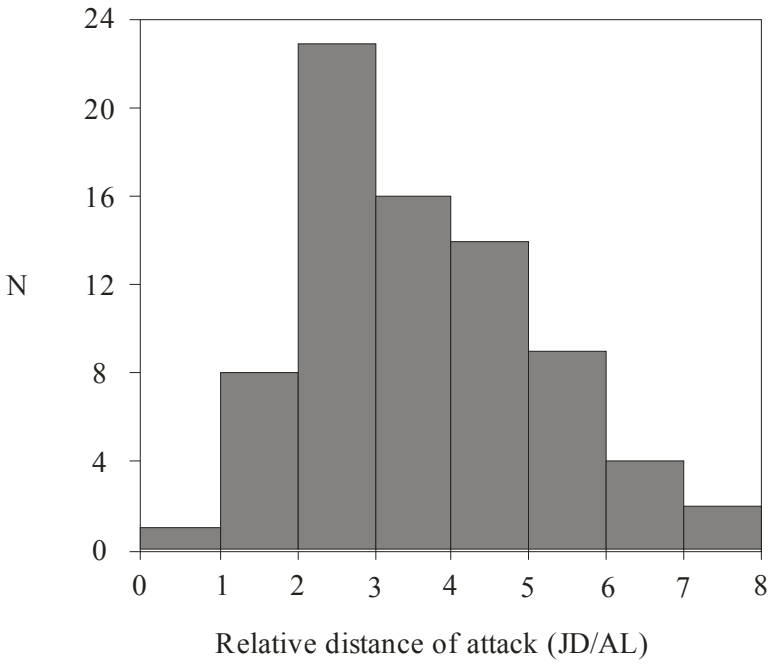

Fig. 3. The relative distance of attack (DA/AL) of *Yllenus arenarius* hunting flies. DA – distance of attack; AL – spider's abdomen length.

4. DISCUSSION

The general pattern of hunting flies by *Y. arenarius* seems to express a fairly universal mode of approach and capture prey that can efficiently escape. There is

also a high degree of resemblance between the predatory behaviour of *Y. arenarius* and the behaviour of other non-specialized salticids approaching comparable prey (DILL 1975; FORSTER 1977, 1982; EDWARDS, JACKSON 1993, 1994; BEAR, HASSON 1997).

There is a very close similarity between the order and presence of particular behavioural units in predatory encounters of *Y. arenarius* with flies and with other prey characterized by high ability to escape (Homoptera, Orthoptera), which this spider was tested with (BARTOS 2002, 2007, 2008). All behavioural elements observed in this study were also present in experiments with Homoptera and Orthoptera. Most of them occurred with similar frequencies (BARTOS 2000, 2007). There were also no differences in the distance of attack between Diptera, Homoptera and Orthoptera (BARTOS 2002). All these similarities suggest that there is a common strategy of hunting all the three types of prey. Even though there is no apparent similarity between insects from the three taxa they are hunted in a common way that seems to minimize the risk of detection of the predator by the prey (BEAR, HASSON 1997; BARTOS 2000, 2007).

Behavioural adaptations that may minimize the risk of detection the predator before attack were present at the stage of late approach and jumping distance. The approach to prey was fairly uniform at the beginning of the hunting sequence, when the spider was at a long distance from its prey. When it reduced the distance to about five body lengths (10 abdomen lengths) two modes of approach were observed (Fig. 2). Both types of approach differed according to the spider's velocity and visibility to the prey.

In stealthy approach (solid line in Fig. 2) the spider's velocity was significantly reduced with decreasing distance to prey. In close vicinity of the prey the spider moved very slowly stalking the prey or even froze in moments, when the prey stopped moving, which may be explained as hiding the spider's presence from the prey. Such behaviour was probably the case of exploiting general insect sensory limitation to perceive motion only when staying still (PEARSON 1988; LAND, NILSSON 2002). Movement masking has already been reported for *Y. arenarius* hunting prey with high ability to escape and discussed elsewhere (BARTOS 2007).

Another type of approach (dashed line in Fig. 2) was characterized by a high spider's velocity that was not reduced in the vicinity of the prey (rapid approach) or by sideways movements of the spider (orientation sideways). Both behaviours were probably highly visible to the prey and, as such, opposite to stealthy approach according to the risk of detection the spider by the prey. This makes the type of approach especially interesting. There is a question of any possible advantages of such risky behaviour that results in increased probability of prey escape. It is possible that in cases, when the prey very often moves from one place to another and has a high motility when on the surface, rapid movement towards such prey and immediate attack may be more effective than slow stalk. In case of Diptera rapid approach was a rare behaviour, but in cases of hunting other prey with high abilities to escape it was more common (BARTOS 2000, 2007)

The distance of attack in case of Diptera was comparable to distances for Homoptera and Orthoptera (BARTOS 2002). All distances had also similar distributions. The distances are significantly right-skewed with a distinct mode range and very few measurements shorter than the mode range. It suggests that the range may be the optimal distance of attack and both, shorter and longer distances may be suboptimal. It seems likely that close approach may increase the risk of prey escape due to predator's detection. Attack from a longer distance seems to be less risky, as detection of the predator is lower, it may, however, decrease the chances of firm prey grasping and as a result make the prey escape more likely.

Acknowledgments

This research was supported by Polish Ministry of Scientific Research and Information Technology (grant 3P04F05822) and research grant form University of Łódź. Voucher specimens of *Y. arenarius* have been deposited in the Arachnological Collection of the Department of Zoology, University of Podlasie, Siedlce, Poland.

5. REFERENCES

BARTOS, M. 2000. The life cycle and hunting strategy of *Yllenus arenarius* (Araneae, Salticidae). PhD Thesis, University of Łódź.

BARTOS, M. 2002. Distance of approach to prey is adjusted to the prey's ability to escape in *Yllenus arenarius* Menge (Araneae, Salticidae). In: S. TOFT, N. SCHARFF (eds), European Arachnology 2000. Aarhus University Press, Aarhus, pp. 33–38.

BARTOS, M. 2004. The prey of *Yllenus arenarius* (Araneae, Salticidae). Bull. Br. arachnol. Soc. 13: 83–85.

BARTOS, M. 2007. Hunting prey with different escape potentials – alternative predatory tactics in a dune-dwelling salticid. J. Arachnol. 35: 499–509.

BARTOS, M. 2008. Alternative predatory tactics in a juvenile jumping spider. J. Arachnol. 36: 300–305.

BARTOS, M. 2011. Partial dietary separation between coexisting cohorts of *Yllenus arenarius* (Araneae, Salticidae). J. Arachnol. (in press).

BEAR, A., HASSON, O. 1997. The predatory response of a stalking spider. Anim. Behav. 54: 993–998.

DILL, M.L. 1975 Predatory behavior of zebra spider, Salticus scenicus (Araneae: Salticidae). Can. J. Zool. 53: 1287–1289.

EDWARDS, G.B., JACKSON, R.R. 1993. Use of prey specific predatory behaviour by North American jumping spiders (Araneae, Salticidae) of the genus *Phidippus*. J. Zool. 229: 709–716.

EDWARDS, G.B., JACKSON, R.R. 1994. The role of experience in the development of predatory behaviour in *Phidippus regius*, a jumping spider (Araneae, Salticidae) from Florida. New Zealand. J. Zool. 21: 269–277.

FORSTER, L. 1977. A qualitative analysis of hunting behaviour in jumping spiders (Araneae: Salticidae). N. Z. J. Zool. 4: 51–62.

HARLAND, D.P., JACKSON, R.R., MACNAB, A.M. 1999. Distances at which jumping spiders distinguish between prey and conspecific rivals. J. Zool. 247: 357–364.

HARLAND, D.P., JACKSON, R.R. 2000. 'Eight-legged cats' and how they see - a review of recent research on jumping spiders (Araneae: Salticidae). Cimbebasia 16: 231–240.

HARLAND D.P., JACKSON R.R. 2004. *Portia* perceptions: the *Umwelt* of an araneophagic jumping spider. In: F.R. PRETE (ed.). Complex Worlds from Simpler Nervous Systems. MIT Press, Cambridge, Massachusetts, pp. 5–40.

JACKSON, R.R., POLLARD, S. 1996. Predatory behaviour of jumping spiders. Annu. Rev. Entomol. 41: 287–308.

LAND, M.F., NILSSON, D-E. 2002. Animal Eyes. Oxford University Press, New York.

LI, D., JACKSON, R.R. 1996. Prey-specific capture behaviour and prey preferences of myrmicophagic and araneophagic jumping spiders (Araneae: Salticidae). Rev. Suisse Zool. vol. hors série: 423–436.

LI, D., JACKSON, R.R. 2003. A predator's preference for egg-carrying prey: a novel cost of parental care. Behav. Ecol. Sociobiol. 55: 129–136.

LOGUNOV, D.V., MARUSIK, Y.M. 2003. A revision of the genus *Yllenus* Simon, 1868 (Arachnida, Araneae, Salticidae). KMK Scientific Press Ltd., Moscow.

MERKENS, S. 2000. Epigeic spider communities in inland dunes in the lowlands of Northern Germany. In: S. TOFT, N. SCHARFF (eds), European Arachnology 2000. Aarhus University Press, Aarhus, pp. 215–222.

PEARSON, D.L. 1988. Biology of tiger beetles. Annu. rev entomol 33 123-47.

PEASLEE, A.G., WILSON, G. 1989. Spectral sensitivity in jumping spiders (Araneae, Salticidae). J. Comp. Physiol. 164: 359–362.

PLATNICK, N.I. 2011. The world spider catalog, version 11.5. American Museum of Natural History, online at http://research.amnh.org/iz/spiders/catalog. DOI: 10.5531/db.iz.0001.

RICHMAN, D.B., JACKSON, R.R. 1992. A review of the ethology of Jumping Spiders. Bull. Brit. arachnol. Soc. 9: 33–37.

TARSITANO, M.S., JACKSON, R.R. 1997. Araneophagic jumping spiders discriminate between detour routes that do and do not lead to prey. Anim. Beh. 53: 257–266.

WILCOX, R.S., JACKSON, R.R. 1998. Cognitive abilities of araneophagic jumping spiders. In: I. PEPPERBERG, A. KAMIL, R. BALDA (eds). Animal Cognition in Nature. Academic Press, New York, pp. 411–434.

Acta Universitatis Lodziensis
———— 2010 ————
Folia Biologica et Oecologica 6: 81–101
(Acta Univ. Lodz, Folia Biol. Oecol.)

ADRIANNA WOJTAL-FRANKIEWICZ & PIOTR FRANKIEWICZ

Department of Applied Ecology, University of Łódź, Banacha 12/16, 90-237 Łodź, Poland, adwoj@biol.uni.lodz.pl

THE INFLUENCE OF THE ZEBRA MUSSEL (*DREISENA POLYMORHPA*) ON MAGNESIUM AND CALCIUM CONCENTRATION IN WATER

Abstract: In this study we examined changes in magnesium and calcium ion concentrations depending on Zebra Mussel biomass, pH values and temperature. We performed field experiments in years with different weather conditions using twelve 200 litre polycarbonate containers filled with 150 litres of non-filtered water from lowland, eutrophic reservoirs. Three treatments of the experiment were represented by: Phyto control with non-filtered water, Phyto+Dreis A with Zebra Mussel biomass of 500 g/m^2**,** and Phyto+Dreis B with Zebra Mussel biomass of 1.000 g/m^2**.** Magnesium and calcium ions concentrations were analyzed on an ion chromatograph (Dionex-1000). Results indicated a significant reduction in magnesium and calcium ion concentrations by Zebra Mussels (independent of mussel biomass), especially in the year with higher and more stable average temperatures. Mg concentration was significantly negatively correlated with temperature in this year. In both years of study the magnesium and calcium ion concentrations were negatively correlated with pH. Analyses of the Zebra Mussel's impact on magnesium and calcium loss from water, linked with the influence of physical factors (temperature and pH), may be valuable for the management of invaded ecosystems.

Key words: invasive species, Mg, Ca, temperature, algal blooms

DOI: 10.2478/v10107-009-0009-y

1. INTRODUCTION

The invasive Zebra Mussel species *Dreissena polymorpha* Pallas is known as an organism that tolerates a wide range of environmental conditions, which results in its high dispersal capacity (STAŃCZYKOWSKA 1983; O'NEILL 1996; LEWANDOWSKI 2001; CASAGRANDI *et al.* 2007). It has, in fact, invaded numerous European and North American inland waters (STRAYER 1991), usually causing dramatic changes in the physical and biological structure of the infested ecosystems (MACISAAC 1996; KARATAYEV *et al.* 2002). However, as the Zebra Mussel originates from the Caspian and Black Seas, colonization of the freshwater environments requires many physiological adaptations. Nevertheless, the water chemistry demands of the Zebra Mussel are extraordinarily complex and not observed in other freshwater bivalves (HOROHOV *et al.* 1992). Their tolerance to decreased Na, Cl, K, and particularly Mg concentrations in water is very low (DIETZ *et al.* 1994), and studies show that the Zebra Mussel is absent in Mg-poor lakes (HALLSTAN *et al.* 2010). RAMCHARAN and co-workers (1992) additionally classified calcium concentration and water pH as the important factors for the success of Zebra Mussel invasion. Identification of these factors may be crucial for limiting and controlling Zebra Mussel distribution. However, the determination of *D. polymorpha*'s influence on the concentration of ions essential for this species' survival may be also valuable for the management of the invaded water bodies. Effective control programs should focus not only on local Zebra Mussel population dynamics but also on the exploration of complex physical, chemical and biological changes impacted by Zebra Mussel activity in monitored ecosystems (O'NEILL 1996; KARATAYEV *et al.* 2002).

We performed outdoor experiments in years with different weather conditions: in 2007 summer temperature was generally low and fluctuating, but in 2008 summer was warm with relatively stable temperatures. In this study we examined how the presence of the Zebra Mussel contributed to changes in magnesium and calcium ion concentrations in water and whether these changes were correlated with water temperature and pH. Research was conducted into the aspect

of water quality, because we were interested whether, and to what extent, Zebra Mussels may be Mg-competitors for bloom-forming algae and cyanobacteria.

2. MATERIALS AND METHODS

The experiment was conducted from 23 June to 13 September 2007 and 22 June to 18 September 2008 in the Field Station of the University of Łodź located near Sulejów Reservoir. Water and Zebra Mussels used in the experiment were collected from the Sulejów Reservoir (51°22'-51°28'N, 19°51'-20°01'E) situated on 138.9 km of the Pilica River (the Vistula River catchment) in central Poland. The Sulejów Reservoir is a 37-year old, shallow (mean depth is 3.3 m) and eutrophic ecosystem, invaded by Zebra Mussel (ABRASZEWSKA 2006). During summer, the cyanobacteria blooms forming mainly by *Microcystis aeruginosa* (Kutzing) are usually observed (TARCZYŃSKA *et al.* 2001).

Zebra Mussels were collected in the shallow littoral part of the reservoir. In the laboratory, colonies were cleaned, weighed and placed in aquaria with reservoir water for acclimatization. After 24 h, Zebra Mussels were used in the experiment. Filtering activity of animals was monitored every day. All Zebra Mussels were again weighed and released back to the reservoir after the end of the experiment.

The experiment was conducted under natural light and temperature conditions in twelve 200 l polycarbonate containers (115 cm height, 60 cm diameter) filled with 150 l of non-filtered water from Sulejów Reservoir. There were three treatments prepared of the experiment (in four replicates):

- Phyto (control with non-filtered water from the reservoir);
- Phyto+Dreis A (non-filtered water + 175 g of Zebra Mussel colonies, which corresponds to a biomass of 500 g/m^2);
- Phyto+Dreis B (non-filtered water + 350 g of Zebra Mussel colonies, which corresponds to a biomass of 1.000 g/m^2).

The containers were put into a concrete ditch filled with water to 2/3 the height of the containers to buffer temperature fluctuations. Before each sampling occasion, water in all containers was mixed.

2.1. Physical water analyses

Water temperature (°C), pH and total dissolved oxygen - TDO (mg/l) were measured weekly in each container at 10:00 a.m. using WTW 340i/SET multisensors.

2.2. Magnesium and calcium ion concentrations

Magnesium (Mg) and calcium (Ca) ion concentrations were analysed on Dionex-1000, which consists of two ion chromatographies IC, separated for anions and cations. Each IC (Dionex Corporation, ICS-1000) consists of a pump, eluent, guard column (CG18 for cation and AG18 for anions), an analytical column (IonPac CS18 for cation, IonPac AS18 for anions), and an electrolytic suppressor (CSRS-ULTRA II cation electrolytic suppressor and ASRS – ULTRA II anion electrolytic suppressor) to stabilize the baseline. The analyses were performed by using 16 mM methanesulfonic acid (Fluka) for the cation analysis and a mixture of 4.5 mM sodium carbonate and 1.4 mM sodium bicarbonate for the anion system prepared from the AS22 Eluent Concentrate (produced by Dionex Corporation). Both ICs were operated in isocratic elution in 30 ºC at a flow rate of 1ml/min. Cation measurements were performed using a 25 µl injection loop and anion using a 450 µl injection loop. For ion identification, combined standards (Seven Anion Standard II, Dionex Six Cation Standard produced by Dionex Corporation) were used.

2.3. Concentration of chlorophyll *a*

For each treatment, the concentration of chlorophyll *a* (Chl *a*) (µg/l) was measured immediately after sampling in a 1-liter water sample using a bbe Algae Online Analyser (AOA, Version 1.5 E1, bbe-Moldaenke company Kiel, Germany). The measurement principle of bbe AOA is based on the determination of the fluorescence spectrum and fluorescence kinetics of the algae (www.bbe-moldaenke.de). This online analyser is recognised as reliable for chlorophyll *a* measurement (CAGNARD *et al.* 2006) and is a useful tool for monitoring phytoplankton community composition, especially as an early warning system for

the detection of harmful algal blooms (IZYDORCZYK *et al.* 2009; RICHARDSON *et al.* 2010).

2.4. Statistical methods

Testing for the treatment effect on the magnesium ion concentration in water, in order to adjust for the effects of both magnesium uptake by algae and pH variability, we applied an analysis of covariance (ANCOVA) with treatments as categorical factors, magnesium concentrations as the dependent factors, and chlorophyll *a* concentrations and pH values as covariate. To test for the treatment effect on both the oxygen and calcium ion concentrations we used an ANOVA with treatments as categorical factors and calcium and oxygen concentrations as dependent factors. Pearson's correlation coefficient was applied for analysing relationships between magnesium and calcium ions concentrations and pH and temperature.

3. RESULTS

3.1. Zebra Mussel analyses

We observed permanent filtering activity of Zebra Mussels in all days of the experiment. In both study years, the entire Zebra Mussel weight increased during the experiment (Fig. 1): in 2007 - from 175 to mean 191.21 g (SD=5.48) in the Phyto+Dreis A, and from 350 to mean 367.41 g (SD=4.67) in the Phyto+Dreis B treatment; in 2008 - from 175 to mean 196.73 g (SD=3.34) in the Phyto+Dreis A, and from 350 to mean 381.6 g (SD=5.87) in the Phyto+Dreis B treatment.

3.2. Physical water analyses

We did not find any significant differences in water temperature and oxygen concentration between the three types of treatments neither in 2007 nor 2008. In 2007 mean temperature was 14.9^{o}C (Fig. 2a), ranging from 11.9^{o}C to 19.7^{o}C. Thus, despite the buffering role of water in the concrete ditch, temperature fluctuations were high. These fluctuations were due to variable weather conditions. In 2008, mean temperature was much higher at 18.3^{o}C and fluctuated from 17.5^{o}C

to 22.9°C between 22 June and 4 September before decreasing to 9.5°C in all treatments during the last two weeks of the experiment (Fig. 2b).

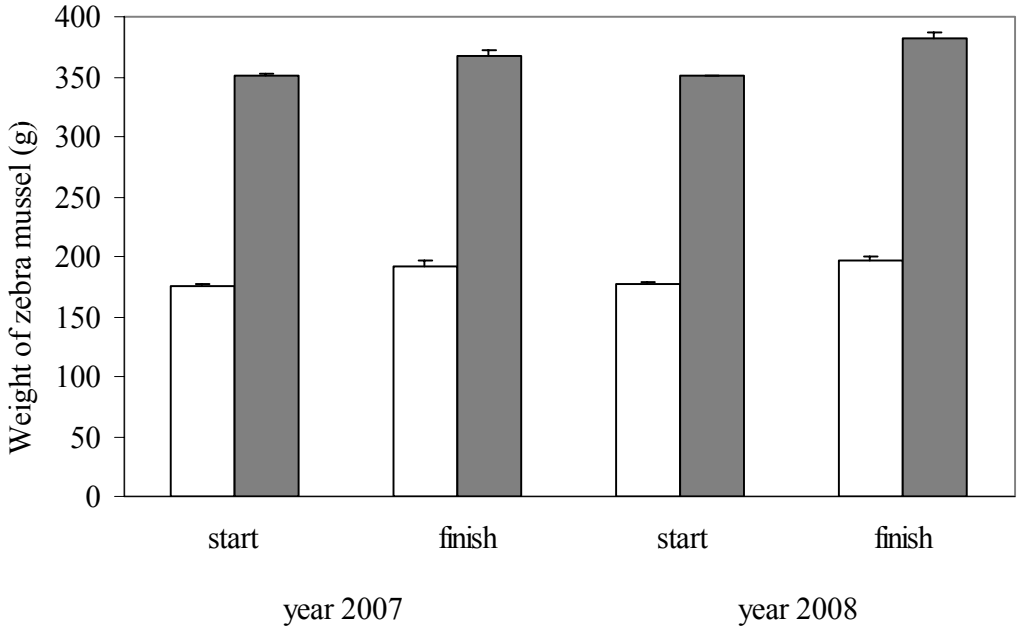

Fig. 1. Weight of Zebra Mussels (g) at start and end of the experiment. White bars – Phyto+Dreis A; grey bars – Phyto+Dreis B treatment. Bars represent means of three replicates ±SD.

In 2007 total dissolved oxygen concentration varied from 9.24 to 10.54 mg/l in July, from 8.02 to 10.83 mg/l in August and from 8.12 to 10.88 mg/l in September. In 2008 the mean values of total dissolved oxygen concentration were higher than in the previous year and fluctuated from 8.90 to 11.70 mg/l in July, from 10.34 to 13.76 mg/l in August and from 9.30 to 14.39 mg/l in September.

In 2007 pH values varied in all treatments (Fig. 3a) in the range of 8.53-9.39 in Phyto and 8.53-9.6 in mussel-containing treatments, but the differences were not statistically significant (ANOVA $F_{2,9}$=0.91; P=0.44). However, in 2008 pH differed significantly between treatments (ANOVA $F_{2,9}$=102.72; P<0.001).

The lowest pH was observed in Phyto (8.97-8.30 and the last value 7.47). In Zebra Mussel treatments pH values fluctuated similarly (8.64-9.20) during the first

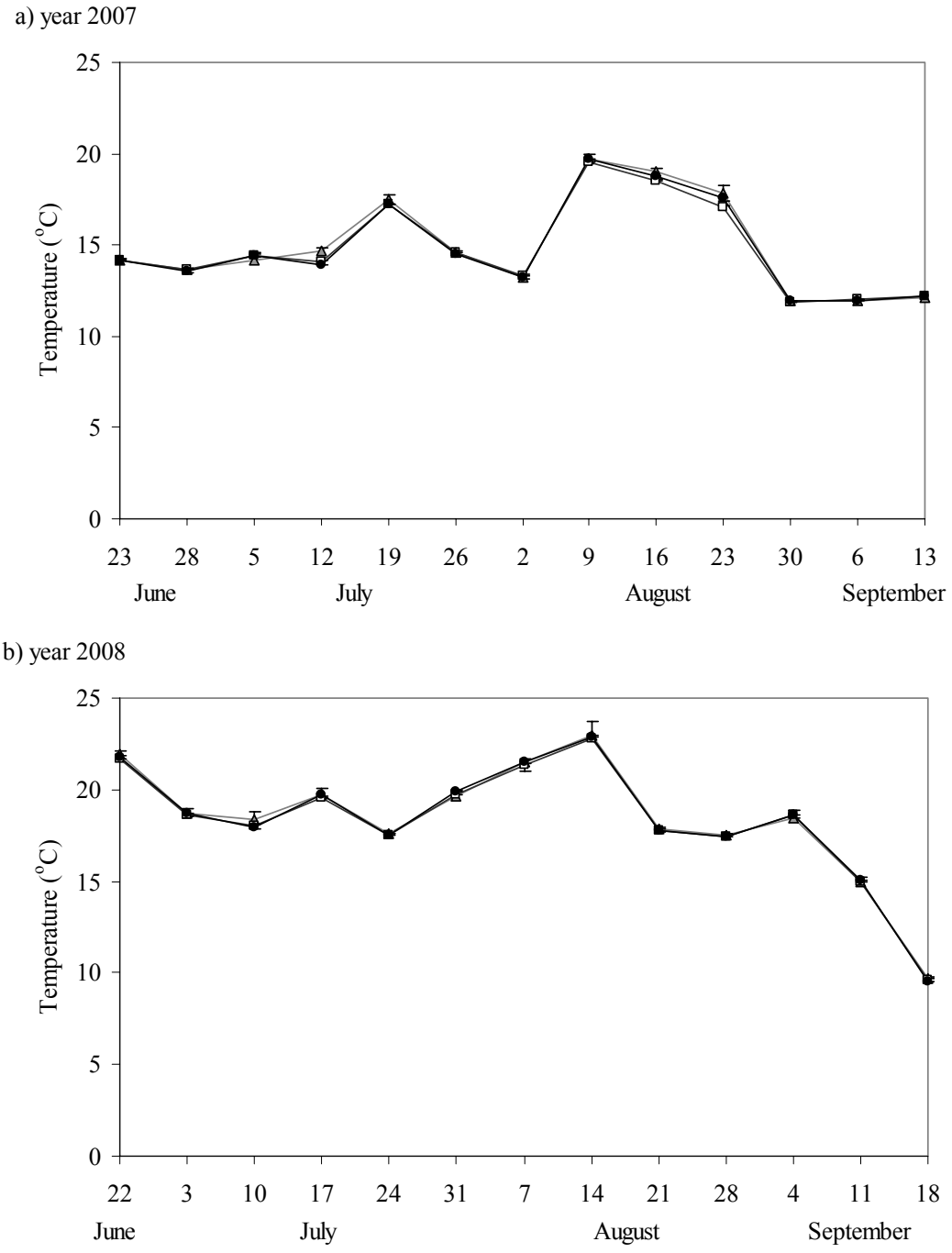

Fig. 2. Water temperature (°C) in: a) year 2007, and b) year 2008. Grey triangles – Phyto; white squares – Phyto+Dreis A; black circles – Phyto+Dreis B. Points represent means of three replicates ±SD.

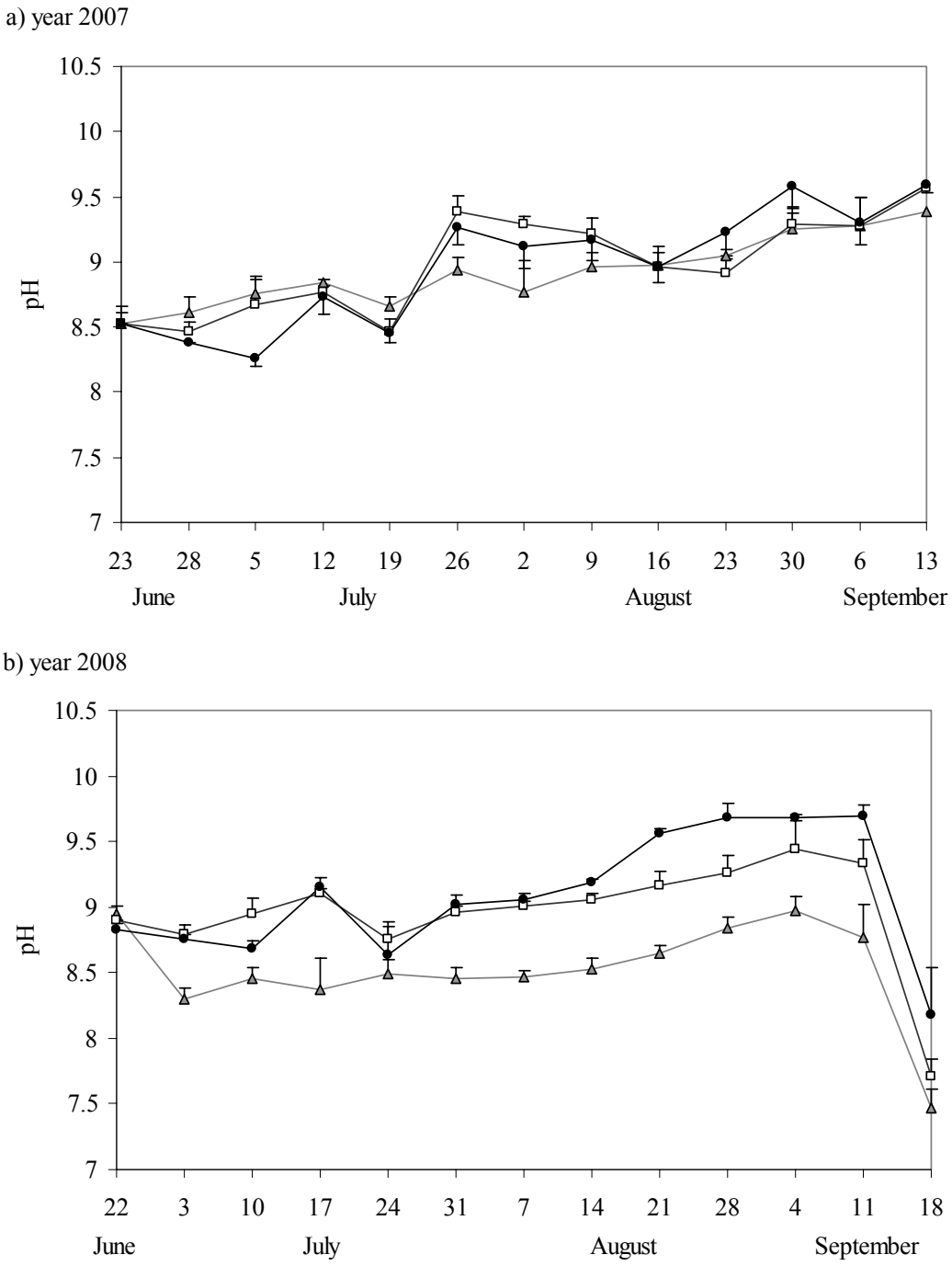

Fig. 3. Water pH in: a) year 2007, and b) year 2008. Grey triangles – Phyto; white squares – Phyto+Dreis A; black circles – Phyto+Dreis B. Points represent means of three replicates ±SD.

half of the experiment. From 14 August pH significantly increased in Phyto+Dreis B, achieving a maximal value of 9.70 (Fig. 3b). In the last week of the experiment pH decreased rapidly in all treatments, which was probably connected with the slowdown of the biological processes due to considerable fall in temperature. Direct effect of temperature on pH was not observed in this study.

3.3. Concentration of magnesium and calcium ions

The values of both magnesium and calcium ion concentrations were substantially lower in 2007 than in 2008. Analysis of covariance showed a significant influence of chlorophyll *a* concentrations on magnesium concentrations in 2007 but not in 2008 (ANCOVA: $F_{1,34}$=6.78; P=0.014 and $F_{1,34}$=0.202; P=0.656, respectively), yet magnesium concentrations differed significantly between treatments in both 2007 (ANCOVA $F_{2,34}$=9.54; P<0.001) and 2008 (ANCOVA $F_{2,34}$=4.71; P=0.016), showing the dominant role of the Zebra Mussel in reducing the quantity of these ions. In both cases, the values were higher in Phyto than in the Zebra Mussel treatments (Fig. 4a,b). In 2007, Mg concentration in Phyto ranged 0.52-0.62 mg/l. In mussel treatments magnesium concentration fluctuated similarly until the beginning of August, achieving the minimal values of 0.36 and maximal of 0.51 mg/l. In the Phyto+Dreis B treatment, the concentration of Mg ion rapidly decreased to 0.19 mg/l (Fig. 4a). In 2008, differences of Mg ion concentration between treatments appeared earlier and were clearer than in 2007. During the first week of the experiment, values of magnesium concentration suddenly decreased in all treatments. Then, they stabilised in the range 0.87-1.10 mg/l. In mussel treatments Mg concentrations started to decrease significantly from 17 July and fluctuated in the range 0.75-0.46 mg/l in Phyto+Dreis A and 0.41-0.18 mg/l in Phyto+Dreis B (Fig. 4b).

Calcium ion concentrations, analogically to magnesium, differed significantly between control (Phyto) and mussel treatments both in 2007 (ANOVA $F_{2,9}$=24.74; P<0.001) and in 2008 (ANOVA $F_{2,9}$=56.19; P<0.001).

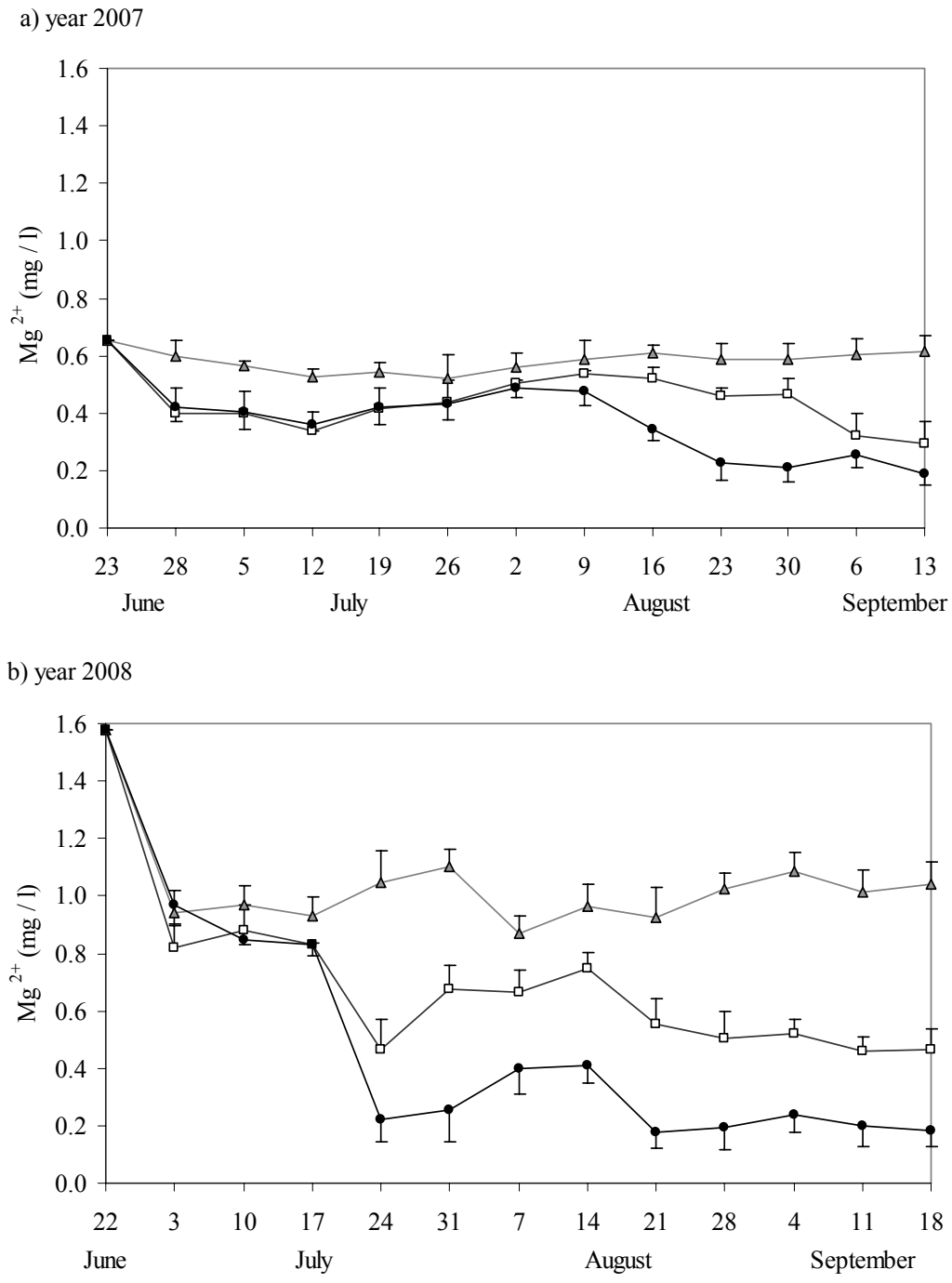

Fig. 4. The concentrations of magnesium ion (mg/l) in three treatments of the experiment in: a) year 2007, and b) year 2008. Grey triangles – Phyto; white squares – Phyto+Dreis A; black circles – Phyto+Dreis B. Points represent means of three replicates ±SD.

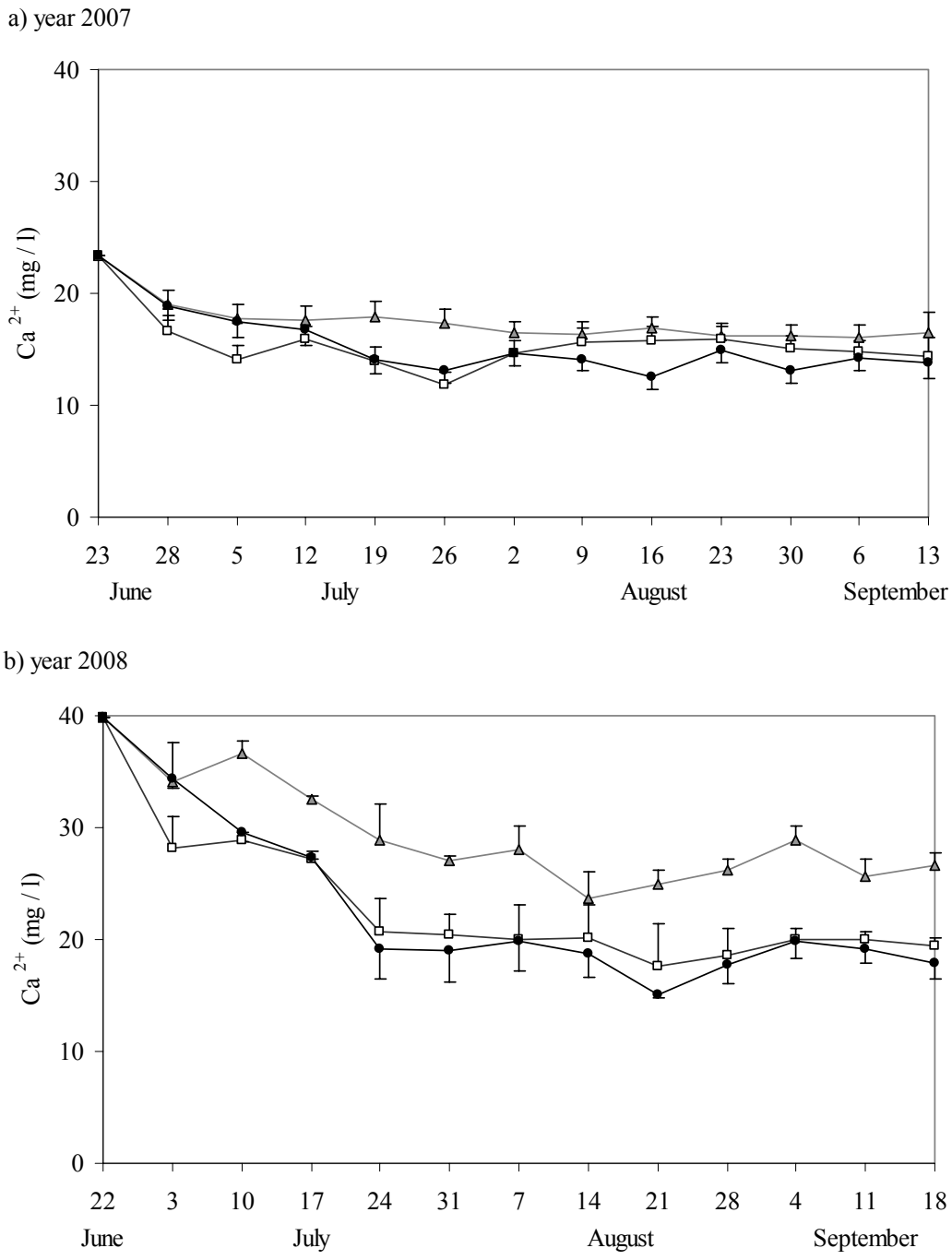

Fig. 5. The concentrations of calcium ion (mg/l) in three treatments of the experiment in: a) year 2007, and b) year 2008. Grey triangles – Phyto; white squares – Phyto+Dreis A; black circles – Phyto+Dreis B. Points represent means of three replicates ±SD.

However, the differences in Ca concentration between treatments seemed to be less visible in 2007; they ranged from 18.9-15.05 mg/l in Phyto, 18.9-11.77 mg/l in Phyto+Dreis A, and 18.9-12.55 mg/l in Phyto+Dreis B (Fig. 5a). In 2008, we observed the decreasing tendency in Ca ion concentration in all treatments, but it was the most pronounced in mussel treatments (Fig. 5b). In the control, the calcium concentration fluctuated from 36.67 to 23.65 mg/l. From 24 July, Ca concentration stabilised both in Phyto+Dreiss A and Phyto+Dreiss B treatments in the ranges 20.71-17.6 and 19.91-15.07 mg/l respectively.

3.4. Physical-chemical correlations

Values of Pearson's correlation coefficient indicated a significant positive relationship between magnesium and calcium ion concentrations in both 2007 ($r=0.632$; $P<0.001$) and 2008 ($r=0.889$; $P<0.001$). In 2007 magnesium and calcium concentrations were negatively correlated with pH ($r=-0.33$; $P=0.040$ and $r=-0.582$; $P<0.001$, respectively). We did not find significant correlations of either Mg or Ca with temperature in this year.

In 2008 negative correlations of both magnesium ($r=-0.392$; $P=0.014$) and calcium concentration ($r=-0.348$; $P=0.030$) with pH were also observed. Magnesium ion concentrations in mussel treatments were positively correlated with temperature ($r=0.45$; $P=0.023$), whereas we did not find such a correlation in the control ($r=0.157$; $P=0.610$). There was also no correlation between calcium and temperature.

3.5. Concentration of chlorophyll *a*

At the start of the experiment, chlorophyll *a* concentration amounted to 22.91 μg/l in 2007 and 44.26 μg/l in 2008. Despite such differences, the changes of Chl *a* dynamics were similar in both study years (Fig. 6a,b). During the first month of the study, Chl *a* concentrations decreased in all treatments. Subsequently, in Zebra Mussel treatments, the biomass of algae started to increase and achieved maximal concentrations of chlorophyll *a* in the beginning of September 2007 as well as in 2008 (in Phyto+Dreis B: 78.10 and 76.50 μg/l in 2007 and 2008, respectively).

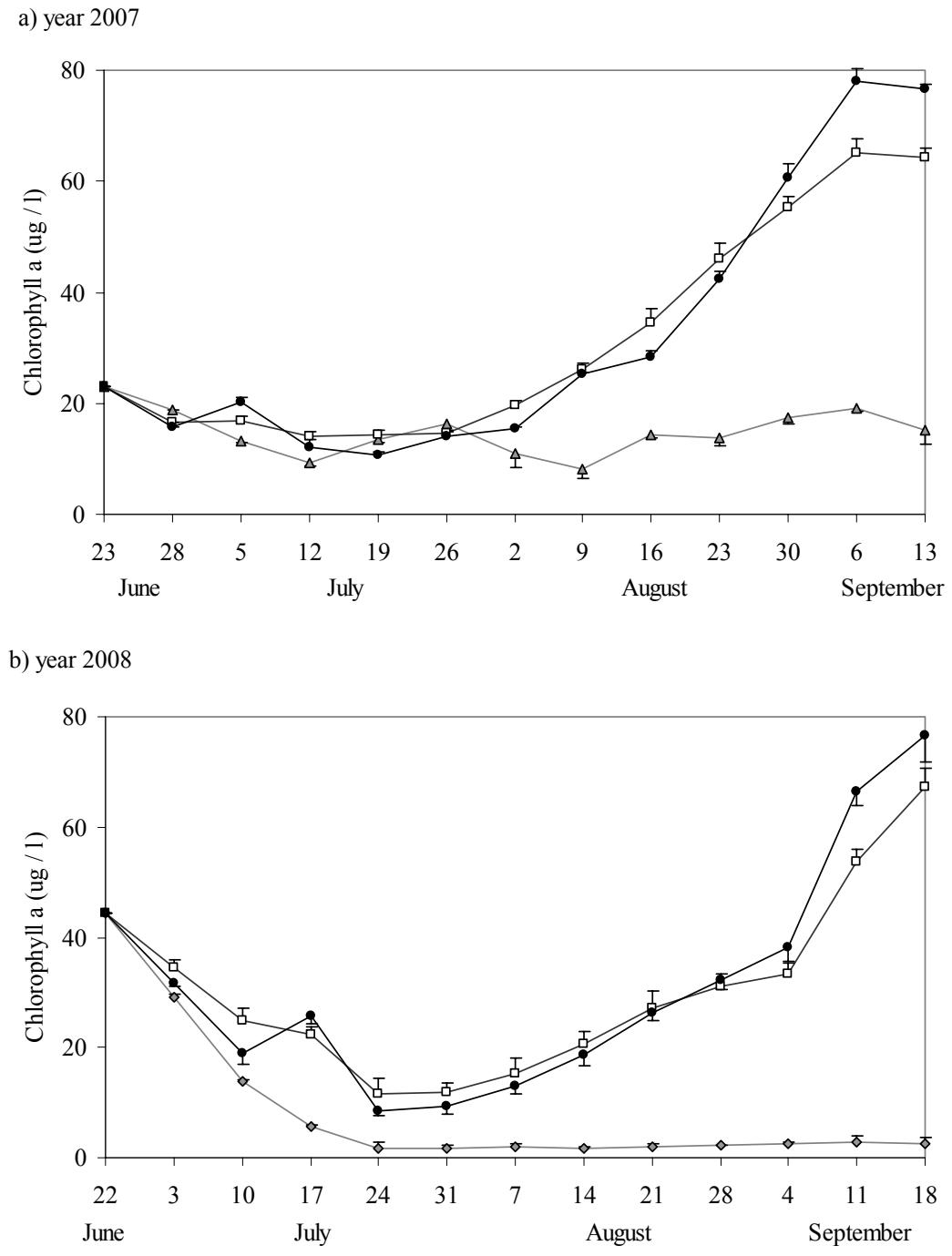

Fig. 6. The chlorophyll *a* concentrations (μg/l) in three treatments of the experiment in: a) year 2007, and b) year 2008. Grey triangles – Phyto; white squares – Phyto+Dreis A; black circles – Phyto+Dreis B. Points represent means of three replicates ±SD.

In the control, Chl *a* concentration was low until the end of the study in both years (15.10 μg/l in 2007 and 2.68 μg/l in 2008; Fig. 6a,b). These differences between lower Chl *a* concentrations in the control and higher Chl *a* concentrations in both Zebra Mussel treatments were significant (ANOVA $F_{2,36}$=3,93; P<0.029 and $F_{2,36}$=7,42; P<0.002 in 2007 and 2008, respectively).

4. DISCUSSION

4.1. Magnesium and calcium roles and dynamics

Magnesium is one of the fundamental cations of the mineral complex in all animal and plant tissues, insuring the correct course of catabolic and anabolic processes (PLESHCHITSER 1955). It has an unusual importance for Zebra Mussels, as many studies have shown that this species cannot survive in Mg-deficient water (DIETZ *et al.* 1994). Elevated Mg/Ca molar ratios in the surrounding water favour the precipitation of aragonite $CaCO_3$-deposits over calcite, which indicates the crucial, regulatory role of magnesium in Zebra Mussel shell production (CHECA *et al.* 2007; HALLSTAN *et al.* 2010). DIETZ and co-workers (1994) have also indicated the exceptional importance of magnesium in Zebra Mussel osmoregulation. The sensitivity of Zebra Mussels to Mg deficits in water is probably an effect of the limited ability of the organism to reduce its rate of magnesium loss, which results from a high passive permeability to Mg by mantle or gill epithelia and substantial urinary loss of Mg (DIETZ *et al.* 1994). Severe decreases in the amount of magnesium ions in Zebra Mussel hemolymph and tissues are especially pronounced during stress (MARTEM'YANOV 2000). Considering, the fact that the Mg/Ca molar ratio in lakes is from 7 to even 43 times lower than in oceans, magnesium becomes a limiting factor in the physiology and shell production of Zebra Mussels in freshwater ecosystems (HALLSTAN *et al.* 2010). Immense demands of Zebra Mussels for magnesium are shown in our study. The presented results, especially from the year 2008, indicated a significant influence of Zebra Mussels on magnesium ion concentrations in water (Fig. 4). Analyzing Mg concentration changes in 2008 (Fig. 4b), it seems that the impact of mussels was abundance-dependent, as Mg loss was proportional to Zebra Mussel biomass. However, these differences were not

statistically significant. Furthermore, in both study years, concentrations of magnesium held to the same level of 0.2 mg/l in Phyto+Dreis B treatments during the last 4-5 weeks of the experiment (Fig. 4), despite the different physical conditions.

In 2008 changes of Mg concentrations in water were correlated with temperature, but only in Zebra Mussel treatments (not in control), which may show that physiological processes of mussels connected with uptake or/and loss of Mg are reliant on temperature. In 2007, we observed similar tendencies, but they were not significant, probably because much lower mean temperature (Fig. 2a) decreased the biological activity of the Zebra Mussels. Due to this effect, the influence of *D. polymorpha* on changes in Mg concentration in this year was not as clear as it was in 2008.

In our study both magnesium and calcium ion concentrations were negatively correlated with pH, which was reflected in the observed trends; concentration of Mg and Ca decreased and pH values increased during the experiment (Fig. 3, 4, 5). In both Zebra Mussel treatments, pH increased during the daytime due to the intensive uptake of carbon dioxide by algae during photosynthesis (Fig. 6). The range of pH influences the physiological ion balance of Zebra Mussels. VINOGRADOV and co-workers (1993) indicated that loss of calcium to the external environment significantly increases at pH values below 7.0. This process may result in Zebra Mussel mortality. Conversely, the increase of pH simultaneously causes an increased influx of calcium to the Zebra Mussel's body (VINOGRADOV *et al.* 1993), a finding that may elucidate our results. In 2008, in mussel treatments, pH exceeded the value of 9.6, probably because of the high rate of CO_2 uptake by intensively photosynthesising green algae. However, despite such a pH value being recognised as the upper tolerance limit of Zebra Mussels, (BOWMAN, BAILEY 1998) we observed permanent filtering activity of Zebra Mussels with no signs of mortality. Increase of Zebra Mussel weight on the end of the study in both years (Fig. 1) confirms that animals stayed alive and physiological active in the experiment duration.

The abundance of calcium is reported by some authors (e.g. RAMCHARAN 1992) to be the main predictor for the occurrence of Zebra Mussels, which reflects its importance for the mussel's viability. Calcium is involved in muscular contractions, cellular cohesion, nervous functions and the maintenance of pH (CHÉTAIL, KRAMPITZ 1982), and of course, it is indispensable to shell growth in the form of calcium carbonate (PIECHOCKI, DYDUCH-FALNIOWSKA 1993). In our experiment, calcium loss was significantly larger in mussel treatments than in the controls, but this effect was not as clear as it was for magnesium (Fig. 4, 5). Results were again more obvious in 2008 than in 2007; in mussel treatments calcium concentrations decreased from 39.87 to mean 19 mg/l and oscillated around this level to the end of study (Fig. 5b). Zebra Mussels require Ca ion concentrations greater than 12 mg/l to establish a significant population, which is considerably higher than what is required by other bivalve molluscs (typically 3-4 mg/l) (HEATH 1993). The upper Ca limit is not unambiguously known, e.g. O'NEILL (1996) indicates a high colonisation potential of Zebra Mussels over a calcium range from 25 to even >125 mg/l. Even though Zebra Mussels are normally found in water with moderate to high calcium concentrations, they will survive in Ca-poor environments, providing the bathing fluid contains magnesium in minimal amounts (DIETZ *et al.* 1994). Moreover, mussels survive Ca-deficiency by mobilising calcium from their shells in order to maintain necessary levels of calcium in hemolymph (DIETZ *et al.* 1994). This fact shows that calcium is not a limiting factor in the Zebra Mussel's biology, as is magnesium.

4.2. Reduction of the excessive magnesium input to freshwater ecosystems

The important sources of magnesium in freshwater ecosystems are fertilizers used in agriculture and organic pollutants from farms, such as animal faeces and herbal food residue (SAPEK 2007). Magnesium from these sources gets to the lakes, reservoirs and rivers through ground water or/and surface flow from farming regions. Although, magnesium is not considered a pollutant, reduction of excessive Mg input to water bodies may be important for water quality, because magnesium, a main component of chlorophyll, has a stimulating effect on algal and cyanobacterial

growth (e.g. TUBEA *et al.* 1981; UTKILEN 1982). However, magnesium may be only one of the factors contributing to algal blooms – the most important seem to be nitrogen and phosphorus, both of which amplify the symptoms of eutrophication. In our experiments the highest values of chlorophyll *a* were observed in treatments with Zebra Mussels (Fig. 6), where, due to a mollusc biological activity, Mg concentration was low but nutrient loads excreted by Zebra Mussels (P and N) were high (results in WOJTAL-FRANKIEWICZ, FRANKIEWICZ 2010) - what indicate that Zebra Mussel may increase water trophy. In order to avoid such a negative effect of Zebra Mussels on water quality in natural ecosystems, strong control of their population size is necessary (WOLNOMIEJSKI, WOŹNICZKA 2008; WIŚNIEWSKI, personal communication). This control may contribute to decrease of an internal input of nutrients and N:P ratio in water (WOJTAL-FRANKIEWICZ, FRANKIEWICZ 2010). For restoring and controlling nutrient input from the catchment area (external input) a complex strategy should be used, according to the interdisciplinary concept for sustainable water resources management (ZALEWSKI 2000). Reduction of magnesium and phosphorus loads is possible by the optimization of agricultural practices and creation of ecotone buffers, where nutrients are transformed into plant biomass. It is especially necessary in the case of improper agricultural management, e.g. monocultural production of crop and livestock, which often destroys the process of residue recycling (PETERSEN 2001). In such cases, the construction or preservation of wetlands at the shore line is crucial for protection against pollutants that inevitably flow from the environment. Moreover, ecotones serve as a productive habitat for species present in adjacent ecosystems, increasing biodiversity and thereby the resistance and resilience of water ecosystems against human impact (SANTIAGO-FANDINO, NEATE 2002). However, this strategy so effective for pollution reduction may be not quite useful for *D. polymorpha* control. This is because submerged macrophytes are the important substrate for settling of Zebra Mussel's planktonic larvae (veligers) and their availability is one of the main factors responsible for raising the abundance of Zebra Mussel population (KOBAK, WIŚNIEWSKI 1998).

In conclusion, Zebra Mussels are able to considerably influence the pool of available magnesium in water of inhabited ecosystems. As the presented results indicate, Zebra Mussels have a higher rate of magnesium uptake than do phytoplankton, and they seem to be important Mg competitors for algae and cyanobacteria. Translocation of magnesium in Zebra Mussel tissues and shells may significantly reduce amount of Mg accessible for phytoplankton. On the other hand, during times of high phytoplankton abundance, intensive photosynthesis causes an increase of pH, which reduces the level of soluble magnesium in water (ROGER, KULASOORIYA 1980) and may limit the availability of Mg for Zebra Mussels. Perhaps, limitation of magnesium inflow to freshwater ecosystems may contribute to the reduction of Zebra Mussel expansion, but this process depends on many factors and requires detailed research. We believe that knowledge about impact of Zebra Mussels on magnesium dynamics may be useful for studies focused on methods of controlling not only this invasive species, but also algal blooms.

5. REFERENCES

ABRASZEWSKA, A. 2006. Dynamics of the occurrence of *Dreissena polymorpha* (Pallas,1771) in the Sulejów Reservoir in 1997-2005. In: B.M. POKRYSZKO (ed.), The 22th Polish Malacological Seminar. Folia Malacol. 14: 85.

BOWMAN, M.F., BAILEY, R.C. 1998. Upper pH tolerance limit of the Zebra Mussel (*Dreissena polymorpha*). Can. J. Zool. 76: 2119–2123.

CASAGRANDI, R., MARI, L., GATTO, M. 2007. Modelling the local dynamics of the Zebra Mussel (*Dreissena polymorpha*). Freshwat. Biol. 52: 1223–1238.

CAGNARD, O., BOUDIN, I., LEMOIGNE, I., CARTNICK, K. 2006. Assessment of emerging optic sensors (fluoroprobes) for algae on-line monitoring. American Water Works Association WQTC Conference, Denverss, pp. 1–10.

CHECA, A.G., JIMENEZ-LOPEZ, C., RODRIGUEZ-NAVARRO, A., MACHADO J. P. 2007. Precipitation of aragonite by calcitic bivalves in Mg-enriched marine waters. Mar. Biol. (Berl) 150: 819–827.

CHÉTAIL, M., KRAMPITZ, G. 1982. Calcium and skeletal structures in mollusks: concluding remarks. Malacologia 22: 337–339.

DIETZ, T.H., LESSARD, D., SILVERMAN, H., LYNN, J.W. 1994. Osmoregulation in *Dreissena polymorpha*: the importance of Na, Cl, K, and particularly Mg. Biol. Bull. 187: 76–83.

HALLSTAN, S., GRANDIN, U., GOEDKOOP, W. 2010. Current and modeled potential distribution of the zebra mussel (*Dreissena polymorpha*) in Sweden. Biol. Invasions 12: 285–296.

HEATH, R.T. 1993. Zebra Mussel Migration To Inland Lakes And Reservoirs: A Guide For Lake Managers, Kent State University, Ohio Sea Grant College Program, online at http://www.sgnis.org/publicat/managers.htm.

HOROHOV, J., SILVERMAN, H., LYNN, J.W., DIETZ, T.H. 1992. Ion transport in the freshwater zebra mussel, *Dreissena polymorpha*. Biol. Bull. 183: 297–303.

IZYDORCZYK, K., CARPENTIER, C., MRÓWCZYŃSKI, J., WAGENVOORT, A., JURCZAK, T., TARCZYŃSKA, M. 2009. Establishment of an Alert Level Framework for cyanobacteria in drinking water resources by using the Algae Online Analyser for monitoring cyjanobacterial chlorophyll *a*. Water Res. 43: 989–996.

KARATAYEV, A.Y., BURLAKOVA, L.E., PADILLA, D.K. 2002. Impacts of zebra mussels on aquatic communities and their role as ecosystem engineers. In: E. LEPPAKOSKI, S. GOLLASCH, S. OLENIN (eds), Invasive aquatic species of Europe. Distribution, impacts and management, Kluwer Academic Publishers, pp. 433–446.

KOBAK, J., WIŚNIEWSKI, R. 1998. Larval settlement and distribution of juveniles of zebra mussel (*Dreissena polymorpha* Pall., Bivalvia) on selected artificial substrates. AUNC Limnological Papers 20: 25–54.

LEWANDOWSKI, K. 2001. Development of populations of *Dreissena polymorpha* (Pall.) in lakes. Folia Malacol. 9: 173–216.

MACISAAC, H.J. 1996. Potential abiotic and biotic impacts of Zebra Mussels on the inland waters of North America. Am. Zool. 36: 287–299.

MARTEM'YANOV, V.I. 2000. The dynamics of the sodium, potassium, calcium, magnesium contents in the fresh water mollusc zebra mussel *Dreissena polymorpha* during stress. J. Evol. Biochem. Physiol. 36: 41–46.

O'NEILL, CH.JR. 1996. The zebra mussel impacts and control, New York Sea Grant, Cornell University, State University of New York; Cornell Cooperative Extension, Information Bulletin 238: 1–62.

PETERSEN, H-G. 2001. Economic aspects of agricultural area management and land/water ecotone conservation. Ecohydrol. Hydrobiol. 1: 97–110.

PIECHOCKI, A., DYDUCH-FALNIOWSKA, A. 1993. Mięczaki (Mollusca). Małże (Bivalvia). Fauna słodkowodna Polski, 7A, PWN, Warszawa, pp. 1–204.

PLESHCHITSER, A.L. (GOR'KII) 1955. Biological role of magnesium. Adv. Contemp. Biol. XL 1: 52–67.

RAMCHARAN, C.W., PADILLA, D.K., DODSON, S.I. 1992. Models to predict occurrence and density of the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 49: 2611–2620.

RICHARDSON, T.L., LAWRENZ, E., PINCKNEY, J.L., GUAJARDO, R.C., WALKER, E.A., PAERL, H.W., MACINTYRE, H.L. 2010. Spectral fluorometric characterization of phytoplankton community composition using the Algae Online Analyser. Water Res. 44: 2461–2472.

ROGER, R.A., KULASOORIYA, S.A. 1980. Blue-green algae and rice, Los Baños, Laguna, IRRI. pp. 1–112.

SANTIAGO-FANDINO, V., NEATE, J. 2002. Phytotechnologies and ecohydrology: A comprehensive approach to watershed management. Ecohydrol. Hydrobiol. 2: 39–47.

SAPEK, A. 2007. Magnesium circulation in environment against the background of oxygen concentration in countryside water resources. Ochrona Środowiska i Zasobów Naturalnych 31: 317-322, Dział Wydawnictw IOŚ.

STAŃCZYKOWSKA, A. 1983. Inwazje zwierząt. Przyr. Pol. 5: 20-21.

STRAYER, D.L. 1991. Projected distribution of the zebra mussel, *Dreissena polymorpha*, in North America. Can. J. Fish. Aquat. Sci. 48: 1389–1395.

TARCZYŃSKA, M., ROMANOWSKA-DUDA, Z., JURCZAK, T., ZALEWSKI, M. 2001. Toxic cyanobacterial blooms in drinking water reservoir – causes, consequences and management strategy. Wat. Sci. Technol.: Water Supply 1: 237–246.

TUBEA, B., HAWXBY, K., MEHTA, R. 1981. The effects of nutrient, pH and herbicide levels on algal growth. Hydrobiologia 79: 221–227.

UTKILEN, H.CH. 1982. Magnesium-limited growth of the cyanobacterium *Anacystis nidulans*. J. Gen. Microbiol. 128: 1849–1862.

WOJTAL-FRANKIEWICZ, A., FRANKIEWICZ, P. 2010. The impact of pelagic (*Daphnia longispina*) and bentic (*Dreissena polymorpha*) filter feeders on chlorophyll and nutrient concentration. Limnologica, DOI:10.1016/j.limno.2010.09.001.

WOLNOMIEJSKI, N., WOŹNICZKA, A. 2008. A drastic reduction in abundance of *Dreissena polymorpha* Pall. in the Skoszewska Cove (Szczecin Lagoon, River Odra estuary): effects in the population and habitat. Ecol. Questions 9: 103–111.

VINOGRADOV, G.A., SMIRNOVA, N.F., SOKOLOV, V.A., BRUZNITSKY, A.A. 1993. Influence of chemical composition of the water on the mollusk *Dreissena polymorpha*. In: T.F. NALEPA, D.W. SCHLOESSER (eds), Zebra Mussels: biology, impacts, and control, Lewis Publishers, Boca Raton, Florida, pp. 283–293.

ZALEWSKI, M. 2000. Ecohydrology the scientific background to use ecosystem properties as management tool toward sustainability of freshwater resources. Guest editorial Ecol. Eng. 16: 1–8.

Acta Universitatis Lodziensis
——— 2010 ———
Folia Biologica et Oecologica 6: 103–115
(Acta Univ. Lodz, Folia Biol. Oecol.)

PIOTR MINIAS[1], KRZYSZTOF KACZMAREK[2], RADOSŁAW WŁODARCZYK[1] & TOMASZ JANISZEWSKI[1]

[1] Department of Teacher Training and Biodiversity Studies, University of Łódź, Banacha 1/3, 90-237 Łódź, Poland, pminias@op.pl

[2] Medical University of Łódź, Kościuszki 4, 91-425 Łódź, Poland

PATTERN OF POST-JUVENILE MOULT IN COMMON SNIPE (*GALLINAGO GALLINAGO)* AND ITS IMPLICATIONS FOR AGEING OF THE SPECIES

Abstract: External ageing of Common Snipe (*Gallinago gallinago*) still engenders considerable problems. To improve precision of age determination on the basis of plumage characteristics a scheme of post-juvenile moult was investigated in approximately 1200 first-year Common Snipes caught during autumn migration in central Poland. Post-juvenile moult was commenced from body feathers followed by moult of rectrices, lesser/median wing coverts and tertials. Moult sequence showed high inter-individual variability and was started in rectrices (36.9%), wing coverts (25.3%), tertials (8.4%) or simultaneously in several of these tracts of feathers (29.4%). Moult of rectrices was finished before completion of moult of wing coverts and tertials. Moult of tertials finished as the last from all age-indicative tracts of feathers. Consequently, tertials were suggested as the most useful for ageing of first-year Common Snipes in an advanced stage of moult. There was no case of moult of the outermost tertial in first-year birds. The second tertial from distal side of wing was moulted as the last one within this tract of feathers and thereby should be of special interest during plumage examination.

Key words: feather replacement, moult sequence, waders, *Charadrii*, Poland

DOI: 10.2478/v10107-009-0010-5

1. INTRODUCTION

Common Snipe (*Gallinago gallinago*) is one of a few Palaearctic waders which still engender considerable problems in external ageing. Impossibility of age determination is closely associated with moult scheme typical for the species. First-year snipes during the post-juvenile moult replace all tracts of feathers which are, according to the current state of knowledge, diagnostic for age identification. These are namely lesser and median coverts, scapulars, tertials and rectrices (GLUTZ VON BLOTZHEIM *et al.* 1977). As the plumage attained by first-year snipes after conclusion of post-juvenile moult is indistinguishable from the one of adults, all individuals which completed moult during the autumn migration are impossible to age (FOGARTY *et al.* 1977). Apart from standard plumage examination a number of other methods were suggested to be useful in external ageing of Common Snipe. A degree of wear of flight-feathers was surmised to differ between age groups, due to looser structure of vanes in first-year birds, which makes them more prone to abrasion and bleaching (PRATER *et al*. 1977). Although this criterion proved satisfactory in the Great Snipe (*Gallinago media*) and a number of other wader species (SÆTHER *et al.* 1994), in Common Snipe such contrast is extremely difficult (or usually not possible) to notice during standard bird examination and strongly depends on light conditions. Thus, the method is highly erroneous and should rather not be applied during fieldworks (STRANDGAARD 1986). Another approach to ageing methodology employed in *Gallinago* genus was discriminant analysis of morphological measurements. Nevertheless, distcriminant functions were developed only for the Wilson's Snipe (*G. delicata*), in which the most accurate equations allowed correct ageing of 66-82% of males and 73-84% of females (MCCLOSKEY, THOMPSON 2000). In the case of Eurasian subspecies of Common Snipe (*G. g. gallingo*) discriminant functions were so far proved useful only in sex identification (GREEN 1991). Colour of bill and legs was also suggested to be age-dependent and consequently proposed as a reliable ageing criterion (TUCK 1972). This method however appeared to be relatively accurate only at the beginning of migration period, but it failed to be applicable later, as the moult progressed (WHITEHEAD 1965; HOFFPAUIR 1969). Lack of any universal ageing criteria means that by the

time some new methods are developed, standard plumage examination procedures will have to prevail. Therefore, maximization of efficiency of snipe ageing on the basis of plumage characteristics seems indispensable in the field studies on this species. In Common Snipe colours and patterning of feathers are extremely complex and show high inter-individual variation, which further impedes ageing attempts. Thereby detailed knowledge on the moult sequence of this species is needed, as it would indicate potentially most age-revealing tracts of feathers. Only under these conditions effective examination of bird plumage will be possible and the rate of individuals with undetermined age is likely to reach irreducible minimum. The data on the moult of Common Snipe are scarce and descriptive, usually confined to a phenological approach (OAG MÜNSTER 1975; DEVORT 1997; ROUXEL 2000). The aim of this study is to provide quantitative data on the post-juvenile moult of Common Snipe and to improve precision of existing methods of its external ageing.

2. METHODS

Common Snipes were caught at Jeziorsko reservoir (51°73'N, 18°63'E) in central Poland. Walk-in traps and mist-nets were used, but their number and localization was variable and dependent on the water level (BARGIEL *et al.* 1998). The data were collected during the years 2004-2007. A study period during all the years of research lasted from the beginning of July until the end of September. The time of fieldworks covered the main autumn migration wave of Common Snipe through central Poland (JANISZEWSKI *et al.* 1998; TOMIAŁOJĆ, STAWARCZYK 2003). Moult phenology of snipes migrating through Jeziorsko reservoir was described in details by MINIAS and others (2010). All birds were ringed and measured according to the standard procedures (BUSSE 2000). In total, 1383 snipes were caught throughout all years of study. Age determination was based on the combination of standard diagnostic features, including patterning of tertials, lesser/median coverts, scapulars and the shape of the outermost rectrices (PRATER *et al.* 1977; CHYLARECKI 1985; KACZMAREK *et al.* 2007; WŁODARCZYK *et al.* 2008). 1198 of snipes were aged as first-year birds, 150 as adults and 35 individuals were not possible to be aged. 549 of first-year snipes were in an active moult.

Moult data were recorded with usage of special moult cards. Tertials (TS) were numbered from distal (T1) to proximal (T5) side of the wing. Rectrices (RS) were divided into two symmetrical parts (L-left, R-right) and were numbered from outer (R1) to inner (R7) side of the tail. Each rectrix and tertial was denoted a moult score in the 5-point BTO scale (GINN, MELVILLE 1983). Lesser and median coverts were divided into three distinct groups of old (before shedding), growing and new feathers. Percentage of each category was recorded with a precision of 5%. The fraction of growing body feathers (BF) was recorded within four different parts of body: head, back (including scapulars), belly feathers and tail coverts with the same precision. Moult indexes of tertials and rectrices were calculated as a sum of all moult scores of individual feathers (SNOW 1967; GINN, MELVILLE 1983). Moult index of wing coverts (WC) was expressed as a percentage of growing and new feathers altogether. Moult sequence within a given tract (RS, TS) was presented with the means of moult scores of particular feathers. Data from all individuals moulting the feather tract served for this purpose. Dependently on the advancement of moult following groups of first-year birds were distinguished: individuals before moult, individuals starting to moult (i.e. moulting only body feathers), and individuals in an intensive stage of moult (i.e. moulting lesser/median wing coverts, tertials or rectrices). In order to define a commencement of moult in different tracts of feathers following categories were assigned: rectrices - moult index <4, tertials - moult index <2, wing coverts - moult index <20.

Fractions of growing body feathers in different tracts were compared with usage of Friedman ANOVA. Kruskal-Wallis ANOVA was used in order to test the differences between moult scores of particular rectrices and tertials. Spearman correlations were used to check for the relationship between intensity/advancement of moult of different tracts of feathers. Interrelationship of moult progression of basic tracts of feathers (rectrices, tertials and wing coverts) was investigated with usage of homogeneity-of-slopes model. Statistical analyses followed ZAR (1996).

3. RESULTS

3.1. General moult scheme

Moult was commenced in body feathers, during replacement of which birds started an intensive moult including lesser/median wing coverts, tertials and rectrices. Sequence of intensive post-juvenile moult showed high inter-individual variability. Data from 95 first-year Common Snipes caught just after commencement of moult showed that most frequently it was started in rectrices (36.9%) or wing coverts (25.3%), however there was also a small fraction of birds which started an intensive moult from tertials (8.4%). 24.1% of birds commenced intensive moult simultaneously in two of these tracts of feathers (rectrices and tertials most frequently – 10.5%). Moreover, 5.3% of birds started intensive moult in all three tracts of feathers at the same time. Moult indexes of wing coverts and of tertials correlated significantly with moult index of rectrices (wing coverts: $r=0.37$, $n=152$, $P<0.001$; tertials: $r=0.45$, $n=152$, $P<0.001$).

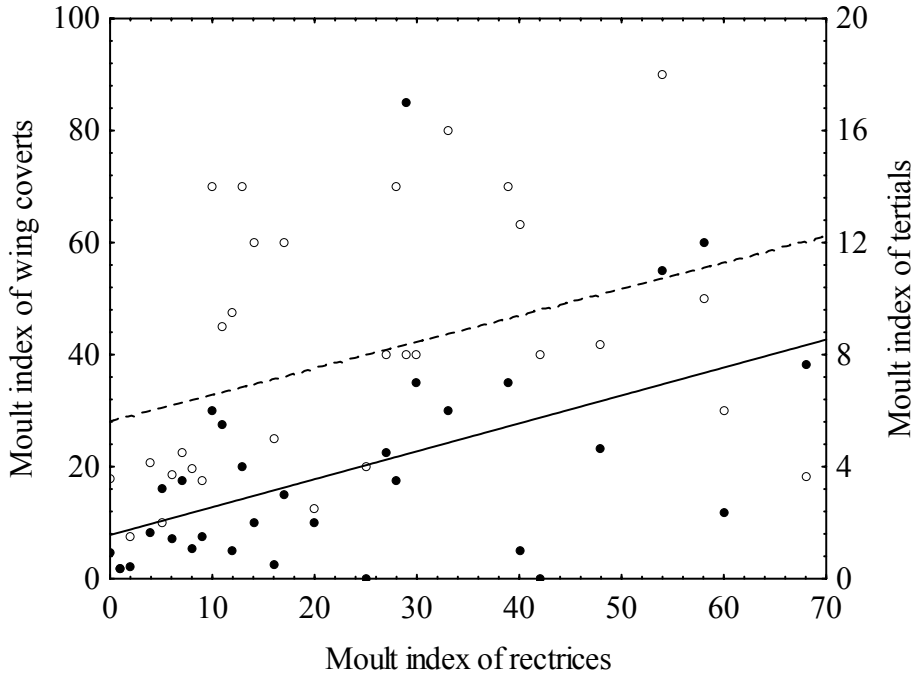

Fig. 1. Dependence of moult of lesser/medium wing coverts (white circles, dashed line) and tertials (black circles, solid line) on advancement of moult of rectrices in first-year snipes.

In general, moult of rectrices was finished before completion of moult of wing coverts and tertials (Fig. 1). Moult of tertials was proceeding at the similar pace as moult of wing coverts (homogeneity-of-slopes model: F=0.69, df=1, P=0.42), however, due to the late shedding of tertials this tract of feathers was the last to be completely moulted.

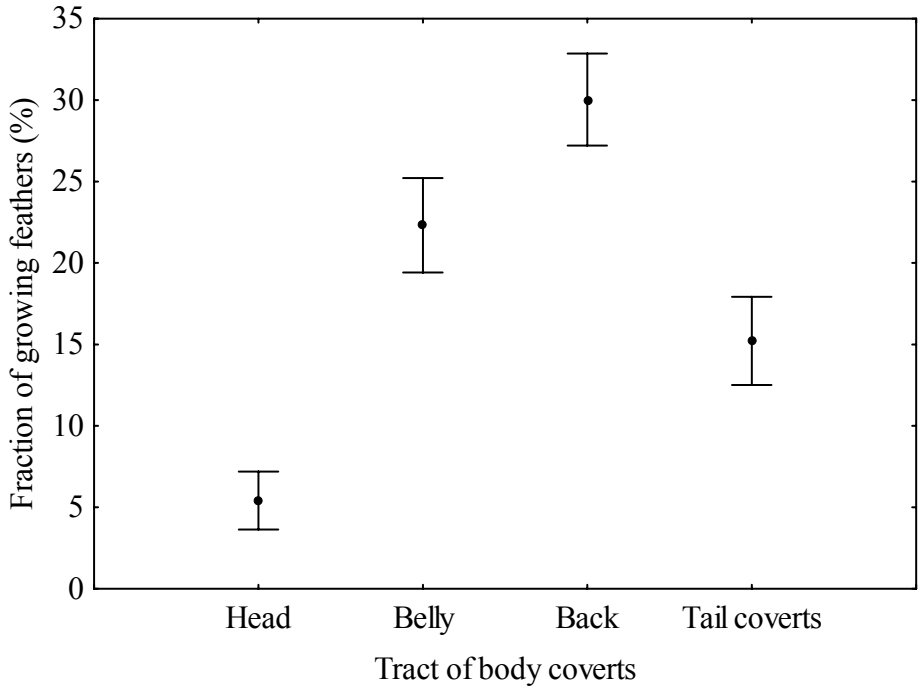

Fig. 2. Intensity of moult of different tracts of body feathers expressed by the fraction of feathers growing simultaneously. Central point – mean; whiskers – 1.96*SE.

3.2. Body feathers

Distinguished tracts of body feathers (i.e. head, back, belly feathers and tail coverts) were moulted with different intensity, as there were significant differences in the fraction of feathers growing simultaneously in each tract (Friedman ANOVA: χ^2=450.86, df=3, P<0.001, Fig. 2). Head feathers were moulted with the lowest intensity (on average 5.4% of feathers growing simultaneously) in contrast to the back feathers, which were moulted in the most intensive way among distinguished

tracts (30.0% of feathers growing simultaneously). Moult of body feathers intensified when replacement of other tracts of feathers (i.e. tertials, rectrices or wing coverts) was commenced as significant increase in the fraction of growing body feathers was recorded at this point (Mann-Whitney U-test: $Z=7.05$, $n_1=173$, $n_2=249$, $P<0.001$). Intensity of moult of tail coverts was associated with intensity of moult of rectrices, which was expressed as a number of simultaneously growing feathers ($r=0.41$, $n=149$, $P<0.001$).

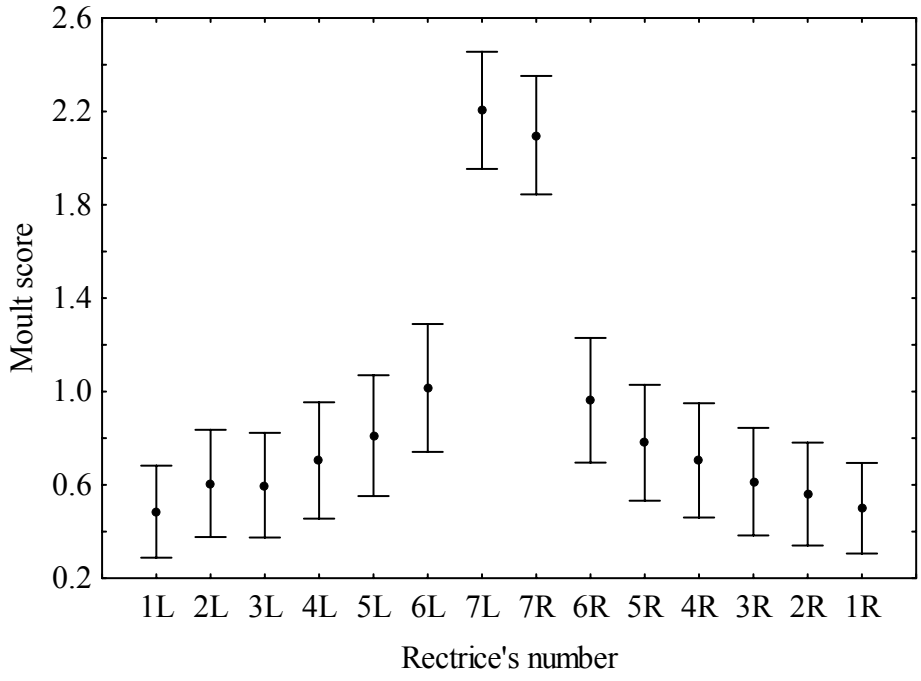

Fig. 3. Sequence of moult of rectrices in first-year snipes expressed by moult scores of particular feathers (L – left side of the tail, R – right side of the tail). Central point – mean; whiskers – 1.96*SE.

3.3. Rectrices

There were significant differences in the mean moult scores of particular rectrices (Kruskal-Wallis ANOVA: $H_{13,1848}=401.57$, $P<0.001$), which implied different times of their shedding. Moult of rectrices usually started from one (76% of cases of centrifugal moult, n=110) or significantly less frequently from a few (24%,

χ^2=60.07, df=1, P<0.001) central pairs of feathers and was followed by centrifugal or irregular moult of the whole tail (Fig. 3). Differences in the moult scores were especially marked between central pair of rectrices and the rest of the feathers from this tract (Kruskal-Wallis multiple comparisons: all cases P<0.001), since many birds finished growth of both central feathers before continuation of tail moult. Regular centrifugal moult was recorded in 87% of cases (n=126) and was significantly more frequent than all other types of moult (χ^2=54.48, df=1, P<0.001). Simultaneous moult was recorded only in 2% of individuals and 11% of birds showed irregular moult.

3.4. Tertials

There was no case of moult of the outermost tertial (T1) in first-year birds, so it was excluded from further analyses. Mean moult scores of particular tertials differed significantly (Kruskal-Wallis ANOVA: $H_{3,248}$=53.28, P<0.001).

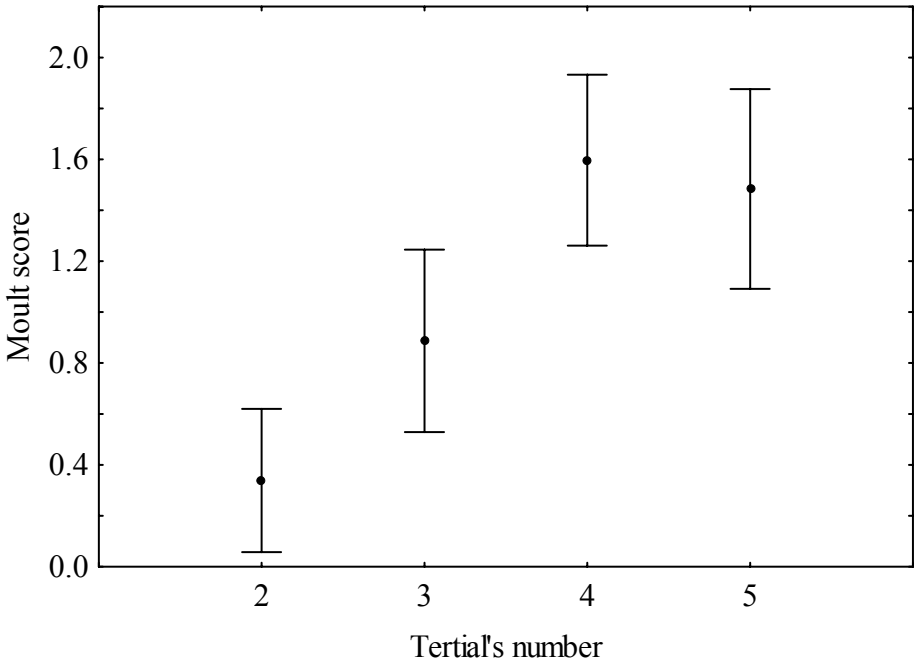

Fig. 4. Sequence of moult of tertials in first-year snipes expressed by moult scores of particular feathers. Central point – mean; whiskers – 1.96*SE.

Following schemes of moult of tertials were distinguished (n=70): moult starting from inner tertials and proceeding outwards (47%), moult starting from outer tertials and proceeding inwards (9%), centrifugal moult starting from middle tertials and proceeding in both directions (44%). In general, two innermost tertials (T4-5) were moulted in the first place and T2 was moulted as the last feather from this tract (Fig. 4). Inter-wing asymmetry in moult of tertials was recorded in 15% of individuals.

4. DISCUSSION

Although sequence of post-juvenile moult of Common Snipe appeared to be individually variable, some general remarks upon the algorithm of ageing procedures of this species can be drawn from our results. Primarily, tertials turned out to be the most useful for ageing of first-year Common Snipes in an advanced stage of moult, as the juvenile feathers in this tract are usually retained longer than age-diagnostic feathers from other tracts. This is particularly valid in the late part of the autumn, when the number of individuals with few retained juvenile feathers is rapidly growing. Outer tertials should be of special interest during age identification procedures, as in most of the cases they are shed as the last ones from this feather tract. The difference between both generations of tertials concerns the shape of subterminal band which in juveniles is parallel to the edge of the feather, whereas in adult-type feathers appears to be rather irregular (WŁODARCZYK *et al.* 2008). Such age-specific patterning is lacking on the outermost tertial, so even though it is not changed during post-juvenile moult, attention should be focused on the second tertial from the distal side of wing. Both wings should be examined in case of any uncertainties, since first-year snipes appeared to show relatively high inter-wing asymmetry of the moult of tertials. Lesser and median wing coverts might be also useful in age determination of birds in advanced moult stage. Contrastingly, rectrices occurred to be of limited usefulness for ageing purposes. Their moulting in first-year birds is usually finished well before all wing coverts and tertials are changed. Nevertheless, due to high frequency of regular centrifugal moult of tail, the outermost rectrices should be primarily examined. This is particularly advisable, as the age-specific differences are most strongly pronounced in this pair of rectrices.

This is manifested by much shorter length and lack of characteristic incision in the inner web of juvenile feathers (PRATER *et al.* 1977). As snipes start post-juvenile moult from body feathers, scapulars are likely to be shed fairly quickly and soon replaced with adult-type fathers, which makes them useless in age identification of birds during later parts of autumn.

Since moult progression of any individual may be considered only in relation to the stage of migration and the time of the season, phenological studies on the moult stage at different stopover sites are desirable. In waders, length of migration is known to be one of the main determinants of moult onset (HOLMES 1971). Long-distance migrants, like Little Stint (*Calidris minuta*), Curlew Sandpiper (*Calidris ferruginea*) or Wood Sandpiper (*Tringa glareola*), usually do not start moulting before they reach wintering grounds in the temperate climatic zones of the southern hemisphere (MIDDLEMISS 1961; ELLIOTT *et al.* 1976). By contrast, waders which cover relatively short distances during the autumn migration start and in many cases may finish moult still being *en route*. Such situation is typical for Common Snipe and many other species with short migration distance, including, for instance, some populations of Dunlin (*Calidris alpina*), Purple Sandpiper (*Calidris maritima*) or Grey Plover (*Pluvialis squatarola*) (HOLMES 1966; BENGTSON 1970; SUMMERS *et al.* 2004; SERRA *et al.* 2006). At Jeziorsko reservoir first-year snipes which started moult in body feathers were recorded since the middle of July, whereas intensively moulting birds appeared at the beginning of August (MINIAS *et al.* 2010). A fraction of completely moulted first-year snipes did not exceed 10% of all caught birds at the end of September (unpublished data). Contrastingly, as much as 52% of first-year birds did not yet begin post-juvenile moult at this time of year (MINIAS *et al.* 2010). This implicates that the majority of first-year birds finish post-juvenile moult on the further stages of migration or at wintering grounds. Similar situation was found during the studies in western Germany (OAG MÜNSTER 1975), where approximately half of first-year snipes caught in the middle of September did not start moulting. The fraction was, however, rapidly falling to 10% at the beginning of October. In Denmark individuals with juvenile feathers were still recorded in November (GLUTZ VON BLOTZHEIM *et al.* 1977). In conclusion, our results suggest that at least until the

end of September only minor fraction of first-year snipes is not possible to be aged on the basis of plumage characteristics during autumn passage through Central Europe. Majority of first-year birds migrating through this part of continent finish post-juvenile moult at the beginning of October occasionally retaining some juvenile feathers until November (GLUTZ VON BLOTZHEIM *et al.* 1977). At this time of autumn migratory period existing ageing techniques start to be inapplicable in Common Snipe, what creates an urgent need for their further improvement.

Acknowledgements

We thank all participants of the fieldworks at the Jeziorsko reservoir, especially Rafał Bargiel, Anna Kleszcz and Łukasz Krajewski.

5. REFERENCES

BARGIEL, R., WŁODARCZYK, R., KALIŃSKI, A. 1998. Catching waders at the Jeziorsko reservoir (western Poland). Ring 20: 77–82.

BENGTSON, S.A. 1975. Timing of the moult of the Purple Sandpiper *Calidris maritima* in Spitsbergen. Ibis 117: 100–102.

BUSSE, P. 2000. Bird station manual. Gdańsk.

CHYLARECKI, P. 1985. Oznaczanie wieku u bekasa *Gallinago gallinago*. Biuletyn Polskiej Stacji Ornitologicznej 9: 30–36.

DEVORT, M. 1997. The Common Snipe. Elements for an action plan. OMPO. Paris.

GINN, H.B., MELVILLE, D.S. 1983. Moult in Birds. BTO Guide number 19. Tring: British Trust for Ornithology.

GLUTZ VON BLOTZHEIM, U.N., BAUER, K.M., BEZZEL, E. 1977. Handbuch der Vögel Mitteleuropas. Bd. 7 Akad. Verlagsges. Wiesbaden.

GREEN, R.E. 1991. Sex differences in the behavior and measurements of common snipes (*Gallinago gallinago*) in Cambridgeshire, England. Ringing and Migration 12: 57–60.

ELLIOTT, C.C.H., WALTNER, M., UNDERHILL, L.G., PRINGLE J.S., DICK, W.J.A. 1976. The migration system of the Curlew Sandpiper *Calidris ferruginea* in Africa. Ostrich 47: 191–213.

FOGARTY, M.J., ARNOLD, K.A., MCKIBBEN, L., POSPICHAL, L.B., TULLY, J. 1977. Common Snipe. In: G.C. SANDERSON (ed.), Management of migratory shore and upland game birds. International Association of Fish and Wildlife Agencies. Washington, D.C., USA, pp. 189–209.

HOFFPAUIR, J.W. 1969. The development of external aging and sexing techniques and trapping methods for common snipe (*Gallinago gallinago delicata*) in Louisiana. Thesis, Louisiana State University, Baton Rouge, Louisiana, USA.

HOLMES, R.T. 1966. Breeding ecology and annual cycle adaptations of the Red-backed Sandpipers (*Calidris alpina*). Condor 68: 3–46.

HOLMES, R.T. 1971. Latitudinal differences in the breeding and and schedules of Alaskan Red-backed Sandpipers (*Calidris alpina*). Condor 73: 93–99.

JANISZEWSKI, T., WŁODARCZYK, R., BARGIEL, R., GRZYBEK, J., KALIŃSKI, A., LESNER, B., MIELCZAREK, S. 1998. Avifauna of Jeziorsko reservoir in the years 1989-1996. Not. Ornit. 39: 121–150. [In Polish with English summary].

KACZMAREK, K., MINIAS, P., WŁODARCZYK, R., JANISZEWSKI, T., KLESZCZ, A. 2007. A new insight into the ageing of Common Snipe *Gallinago gallinago* – the value of contrast within the wing-coverts of adults. Ringing and Migration 23: 223–227.

MCCLOSKEY, J.T., THOMPSON, J.E. 2000. Ageing and sexing Common Snipe using discriminant analysis. J. Wildl. Mgmt. 64: 960–969.

MIDDLEMISS, E. 1961. Biological aspects of *Calidris minuta* while wintering in South-West Cape. Ostrich 32: 107–121.

MINIAS, P., KACZMAREK, K., WŁODARCZYK, R., JANISZEWSKI, T. 2010. Adjustments and exploitation of body stores during post-juvenile moult in Common Snipe *Gallinago gallinago*. Waterbirds 33: 116–120.

OAG MÜNSTER. 1975. Migration, moult and biometry of the Snipe (Gallinago gallinago) in the sewage farms Münster. J.Orn. 116: 455–487. [In German with English summary].

PRATER, A.J., MARCHANT J.H., VUORINEN, J. 1977. Guide to the identification and ageing of Holarctic waders. BTO Guide number 17. Tring: British Trust for Ornithology.

ROUXEL, R. 2000. Snipes of the Western Palearctic. Ed. OMPO. Eveil Nature, Saint Yrieix sur Charente, France.

SÆTHER, S.A., KÅLÅS, J.A., FISKE, P. 1994. Age determination of breeding shorebirds: quantification of feathers wear in the Lekking Great Snipe. Condor 96: 959–972.

SERRA, L., CLARK, N.A., CLARK, J.A. 2006. Primary moult, body mass and migration of Grey Plovers *Pluvialis squatarola* in Britain. Ibis 148: 292–301.

SNOW, D.W. 1967. A Guide to Moult in British Birds. BTO Guide noumber 11. Tring: British Trust for Ornithology.

STRANDGAARD, K. 1986. Ageing and sexing of the Common Snipe (*Gallinago gallinago*). Proc. Third European Woodcock and Snipe Workshop. Paris, France, pp. 24–36.

SUMMERS, R.W., UNDERHILL, L.G., NICOLL, M., STRANN, K-B., NILSEN, S.Ø. 2004. Timing and duration of moult in three populations of Purple Sandpipers *Calidris maritima* with different moult/migration patterns. Ibis 146: 394–403.

TOMIAŁOJĆ, L., STAWARCZYK, T. 2003. Avifauna of Poland. Distribution, numbers and changes. PTPP "pro Natura" Wrocław. [In Polish with English summaries]

TUCK, L.M. 1972. The snipes: a study of a genus *Capella*. Canadian Wildlife Service Monograph Series 5.

WHITEHEAD, C.J. 1965. Foods and feeding habitats of the common snipe (*Capella gallinago delicata*) in Cameron Parish, Louisiana, with ecological notes and a discussion of methods of sexing and aging. Thesis, Louisiana State University, Baton Rouge, Louisiana, USA.

WŁODARCZYK, R., KACZMAREK, K., MINIAS, P., JANISZEWSKI, T. 2008. Ageing and sexing of the Common Snipe *Gallinago gallinago gallinago*. Wader Study Group Bull. 115: 45–49.

ZAR, J.H. 1996. Biostatistical Analysis, 3rd ed. Prentice-Hall, London.

Acta Universitatis Lodziensis
——— 2010 ———
Folia Biologica et Oecologica 6: 117–136
(Acta Univ. Lodz, Folia Biol. Oecol.)

ZBIGNIEW WOJCIECHOWSKI, JANUSZ MARKOWSKI, TOMASZ JANISZEWSKI & JANUSZ HEJDUK

Department of Teacher Training and Biodiversity Studies, University of Łódź, Banacha 1/3, 90-237 Łódź, Poland, zbig@biol.uni.lodz.pl

30-YEAR-LONG CHANGES IN TERRESTRIAL VERTEBRATE FAUNA OF NIEBIESKIE ŹRÓDŁA (BLUE SPRINGS) NATURE RESERVE IN TOMASZÓW MAZOWIECKI, CENTRAL POLAND

Abstract: The article focuses on changes in terrestrial vertebrate fauna of Niebieskie Źródła (Blue Springs) Nature Reserve that occurred between two censuses carried out in the area. The first census was carried out in the period of 1968-1970, and the second census in the period of 1998-1999. In 1998-1999 the occurrence of four species of amphibians, 41 of birds and 15 of mammals was recorded. In comparison with investigations from 1968-1970 two species of amphibians, two species of mammals, and nine species of birds were found to have disappeared from the reserve. In the census carried out in 1998 and 1999 we recorded 22 new species of vertebrates: one new species of amphibian, five species of mammals, and 16 species of birds. The recent observations showed a decline in the abundance of amphibian species in the reserve, especially in the closest vicinity to the urban development around the city of Tomaszów Mazowiecki. The quantitative data on birds showed an increase in the numbers and abundance, which was mainly pronounced in the group of forest species, especially cavity and shrub nesters. We suggest that these changes are directly related to the succession of forest vegetation and its developing vertical structure, as well as to an increase in the forestation of the reserve area.

Key words: diversity, species richness, terrestrial vertebrate community, bird community changes, nature reserve

DOI: 10.2478/v10107-009-0011-4

1. INTRODUCTION

A nature reserve, by definition, represents entire environmental variation and should be set up in response to certain scientific criteria. Following these assumptions, the regional complementary and representative reserve network should be formed (BENNETT 1994). In practice most reserves have been formed on the basis of the richest-in-species areas, however, other criteria, such as aesthetic or even socio-economic are also taken into consideration (EMNEBORG, GÖTMARK 2000; RODRIGUES *et al.* 2004). In human-dominated environments nature reserves have played an important role in the conservation of biodiversity (FORMANN 1995; MARGULES, PRESSEY 2000).

The Łódź Voivodeship (18.219 km^2), Central Poland, is the area that is heavily transformed and degraded by human activity. This is confirmed by the lowest index of forestation in the country (20.6%) and by large area of degradation due to the strip mines and electricity power station situated in Bełchatów Industrial Region. Despite the circumstances, there are 89 nature reserves in Łódź Voivodeship, covering a total area of 7 405.06 ha, which constitute 0.4% of the Voivodeship (WĘŻYK 2008).

Niebieskie Źródła Nature Reserve (28.77 ha), situated within the town of Tomaszów Mazowiecki, was created in 1961 to protect a rare geological phenomenon in Central Poland, known as limnocrenic springs and their picturesque watershed (MOWSZOWICZ, OLACZEK 1961). The first faunistic data on terrestrial vertebrates of Niebieskie Źródła, although very scarce and imprecise or sometimes even false, were presented in the documentation of the reserve and in the first popular science publications (MOWSZOWICZ, OLACZEK 1961, 1965). In 1967-1970 there was an inventory of aquatic invertebrates, fishes and one terrestrial insect family Syrphidae (Diptera) (WOJTAS, SOSZYŃSKI 1972), as well as of terrestrial vertebrates in the area of the reserve and its closest vicinity was carried out. The presence of seven amphibian species (TRANDA, CICHOWICZ 1972), 35 breeding bird species (MARKOWSKI, WOJCIECHOWSKI 1972) and 12 mammal species (BARTOSZ, MARKOWSKI 1972) was then recorded. The study showed no distinction in the fauna composition but specificity in the community of limnological invertebrates

(OLACZEK, TRANDA 1990) and indicated some characteristic adaptations in their biology, mainly prolongation of life cycles caused by the stable water temperature of about 9°C (TABACKI 1972; TOMASZEWSKI 1972). Irregular ornithological observations were carried out and published by MARKOWSKI (1982) and SOSNOWSKI (1994, 1995) in the following years. Complex zoological investigations that were conducted in 1997-1998 covered various groups of invertebrates (TOŃCZYK *et al*. 2000).

The aim of this study is to examine the changes in the composition of terrestrial vertebrate fauna of the reserve in relation to the succession of plant associations and urbanization pressure.

2. MATERIALS AND METHODS

2.1. Study area

The total area of the reserve is 28.77 ha, including 22.57 ha of forest, 5.72 ha of canals and flooded areas together with the outlet section of the stream, and 0.48 ha of roads. In the flooded areas there are three larger and six smaller islands of the total area of 4.08 ha. On the flat banks of the canals and on the islands, an alderwood (*Alnetum glutinosae*) and fragments of alder-ash carr (*Fraxineto-Alnetum*) are present. These associations occupy a total of 8.22 ha. The tree stands are formed by 40-60-year old Black Alder (*Alnus glutinosa*) trees; small fragments are 60-80 years old. In the undergrowth layer the European Spindle (*Euonymus europeus*), Alder Buckthorn (*Rhamnus frangula*), and Willow (*Salix sp.*) are recorded. The south-eastern bank and interior of two of the islands are overgrown by fresh pine forest (a total of 10.55 ha), where 40-45-year old Scots Pine (*Pinus silvestris*) trees dominate. Higher level of the flooded area is occupied by an over 100-year-old pine stand. In its middle part, after an extirpation of trees in 1974, Pine and Birch (*Betula sp.*) trees were planted over an area of 1.65 ha. Some other islands and the western bank are occupied by fresh mixed forest (a total of 2.70 ha). This stand is dominated by 40 years old pine trees with contributions of Oak (*Quercus sp.*), Linden (*Tilia sp.*), Larch (*Larix sp.*), Sycamore Maple (*Acer pseudoplatanus*), Northern Maple *(Acer platanoides*) and Poplar (*Populus sp.*). Between the road to the Ludwików

Settlement and the eastern bank 25-30-year old planted alder and birch trees with brushwood of alder buckthorn and European Rowan (*Sorbus aucuparia*) occur.

In comparison with the investigations carried out in the period of 1967-1970 essential changes have occurred in the structure of forest associations due to the aging of tree stands and development of layer structure in the tree stratum, bushes, and creeper vegetation. Besides, the afforested area has increased by 4%. Formally only one section (a meadow - 0.7 ha of the area) has been completely planted with trees, but in practice several forest species (Oak, Lime, Sycamore Maple, Northern Maple) were planted in some other parts of the reserve. After the accidental extirpation of 1.65 ha of fresh forest in the early 1970s, a fragment of the respective area was afforested with pine and birch trees. In the years 1994-1995 hydrotechnical works were carried out to remove the organic sediments from the main water basin and canals by the method of hydraulic dredging. The patches of *Glyceria aquatica* and others patches of reeds, as well as plants growing on the shoreline were removed. The shoreline was straightened and strengthened by the Willow.

2.2. Samples

2.2.1. Amphibians

Material was collected from April to August 1998 while penetrating the whole area of the reserve and its closest vicinity. Amphibians were captured and released after marking. Only the records from the area of the reserve were used in the comparisons.

2.2.2. Birds

A combined cartographic method was used for the qualitative and quantitative assessment of nesting avifauna of the reserve (TOMIAŁOJĆ 1980). In the case of Mallard *Anas platyrhynchos* and Tufted Duck *Aythya fuligula* estimation methods followed the instructions of BOROWIEC *et al.* (1981). Estimates of the abundance of the avifauna in the whole area of the reserve were done during nine bird censuses carried out at 10 to 14 day intervals between 9 April and 23 July 1998. The qualitative changes in bird assemblages were estimated by the fauna turnover

index: $T=(C+E)/(S_1+S_2)$ (DIAMOND, MAY 1997), where: C is the number of colonized species, E is the number of extinct species, S_1 and S_2 are the numbers of species in the particular samples. We also calculated bird species diversity (using the Shannon-Wiener index H'), the evenness index (J'), and total biomass (i.e. the sum of body weight of breeding pairs). The differences between H' indices were tested by t^0 formula presented by HUTCHESON (1970). Values of body weights characteristic for different avian species followed the estimates given by SOKOŁOWSKI (1972). Bird species were categorized according to ecological guild nesting substrates: ground nesters, shrub nesters, tree nesters, cavity nesters (FULLER 1995; JOKIMÄKI, HUHTA 1996); and habitat preferences: edge of the forest or open habitat (treated jointly), and interior of the forest (HAAPANEN 1965; GROMADZKI 1970; TOMIAŁOJĆ *et al.* 1981). The differences in abundance of functional guild classification and forest habitat preferences were tested by nonparametric tests: χ^2 and Wilcoxon signed-rank test.

2.2.3. Mammals

The samples of micromammalia were taken overnight. There were two sampling periods: on the night of 14-15 October 1997 (120 trap-nights) and 10-11 September 1998 (120 trap-nights), three lines of 40 live- and snap-traps placed in 10 m intervals. Trap lines were distributed on two islands and in the western and southern parts of the reserve covering the most typical plant associations (alderwoods, mixed forest, pine forest). Besides, on the night of 3-4 September 1999 a sampling of bats in mist-nets was carried out. Additionally, all traits of mammal presence (bites, trails, excrements) were recorded. For comparison with 1968-1970 period the index of trappability was calculated ($IP=n_i/n_t$, where n_i – number of specimens of given species captured, n_t – number of traps).

3. RESULTS

3.1. Amphibians

During the recent studies four species of anura (29 specimens) were recorded, including Green Toad (*Bufo viridis*) – a new species (Table 1). To show

the trends in the abundance we decided to present the number of anuran species visually recorded within the area of the reserve and compare them with earlier data, when all seen individuals were caught for parasitological investigation (CICHOWICZ, ŻUCHOWSKA 1972).

Table 1. The number of amphibians caught for parasitological investigation in 1968-1970 period (CICHOWICZ, ŻUCHOWSKA 1972; TRANDA, CICHOWICZ 1972) and observed in 1997-1998 period.

Species	1968-1970	1997-1998
Rana esculenta	4	-
Rana lessonae	1	-
Rana temporaria	17	8
Rana arvalis	20	9
Bufo bufo	10	6
Bufo viridis	-	1
Total	52	24

Common Toad (*Bufo bufo*): in the breeding season (April 1998) single specimens were recorded in the outflow part of the flooded area and in the outflow stream. Migrating individuals were recorded several times in various parts of the reserve (from April to July 1998).

European Green Toad (*Bufo viridis*) occurred in the closest vicinity of the reserve. One dead specimen for the first time was noticed on 12 May 1998 on the road leading to the Ludwików Settlement.

Common Frog (*Rana temporaria*) was recorded twice in the outlet part of the flooded area in April 1988. Migrating specimens were observed several times in the western part of the reserve between May and July (totally eight individuals were recorded).

Marsh Frog (*Rana arvalis*) was observed several times (from May to July 1998), most frequently in the western part of the reserve and on the islands (totally nine individuals were recorded).

Additionally, a small reservoir in the close vicinity of the western border of the reserve and a series of oxbow lakes on the right side of the Pilica River floodplain were controlled. In total, eight amphibian species including Common Water Frog (*Rana esculenta*), Pool Frog (*Rana lessonae*), Smooth Newt (*Triturus vulgaris*), and Great-crested Newt (*Tristurus cristatus*), were recorded. Except for the European Green Toad, all above listed species were not numerous.

3.2. Birds

The number of species of breeding birds recorded in 1997 was 41. Two species of waterfowl (Mallard and Tufted Duck) were found to nest in the reserve, in the abundance of 12 and 11 pairs, respectively. The abundance, density and domination of the remaining 37 species are presented in Table 2, along with similar data for 1968-1970 (MARKOWSKI, WOJCIECHOWSKI 1972). The data from 1968-1970 were not collected according to the requirements of the cartographic methods and detailed number of breeding pairs was not quantified for Starling (*Sturnus vulgaris*) and Tree Sparrow (*Passer montanus*). A relative scale was used instead. Nevertheless, the field notes from these years (JM and ZW) allowed precise estimation of nesting pair abundance. The calculated biodiversity indices and turnover index are presented in Table 3. An increase in the species richness and important changes in bird assemblages (disappearance of nine and appearance of 16 new species) were recorded, what was supported by the high value of turnover index T=0.36 (Table 3).

Total abundance was almost three times higher in the later study period, and the difference was significant (χ^2_0=75.65, df=1, P<0.001). With regard to the biotope preferences, there was an almost 5-fold increase in the abundance of interior forest bird species, which changed from 44 pairs in 1968 to 209 pairs in 1998. There was an increase in the frequency of this group from 51.1% to 85.5%. Simultaneously, there was a decrease in the number of open habitat or forest-edge bird species from

39 pairs in 1968 to 35 pairs in 1998 and a pronounced decrease in the frequency of this group from 45.3% to 14.2% (Table 4). The differences in the abundance of both groups were significant (χ^2_0= 42.29, df= 1, P<0.001). Apart from that, significant differences were shown for interior forest bird species, as well as their abundance (Wilcoxon-test Z^0=3.07, p=0.002, n=15), density (Wilcoxon-test Z^0=3.11, p = 0.002, n=15), and biomass (Wilcoxon-test Z^0= 2.97, p =0.003, n=15) were higher in 1998.

There was a slight decrease in the following edge species: Icterine Warbler (*Hippolais icterina*), Golden Oriole (*Oriolus oriolus*) and Serin (*Serinus serinus*). Other edge species, such as Whitethroat (*Sylvia communis*), Tree Sparrow (*Passer montanus*) and Yellowhammer (*Emberiza citrinella*) and one species related to open habitats – Crested Lark (*Galerida cristata*), completely retreated from the reserve (Table 2).

Several forest species, such as: Pied Flycatcher (*Ficedula hypoleuca*), Blackbird (*Turdus merula*), Song Trush (*Turdus philomelos*), Wood Warbler (*Phylloscopus sibilatrix*), Nuthatch (*Sitta europaea*), Wren (*Troglodytes troglodytes*), Coal Tit (*Periparus ater*) and Hawfinch (*Coccothraustes coccothraustes*) appeared in the reserve, and several other increased their numbers, i.e. Great Tit *(Parus major*), Blue Tit (*Cyanistes caeruleus*), Blackcap (*Sylvia atricapilla*), Chiffchaff *(Phylloscopus collybita*), Robin (*Erithacus rubecula*), Chaffinch (*Fringilla coelebs*), Jay (*Garrulus glandarius*) (Table 2).

In the respective comparison of density, 2.8 times higher values were obtained for the later study period (Table 2). Particularly interesting is almost 4.5-fold increase in the group of cavity or semicavity nester birds, 4-fold increase in the shrub nester species and about 2- and 2.5-fold increase in tree and ground nester groups (Table 4).

The frequency of shrub nesters increased between both study periods from 2.3% to 13.5% and their biomass increased from 0.11 to 0.74 kg/10 ha. The same trend was recorded in semicavity and cavity nesters. There was an increase from 29.5% to 39.5% in frequency and from 0.85 to 1.63 kg/10 ha in their biomass. A decrease in frequency and biomass was recorded in two other guild nester groups (Table 4). Only the cavity and semicavity nesters were more abundant (Wilcoxon-

Table 2. Selected characteristics of breeding bird community in the Niebieskie Źródła Nature Reserve in years 1968-1970 and in 1998. Asterisk indicates bird species breeding at the border of the reserve

Species	1968–1970			1998		
	Number of pairs	Density of pairs/10 ha	Dominance (%)	Number of pairs	Density of pairs/10ha	Dominance (%)
Parus major	3	1.04	3.48	34	11.82	13.9
Cyanistes caeruleus	1	0.35	1.16	21	7.30	8.6
Turdus pilaris	2	0.70	2.32	19	6.60	7.8
Phylloscopus collybita	4	1.39	4.65	19	6.60	7.8
Fringilla coelebs	6	2.09	6.97	15	5.21	6.2
Phylloscopus trochilus	6	2.09	6.97	14	4.86	5.8
Sylvia atricapilla	4	1.39	4.65	13	4.52	5.4
Sturnus vulgaris	10	3.46	11.63	13	4.52	5.4
Erithacus rubecula	6	2.09	6.97	10	3.46	4.2
Columba palumbus	2	0.70	2.32	8	2.76	3.4
Ficedula hypoleuca	-	-	-	7	2.43	2.9
Turdus merula	-	-	-	6	2.09	2.5
Phylloscopus sibilatrix	-	-	-	5	1.74	2
Sitta europaea	-	-	-	5	1.74	2
Troglodytes troglodytes	-	-	-	5	1.74	2
Luscinia luscinia	2	0.70	2.32	4	1.39	1.6
Garrulus glandarius	1	0.35	1.16	4	1.39	1.6
Muscicapa striata	1	0.35	1.16	4	1.39	1.6
Certhia brachydactyla	1	0.35	1.16	3	1.04	1.2
Coccothraustes coccothraustes	-	-	-	3	1.04	1.2
Hippolais icterina	4	1.39	4.65	3	1.04	1.2
Pica pica	2	0.7	2.32	3	1.04	1.2
Certhia familiaris	1	0.35	1.16	2	0.7	0.8
Chloris chloris	4	1.39	4.65	2	0.7	0.8
Dendrocopos minor	-	-	-	2	0.7	0.8
Oriolus oriolus	3	1.04	4.48	2	0.7	0.8
Lophophanes cristatus	-	-	-	2	0.7	0.8
Poecile montanus	2	0.7	2.32	2	0.7	0.8
Prunella modularis	-	-	-	2	0.7	0.8
Serinus serinus	3	1.04	4.48	2	0.7	0.8
Sylvia borin	-	-	-	2	0.7	0.8
Turdus philomelos	-	-	-	2	0.7	0.8
Carduelis carduelis	-	-	-	2	0.7	0.8
Corvus corone	1	0.35	1.16	1	0.35	0.4
Dendrocopos major	1	0.35	1.16	1	0.35	0.4
Periparus ater	-	-	-	1	0.35	0.4
Sylvia curruca	-	-	-	1	0.35	0.4
Phoenicurus phoenicurus	-	-	-	*	-	-
Emberiza citrinella	3	1.04	4.48	*	-	-
Streptopelia turtur	1	0.35	1.16	-	-	-
Picus viridis	1	0.35	1.16	-	-	-
Sylvia communis	2	0.70	2.32	-	-	-
Motacilla alba	2	0.70	2.32	-	-	-
Passer montanus	4	1.39	4.65	-	-	-
Galerida cristata	1	0.35	1.16	-	-	-
Acrocephalus arundinaceus	1	0.35	1.16	-	-	-
Acrocephalus palustris	1	0.35	1.16	-	-	-
Anas platyrhynchos	6-7			12		
Aythya fuligula	-			11		
Gallinula chloropus	2			-		
Total	86	30.06	99.94	244	84.86	99.9
Total number of species	34			41		

test Z^0=3.37, p=0.018, n=9), had higher density (Wilcoxon-test Z^0=3.37, p=0.018, n=9), and biomass (Wilcoxon-test Z^0=3.37, p=0.018, n=9) in the second period. The differences in the frequency were not significant. Also indices of biological diversity (H') did not differ (t^0=0.24, p=0.82, df=226).

Table 3. Comparison of diversity indices of birds between two periods of study

Diversity indices	1968-1970	1998
Number of species	32	37
H'	3.145	3.229
H'_{max}	3.61	3.47
J'	0.87	0.93

Table 4. Comparison of bird community indices in two study periods in diet and nesting category of guilds (S – number of species, N – number of pair, D – density pairs/10 ha, F – frequency, B – biomass (kg/10 ha)

Ecological groups	years 1968-1970					year 1998				
	S	N	D	F	B	S	N	D	F	B
Nesting guilds										
Ground nesters	5	20	6.96	23.3	0.23	4	48	18.6	19.8	0.37
Shrub nesters	3	8	2.79	2.3	0.11	7	33	11.4	13.5	0.74
Tree nesters	11	29	10.1	33.7	2.00	13	66	24.9	27.0	4.94
Cavity nesters	13	25	8.81	29.5	0.85	13	97	39.5	39.6	1.63
Habitat preferences										
Edge	13	39	13.7	45.3	1.66	10	35	12.2	14.2	1.82
Interior	16	44	15.3	51.1	1.53	27	209	72.7	85.8	5.86
Other	3	3	1.05	3.5	0.06	-	-	-	-	-
Total	32	86	30.6	99.9	3.25	37	244	84.9	100.0	7.68

The recorded increase in the number of species, index of diversity (H'), abundance and biomass of cavity and brush nester (Table 3, 4) could be explained by progressive succession in plant community and stand age. In the first period of

study 44.21% of the forest area in the reserve was covered with young tree plantations (trees up to 20 years old), 1.2% of forest was in age classes of 40-60 years, and 38.48% of the forest area was covered with older Scots Pine stand of about 80 years, characterized by very simple vertical structure (the canopy top of Scots Pine crown and forest floor of mosses and lichens). In 1974 there was a tree clearance of 1.65 ha old Scots Pine stand and the area was immediately afforested by Scots Pine and birch. In the same year other open habitats (1.65 ha) in the western part of the reserve were also afforested. As a result the total forest area slightly increased. In the second period of study no tree stands below 20 years were recorded and some parts of forests were shifting towards older age classes of 80-100 years. Over 55% of the area of tree stand was in the age of 20-40 years and about 15% in the age of 40-60 years. The advancing aging of tree stands and plant succession processes leaded to development of vertical structure, especially of an understorey layer.

3.3. Mammals

In the area of the reserve a total of 15 species of mammals were recorded. During two periods of removal of small mammals, a total of 12 specimens representing four species were obtained (Table 6) and 162 specimens of three bat species were caught. European Mole (*Talpa europaea*) was encountered in the whole area of the reserve, but only in the eastern part of the reserve was found in higher abundance. Common Shrew (*Sorex araneus*) was frequently recorded in the reserve and a total of three specimens were captured (Table 6). Pygmy Shrew (*Sorex minutus*) was recorded for the first time during the second period of investigation, when one specimen was captured. Also three species of bats were recorded for the first time: Daubenton's Bat (*Myotis daubentonii*) (159 specimens captured), Pond Bat (*Myotis dasycneme*) (two males captured) and Brown Long-eared Bat (*Plecotus auritus*) (one specimen captured). European Hare (*Lepus europaeus*) was noted in the reserve in late autumn and winter. Red Squirrel (*Sciurus vulgaris*) was observed several times over a year in various places in the reserve. Striped Field Mouse (*Apodemus agrarius*) was a common species in the alderwood association, two

specimens were captured. Bank Vole (*Myodes glareolus*) was a very common species. Six specimens were captured in alderwoods and in pine forest habitats. Common Vole (*Microtus arvalis*) was recorded when a dead specimen was found at the road to the eastern bank of the watershed (September 1997). Least Weasel (*Mustela nivalis*) was recorded only once, when an individual was seen while crossing the eastern bank in October 1997. The occurrence of Red Fox (*Vulpes vulpes*) was confirmed by the records of its characteristic excrements over the whole area of the reserve for several times. Three specimens of Roe Deer (*Capreolus capreolus*) were seen in the southern island in October 1997. Wild Boar's (*Sus scrofa*) paw prints and rooted areas were noticed several times in the southern part of reserve and on islands in autumn 1997 and spring 1998.

Table 6. Comparison of sampling small mammals in 1968-1970 (BARTOSZ, MARKOWSKI 1972) and in 1997-1998 (n_t – number of trapnights, IP – index of trapability). Asterisk indicates records of dead individuals found in the study area

Species	1968-1970 (n_t=725)		1997-1998 (n_t=240)	
	N	IP	N	IP
Sorex araneus	5	0.007	3	0.012
Sorex minutus	-	-	1	0.004
Apodemus agrarius	3	0.004	2	0.008
Myodes glareolus	40	0.055	6	0.025
Microtus oeconomus	1	0.001	-	-
Microtus arvalis	6	0.008	1*	-
Total	55	0.076	12	0.05

4. DISCUSSION

In comparison with the earlier study, there was no presence of Common Water Frog (*Rana esculenta*) and Pool Frog (*Rana lessonae*), which in 1968-1970

were noted in the area of the reserve in very small numbers (Table 1). Low number of amphibians in the reserve was found in both periods of study. This phenomenon could be explained by the water temperature which fluctuates around 9°C and only in the southern canal water reaches 13-14°C (WOJTAS, SOSNOWSKI 1972). In consequence, some late reproducing species, such as pool frog, cannot lay their eggs frequently (TRANDA, CICHOWICZ 1972). The scarcity of amphibians in the reserve could also be explained by the hydrotechnical works carried out in 1995, which disturbed the previous hydrological system. Increased discharge of cold water (during dredging activity) in all the canals might have forced scarce and scattered individuals of these species to emigrate. However, it is also possible that both species have disappeared much earlier, as a result of a marked decline in their population in the vicinity of the reserve caused by urbanization and disappearance of many shallow and periodic ponds. Remembering to treat these data carefully (they were collected without any formal quantitative method), it is possible to conclude that the abundance of anuran species decreased in this area by at least 50% over 30 years. This decline could be a result of developing built-up area of Ludwików housing estate on the right side of the Pilica River. Declines of amphibians caused by similar reasons were well documented on all continents where the group occurs (BARINAGA 1990; BLAUSTEIN, WOKE 1990; BLAUSTEIN *et al*. 1994) and the same process was so far recorded in Poland (GŁOWACIŃSKI *et al*. 1980; SURA, RYBACKI 1998).

Bird community changes during even-aged succession, especially following clearcutting or fire have been well documented in the literature along the 20th century (KELLER *et al*. 2003). In general, two patterns of the changes were proposed, the first one suggested a linear and unimodal increase (ODUM 1950; HAAPANEN 1965; SHUGERT, JAMES 1973), and the second one a bimodal increase in avian richness during successions (GŁOWACIŃSKI 1981; DEGRAAF 1991; KELLER *et al*. 2003). Many authors have documented the relationship between vertical structure of plant community and bird species richness or composition (MACARTHUR, MACARTHUR 1961; CODY 1985). Increase in avian diversity along with the forest maturation was also frequently presented (JOHNSTON, ODUM 1956; EMLEN 1970;

SHUGART, JAMES 1973; MOSS 1978; GŁOWACIŃSKI 1975, 1981; HELLE, MONKKONEN 1990). JOKIMÄKI, HUHTA (1996) have shown that stand age is positively correlated with a tree volume and consequently with the productivity of forest areas. Therefore its effect on bird assemblages was suggested to be very strong. Primarily, abundance of cavity nesters is known to increase significantly with stand age, because of the greater availability of holes and crevices in mature trees (SMITH *et al*. 1985).

The processes of fauna synurbization occurring in the town of Tomaszów Mazowiecki are probably partially responsible for an increase in the abundance of such species as Blackbird, Starling, Eurasian Jay and Magpie. In 1994 the appearance of breeding tufted duck was recorded (SOSNOWSKI 1995). An abrupt increase in the abundance of this species was related to the process of synurbization, which was preceded by expansion of this species from the south-eastern Europe (HAGEMEIJER, BLAIR 1997; TOMIAŁOJĆ, STAWARCZYK 2003) and its general increase in numbers across the geographical range. Similar reasons were suggested to be responsible for the appearance of Dunnock (*Prunella modularis*) and Thrush Nightingale (*Luscinia luscinia*) in the reserve (HAGEMEIJER, BLAIR 1997; TOMIAŁOJĆ, STAWARCZYK 2003).

The disappearance of the species connected with reeds as Great Reed Warbler (*Acrocephalus arundinaceus*) and Reed Warbler (*Acrocephalus scirpaceus*) occurred much earlier. In the course of the investigations in 1967-1970 period the number of pairs of reed warbler decreased from four to one (MARKOWSKI, WOJCIECHOWSKI 1972). This was related to the rapid reduction of the reed belt in the western part of the reserve and devastation of a small pond at the border of the reserve on the side of the Utrata Village. In consequence, these species did not occur in the reserve after 1971 (MARKOWSKI 1982). Untill the middle of the 1990s the Marsh Warbler (*Acrocephalus palustris*) persisted in the area (SOSNOWSKI 1995). There was, however, no conclusive evidence of breeding. The author mentioned also several other bird species breeding in the reserve in 1990s, i.e. Long-tailed Tit (*Aegithalos caudatus*), Bullfinch (*Pyrrhula pyrrhula*), Linnet (*Carduelis cannabina*), Tawny Owl (*Strix aluco*) and Turtle Dove (*Streptopelia turtur*)

(SOSNOWSKI 1994, 1995). Thorough comparison of these two publications, the first of which is considered as a scientific research publication, while the latter a popular-scientific monograph, brings numerous inconsistencies into light. This inevitably results in limited confidence put in these data. An example of contradictory information on the Water Rail (*Rallus aquaticus*) is that three breeding pairs occurred in the agrocoenoses of the Podoba district and in the Niebieskie Źródła Reserve (SOSNOWSKI 1994). By contrast, in the second publication the author did not mention the Water Rail, but the Common Moorhen (*Gallinula chloropus*) as a species breeding in the area (SOSNOWSKI 1995). SOSNOWSKI (1994) also mentioned two breeding pairs of Eurasian Coot (*Fulica atra*) in the reserve, while in the second publication (SOSNOWSKI 1995) he stated that the Eurasian Coot is a constant guest there and that clutches are produced by only solitary pairs and not every year. As the waters of the reserve are not abundant in submergent vegetation, it is difficult to imagine the breeding of as much as two pairs of coot. In fact some non-breeding Eurasian Coots were noted from September to the end of May (MARKOWSKI, WOJCIECHOWSKI 1972).

The changes in mammal species composition in comparison with the earlier investigations (BARTOSZ, MARKOWSKI 1972) included disappearance of two microtine species: Water Vole (*Arvicola terrestris*) and Root Vole (*Microtus oeconomus*) and the appearance of five others: Pygmy Shrew, Daubenton's Bat, Pond Bat, Brown Long-eared Bat and Wild Boar. Even during the former investigations Water Vole and Root Vole were rare, and their presence was indicated by only single observations of one swimming Water Vole and capturing a solitary specimen of Root Vole (Table 2). The retreat of Water Vole from the area of the reserve was possibly caused by hydrotechnical works that contributed to the eradication of reeds on the eastern bank and in the southern canal, which, in turn, limited food base of the species. The appearance of new mammal species could have different reasons. The appearance of Pygmy Shrew could be attributed to the process of succession and aging of tree stands. PUCEK (1984) stressed that the preferences of this species are confined to the mature deciduous or mixed forests and wet biotopes. A comparison of the results of the catches indicates large similarity of the

composition of small terrestrial mammal species in both periods of study. Presence of three new bat species was recorded for the first time due to an application of a specific mist-net sampling method (HEJDUK *et al.* 1999). The presence of the Wild Boar in the reserve was an effect of an increase in the abundance and progressive synurbization of the populations inhabiting the area around Tomaszów Mazowiecki. The occurrence of wild boar within urban areas in Poland was noticed by MARKOWSKI *et al.* (1998) and MIKOS (2002) and was accompanied by a similar process in other European countries (FISCHER *et al*. 2002, SONZA 2000).

In conclusion, the increase in biodiversity of terrestrial vertebrate species of Niebieskie Źródła Nature Reserve was recorded, mainly in the bird (seven species) and mammal (five species) communities.

5. REFERENCES

BARINAGA, M. 1990. Where have all the froggies gone? Science 247: 1033–1034.

BENNETT, G. 1994. Conserving Europe's natural heritage: towards European ecological network. Graham and Trotman / Martinus Nijhoff.

BARTOSZ, G., MARKOWSKI, J. 1972. Fauna Niebieskich Źródeł. Materiały do fauny ssaków. Zesz. Nauk. Uniw. Łódz. Nauk. matem.- przyr., ser. II, 46: 97–98.

BLAUSTEIN, A.R., WOKE, D.B. 1990. Declining amphibians: a global phenomenon? Trends Ecol. Evol. 5: 203–204.

BLAUSTEIN, A.R., WOKE, D.B., SOUSA, W.P. 1994. Amphibian declines: Judging stability, persistence and susceptibility of populations to local and global extinctions. Cons. Biol. 8: 60–71.

BOROWIEC, M., STAWARCZYK, T., WITKOWSKI, J. 1981. Próba uściślenia metod oceny liczebności ptaków wodnych. Not. Orn. 22: 45–61.

CICHOWICZ, M., ŻUCHOWSKA, E. 1972. Fauna Niebieskich Źródeł. Robaki pasożytnicze płazów. Zeszyty Naukowe Uniwersytetu Łódzkiego. Nauki matematyczno-przyrodnicze, ser. II, 46: 5–11.

CODY, M.L. 1985. Habitat selection in birds. Academic Press, London.

DEGRAAF, R.M. 1991. Breeding bird assemblages in managed northern hardwood forest in New England. In: J.E. RODIEK, E.G. BOLEN (eds), Wildlife and habitats in managed Landscapes. Island Press, Washington DC, pp. 153–171.

DIAMOND, J.M., MAY, R.M. 1977. Species turnover rates on islands: dependence on census interval. Science 197: 266-277.

EMLEN, J.T. 1970. Habitat selection by birds following a forest fire. Ecology 51: 343–345.

EMNEBORG, H., GÖTMARK, F. 2000. The role of threat to areas and initiative from actors for establishment of nature reserve in southern Sweden 1926–1996. Biodivers. Conserv. 9: 727–738.

FISCHER, C., GOURDIN, H., DOBERMAN, M. 2002. Spatial behaviour of wild boar in Genova (Switzerland). Test in the methods and first results. Abstracts of the 4th International Wild Boar Symposium. Lousa Portugal, p. 25.

FORMANN, R.T.T. 1995. Some general principles of landscape and regional ecology. Landscape Ecol. 10: 135–142.

FULLER, R.J. 1995. Bird life of woodland and forest. Cambridge University Press, Cambridge.

GŁOWACIŃSKI Z. 1975. Succession of bird communities in the Niepołomice Forest (Southern Poland). Ekol. Pol. 23: 231–236.

GŁOWACIŃSKI, Z. 1981. Wtórna sukcesja ptaków w dojrzewającym ekosystemie leśnym. (Synteza). Studia Naturae A, 26: 1–64.

GŁOWACIŃSKI, Z., PIENIEK, M., DYDUCH, A., GERTYCHOWA, R., JAKUBIEC, Z., KOSIOR, A., ZEMANEK, M. 1980. Stan fauny kręgowców i wybranych bezkręgowców Polski – wykaz gatunków, ich występowanie, zagrożenie i status ochrony. Studia Naturae A, 21: 1–163.

GROMADZKI, M. 1970. Breeding bird communities of birds in mid–field afforested areas. Ekol. Pol. 18: 1–44.

HAGEMEIJER, W.J.M., BLAIR, M.J. 1997. The EBCC Atlas of European Breeding Birds. T, A D Poyser. London.

HAAPANEN, A. 1965. Bird fauna of the Finish forest in relation to forest succession. Ann. Zool. Fenn. 2: 153–196.

HEJDUK, J., JAROS, R., PAWENTA, W., PŁUCIENNIK, H. 1999. Charakterystyka jesiennego zgrupowania nocka rudego *Myotis daubentonii* w rezerwacie „Niebieskie Źródła" koło Tomaszowa Maz. XII O OKC, Błażejewko.

HELLE, P., MÖNKKÖNEN, M. 1990. Forest succession and bird communities: theoretical aspects and practical implication. In: A. KEAST (ed.), Biogeography and Ecology of Forest Bird Communities. SPB. Academic Publishing, The Hague, pp. 299–318.

HUTCHESON, K. 1970. A test for comparing diversities based on the Shannon formula. J. Theor. Biol. 29: 151–154.

JOHNSTON, D., ODUM, E. 1956. Breeding bird populations in relation to plant succession on the Piedmont of Georgia. Ecology 37: 50–62.

JOKIMÄKI, J., HUHTA, E. 1996. Effects of landscape matrix anad habitat structure on a bird community in Northern Finland: a multiscale approach. Ornis Fenn. 73: 97–113.

KELLER, J.K., RICHMOND, M.E., SMITH, C.R. 2003. An explanation of patterns of breeding bird species richness and density following clearcutting in northeastern USA forests. Forest Ecol. Manag. 174: 541–564.

MACARTHUR, R.H., MACARTHUR, J.W. 1961. On bird species diversity. Ecology 42: 594–598.

MARGULES, C.R., PRESSEY, R.I. 2000. Systematic conservation planning. Nature 405: 243–253.

MARKOWSKI, J. 1982. Ptaki doliny Pilicy – projektowanej strefy krajobrazu chronionego. Ochrona Przyrody 44: 163–217.

MARKOWSKI, J., WOJCIECHOWSKI, Z. 1972. Fauna Niebieskich Źródeł. Materiały do awifauny rezerwatu i okolic. Zesz. Nauk. Uniw. Łódz. Nauk. matem.- przyr., ser. II, 46: 99–109.

MARKOWSKI, J., WOJCIECHOWSKI, Z., KOWALCZYK, J.K., TRANDA, E., ŚLIWIŃSKI, Z., SOSZYŃSKI, B. 1998. Fauna Łodzi. Fundacja Człowiek i Środowisko, Łódź.

MOSS, D. 1978. Diversity of woodland song–bird populations. J. Anim. Ecol. 47: 521–527.

MOWSZOWICZ, J., OLACZEK, R. 1961. Flora naczyniowa rezerwatu „Niebieskie Źródła". Łódzkie Towarzystwo Naukowe. Wydział III, 73, p. 40.

MOWSZOWICZ J., OLACZEK, R. 1965. Niebieskie Źródła. Łódzkie Towarzystwo Naukowe. Łódź.

MIKOS, J. 2002. Management strategy of wild boar in Wejherowo–Gdańsk suburban area, North–Central Poland. Abstracts of the 4th International Wild Boar Symposium. Lousa Portugal, p. 28.

ODUM, E.P. 1950. Bird populations of the Highlands (North Carolina) Plateau region in relation to plant succession and avian invasion. Ecology 31: 587–605.

OLACZEK, R., TRANDA, E. 1990. Z biegiem Pilicy. WP, Warszawa.

PUCEK, Z. 1984. Klucz do oznaczania ssaków Polski. PWN, Warszawa.

RODRIGUES, A.S.L., ANDELMAN, S.J., BAKARR, M.I., BOITANI, L., BROOKS, T.M., COWLLING, R.M., FISHPOOL, L.D.C., DA FONESCA, G.A.P., GASTON, K.J., HOFFMAN, M., LONG, J.S., MARQUET, P.A., PILGRIM, J.D., PRESSEY, R.L., SCHIPPER, J., SECHREST, W., STUART, S.N., UNDERHILL, L.G., WALLER, R.W., WATTS, M.E.J., YAN, X. 2004. Effectiveness of the global protected area network in representing species diversity. Nature 4128: 640–643.

SHUGART, H., JAMES, D. 1973. Ecological succession in Northwestern Arkansas. Auk 90: 62–77.

SMITH, K.W., AVERIS, B., MARTIN, B. 1985. The breeding bird community of oak plantations in the forest of Dean, southern England. Acta Oecol.–Oec. Gen. 8: 209–217.

SOKOŁOWSKI, J. 1972. Ptaki ziem polskich. PWN, Warszawa.

SONZA, B. 2000. Mamiferos urbanos en Zaragoza. Galemys 12: 55–57.

SOSNOWSKI, J. 1994. Ptaki miasta Tomaszowa Mazowieckiego. Muzeum w Tomaszowie Mazowieckim, p. 44.

SOSNOWSKI, J. 1995. Niebieskie Źródła – Groty. Informator turystyczny. Tomaszów Mazowiecki. Urząd Miasta Tomaszowa Mazowieckiego.

SURA, P., RYBACKI, M. 1998. Losy polskich płazów. Wiedza i Życie 8: 38–39.

TABACKI, A. 1972. Fauna Niebieskich Źródeł. Widłonogi (Copepoda). Zesz. Nauk. Uniw. Łódz. Nauk. matem.- przyr. ser. II, 46: 15–19.

TOMASZEWSKI, C. 1972. Fauna Niebieskich Źródeł Chruściki (Trichoptera). Zesz. Nauk. Uniw. Łódz. Nauk. matem.- przyr., ser. II, 46: 59–63.

TOMIAŁOJĆ, L. 1980. Kombinowana odmiana metody kartograficznej do liczenia ptaków lęgowych. Not. Orn. 21: 33–54.

TOMIAŁOJĆ, L., STAWARCZYK, T. 2003. The avifauna of Poland. Distribution, numbers and trends. PPPP ”proNatura”, Wrocław. [In Polish with English summary].

TOMIAŁOJĆ, L., WESOŁOWSKI, T., WALANKIEWICZ, W. 1981 Breeding bird community of a primaevel temperature forest (Białowieża National Park, Poland). Acta Ornithol. 20: 241–310.

TOŃCZYK, G., KLUKOWSKA, M., JURASZ, W., MARKOWSKI, J. 2000. The Niebieskie Źródła nature reserve as subject of scientific research. Acta Univ. Lodz., Folia Limnol. 7: 3–17.

TRANDA, E., CICHOWICZ, M. 1972. Fauna Niebieskich Źródeł. Płazy (Amphibia). Zesz. Nauk. Uniw. Łódz. Nauk. matem.- przyr., ser. II, 46: 97–98.

WOJTAS, F., SOSNOWSKI, J. 1972. Fauna Niebieskich Źródeł. Wstęp. Zesz. Nauk. Uniw. Łódz. Nauk. matem.- przyr., ser. II, 46: 1–3.

WOJTAS, F., SOSZYŃSKI, B. 1972. Fauna Niebieskich Źródeł. Bzygowate (Syrphidae, Diptera). Zesz. Nauk. Uniw. Łódz. Nauk. matem.- przyr., ser. II, 46: 75–80.

WĘŻYK, M. 2008. Ochrona przyrody i krajobrazu. Raport o stanie środowiska w województwie łódzkim w 2007 roku. Wojewódzki Inspektorat Ochrony Środowiska. Łódź, pp. 203–209.

Acta Universitatis Lodziensis
——— 2010 ———
Folia Biologica et Oecologica 6: 137–144
(Acta Univ. Lodz, Folia Biol. Oecol.)

JUSTYNA TRACZ

Department of Geobotany, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland, justyna.tracz@gmail.com

CHANGES OF *DENTARIO GLANDULEOSAE-FAGETUM* FOREST STAND IN ROZTOCZE NATIONAL PARK, SOUTH EASTERN POLAND FROM 1946 TO 2001

Abstract: Roztocze Nation Park (RNP) is located in the central part of Roztocze, a hilly region in south-eastern Poland. The most important type of forest community in RNP is *Dentario glandulosae-Fagetum*. Potential and real vegetation, as well as forest stand maps were used to analyze changes of the age and species structure of beechwood stand from 1946 to 2001. Beechwoods were dominated by two species: fir and beech. During the last 50 years their proportion has entirely changed and the share of oldest groups of stand (over 120 years old) has increased significantly.

Key words: beechwood, Roztocze, forest stand changes

1. INTRODUCTION

1.1 General information

Roztocze is a group of hills in south-east Poland, which ranges from Kraśnik to Lwów (Ukraine). In the north Roztocze borders with the Lublin Upland, and in the south with the Sandomierz Dale. Its highest elevation is 391.5 m above sea level. This region differs from the neighbouring areas. Roztocze is located on the Vistula River and the Bug River watershed and is under the influence of continental climate but due to its altitude annual sum of atmospheric precipitation in the region is higher than the average for Poland. Geological structure of Roztocze is also unique as the region is located in the Teisseyre-Tornquist Zone – the line of contact of East

DOI: 10.2478/v10107-009-0012-3

European craton and paleozoic structures of Western Europe. This region is also interesting due to its geobotanical features. Natural ranges of several tree species have their edges at the territory of Roztocze. These are European Beech (*Fagus sylvatica*), Silver Fir (*Abies alba*), Norway Spruce (*Picea abies*), Large-leaved Lime (*Tilia platyphyllos*) and Sycamore Maple (*Acer pseudoplatanus*). In spite of the borderline range, these species are dynamic and vital in Roztocze region. What is more, two montane associations: *Dentario glandulosae-Fagetum* and *Abietetum polonicum* are present in Roztocze (BURACZYŃSKI 2002; WILGAT 2004)

Roztocze National Park (RNP) is situated in the Central Roztocze. This national nark with area of 8483 ha is predominantly woody and as much as 95.5% of its area is covered with forests. All types of vegetation are well-preserved. Almost 10% of RNP is under the protection of nature reserves. RNP has been established in 1974 and covered an area of 1064.38 ha which was under the surveillance of Kosobudy and Zwierzyniec Forest Districts. The history of nature conservation at the territory of RNP has begun in 1936 when Bukowa Góra reserve has been established (LIS 1979; BRZYSKI 1983; WILGAT 2004).

1.2 Characteristic of beechwoods in Roztocze National Park

Dentario glandulosae-Fagetum is the most important association in Roztocze National Park. It occupies 26% of the Park area and it is one of the montane associations characteristic for the Roztocze region. Natural beechwoods of Roztocze have been changed by an inappropriate forest management – the Scots Pine (*Pinus sylvestris*) has been planted on *Dentario glandulosae-Fagetum* habitats and some parts of stand have been cut completely. Nowadays this type of forest occupies 55% of its natural habitat, the rest of the area is taken up by the substitute association from *Querco-Fagetea* class (IZDEBSKI *et al.* 1992; WILGAT 2004, Fig. 1, Table 1).

Usually *Dentario glandulosae-Fagetum* is thorny and its canopy is multilayer. The area of a tree crown surface is 70-90%. The canopy of most stands is two-layer. Upper-canopy layer is built by European Beech (*Fagus sylvatica*), alone, the mixture of European Beech and Silver Fir (*Abies alba*), or rarely exclusively by Sliver Fir. Single specimen of European Hornbeam (*Carpinus betulus*), Small-

leaved Lime (*Tilia cordata*), Norway Maple (*Acer platanoides*), Sycamore Maple (*Acer pseudoplatanus*), and Wych Elm (*Ulmus glabra*) may also be found in this layer. Lower-canopy layer is dominated by European Beech and European Hornbeam., in some parts with significant share of Silver Fir. European Beech individuals are not robust except the most fertile habitat but Silver Fir specimens are very robust. There are specimens 40 meter high and of 3 meter circumference of beech and fir in the Park.

Understory layer surface is variable and amount from a few to 80 percent. Shrubs and tree seedlings are present mainly in the clearings. Beech saplings are most common in this layer, sometimes fir saplings co-dominate (CZARNECKA 1978; IZDEBSKI *et al.* 1992; WILGAT 2004).

Herb layer surface is 50-90% (in most stands at least 70%). In most natural parts of stands herb development is seasonal. Herb layer surface is largest in spring and in early summer. Herb layer does not occur in the most shaded areas of stands and is thereby characterized by high mosaicity. Moss layer is developed rather poorly. Only in some parts of beechwoods its surface is 10-40% (IZDEBSKI *et al.* 1992).

Some parts of beechwoods have been changed by inappropriate forest management. The Scots Pine has been planted on deciduous forest habitat and clearcutting has been implemented. *Dentario glandulosae-Fagetum* occupies more than half of its potential habitat. Almost 40% of beechwood habitat is occupied by substitute associations from *Querco-Fagetea* class. Small share is occupied by other associations, for example *Querco roboris-Pinetum fagetosum* or *Abietetum polonicum*. In contrast, beechwood occupies almost exclusively its potential habitat. There is also a small share (ca. 0.3%) of beechwood on *Tilio-Carpinetum* habitat (WILGAT 2004, Fig.1).

The Scots Pine is the main species of substitute associations of *Querco-Fagetea* stand. Pine was planted there 50-100 years ago, and nowadays is dominant in the upper-canopy layer. In lower-canopy layer there are deciduous trees (mainly European Beech). European Beech is vivid and very often is large enough to reach upper-canopy layer. In some stands there is large share of Common Aspen (*Populus*

tremula) and admixture of Silver Birch (*Betula pendula*) (IZDEBSKI *et al.* 1992). In understory layer there are Common Hazel (*Corylus avellana*), saplings of European Hornbeam, European Beech and Silver Fir, frequently there are also: Common Dogwood (*Cornus sanguinea*) or Sycamore Maple and Alder Buckthorn (*Frangula alnus*). Herb layer is well developed and its species composition is typical for *Dentario glandulosae-Fagetum* (IZDEBSKI *et al.* 1992).

2. METHODS

Potential vegetation map – scale 1:20 000 (IZDEBSKI *et al.* 1997) and real vegetation map – scale 1:10 000 (IZDEBSKI *et al.* 1991) of Roztocze National Park were compared. Additionally, tree stand maps (scale 1:20 000 for 1946, scale 1:25 000 for 2001) and forest descriptions (parts of Forest Management Plan) were used to analyze species and age structure of forest stand and its changes (listed in the Appendix). Tree stand maps contained data on age and share of dominate species. Data from Forest Management Plans were more precise. Vertical structure of stand, age and species structure of all stand layers and characterisation of understory layer were included in the Forest Management Plans used in the analysis. Corel Draw and ArcView were used to draw maps and prepare statistics. Due to the lack of information about the map projection, maps were overlapped using characteristic points (roads, borders of the National Park, locations of villages, grid of forest departments).

3. RESULTS

3.1. **Forest stand changes**

Species composition of beechwood stand in Roztocze National Park was dominated by two tree species: European Beech and Silver Fir. As a result of natural processes and forest management proportion of fir to beech was considerably altered across the second half of 20th century. In 1946 there was mass domination of fir on 50% of the beechwood area and beech dominated on 25% of the area. In 2001 beech prevailed on 55% of the area, which means that the area of its mass domination has increased 2.2-fold since 1946. In 2001 fir dominated on 34% of the area. Other

significant change in species structure of beechwood was decrease of Scots Pine share – 7.5% in 1946 and ca. 1% in 2001 (Fig 2, Table 2). Statistic of χ^2 test is 0.2457 for 5 degrees of freedom according to share of different tree species in beechwood stands in 1946 and 2001.

In age structure of beechwood in 1946 dominated forest stand of 60-120 years, which occupied 66% of the area. In 2001 the largest area was occupied by forest stand 90-150 years old (ca. 65%). In 1946 tree stand younger than 90 years old occupied almost 55% of the area and in 2001 only 14%. The oldest groups of tree stand (more than 120 years old) occupied 12% of the area in 1946 , but in 2001 it was almost three times larger – 33% (Fig. 3, 4, Table 3). Statistic of χ^2 test is 0.9575 for 7 degrees of freedom according to age structure of beechwood stand in Roztocze National Park

The most significant changes were observed in the age group of 61-90 years. Share of this stand changed from 1946 to 2001 by ca. 24%. Significant change occurred in the age groups of 91-120 and 31-60 years – share of both groups decreased by ca. 12 and 14%, respectively. In contrast, share of the oldest groups (over 120 years) increased. Share of the stands of 121-150 years increased by 4% and those of 151-180 years by 16%. In 2001 stands over 180 years were recorded, but their share were less than 1% (Fig. 3, 4, Table 3).

4. DISCUSSION

This was the first attempt of using forest description to analyze changes of beech stands in Roztocze. Beechwood in Roztocze National Park exists almost exclusively on its own habitat, but it occupies only half of the area. Substitute associations from *Querco-Fagetea* dominate the rest of beechwood potential habitat. Stands of substitute associations from *Querco-Fagetea* consist mostly of Scots Pine. In understory layer of this association there is domination of young beeches. What is more, herb layer composition is typical for *Dentario glandulosae-Fagetum* (WILGAT 2004). If proper forest management is implemented in future these artificially changed parts of the forest could be again classified as typical beechwoods.

One of typical features of beechwood stand in Roztocze is an admixture of Silver Fir. There is a noticeable tendency of fir withdrawal from RNP stand, especially on the edges of beechwood range that neighbour with *Abietetum polonicum circaeaetosum*. In some cases beechwood expanded its range and took over habitat of the fir forest. What is more, there are only old specimens of fir in the beechwood stand and there are no representatives of younger generation. Dynamics of both associations need to be researched thoroughly. KOZŁOWSKA (2007) analysed changes in the montane beech forests in Bieszczady. Most data came from Bieszczady National Park and beech forests from the periods of 1954-1962 and 1994 were compared. In the Bieszczady beech forests Silver Fir was 35% less frequent in 1994 than in 1954-1962, frequency of European Beech was the same in both periods.

There could be, however, another explanation for the decrease of the fir share in beechwood stands. In the forest management plans lands are divided on the basis of tree species mass domination. In some parts of the stands, mass of the single, old and large firs exceed mass of several younger beeches. During the period of over 50 years young beeches considerably increased their mass and outweighed the mass of sparsely distributed old firs. This process took place on 15% of beechwood area.

Obtained results show that beechwood stand in RNP became older over the second half of the 20th century. In 1974 Roztocze National Park was established and since then most valuable parts of Roztocze forests have been protected, which could play an important role in the process of ageing. In conclusion, the analysis of change tendencies in the forests of RNP indicate that beech forests are not endangered, as their area has increased since 1946. In contrary, Silver Fir share in beech forest decreased and the causes of the process should be investigated. Furthermore, I suggest that the substitute associations from *Querco-Fagetea* which exist on beech forest habitat are likely to transform into *Dentario glandulosae-Fagetum*. It is therefore possible that in the protected areas such as Roztocze National Park beech forest will develop in sustainable high forests.

5. REFERENCES

BRZYSKI, B. 1983. Distribution and protection of the terminal localities of beech and fir in Rotocze and adjacent regions (south-east Poland). Ochr. Przyr. 26: 368-393.

BURACZYŃSKI, J. 2002. Roztocze. Środowisko przyrodnicze. Wydawnictwo Lubelskie, Lublin.

CZARNECKA, B. 1978. Characteristics of the Jarugi Forest Reserve in Central Roztocze. Ann. UMCS, Sec. C, 33, 21: 309-331. [In Polish with English summary].

IZDEBSKI, K., CZARNECKA, B., GRĄDZIEL, T., LORENS, B., POPIOŁEK, Z. 1991. Maps of forest plant communities and distribution of rare vascular plant species in the Roztocze National Park. Roztoczański Park Narodowy, Lublin. [In Polish with English summary].

IZDEBSKI, K., CZARNECKA, B., GRĄDZIEL, T., LORENS, B., POPIOŁEK, Z. 1992. Plant communities against the background of the Roztocze National Park habitat conditions. UMCS, Roztoczański Park Narodowy, Lublin. [In Polish with English summary].

IZDEBSKI, K., GRĄDZIEL, T., LORENS, B., POPIOŁEK, Z. 1997. Potencjalna roślinność naturalna Roztoczańskiego Parku Narodowego. Roztoczański Park Narodowy, Zwierzyniec.

KOZŁOWSKA, A. 2007. Changes in montane beech forests in Bieszczady. In: J.M. MATUSZKIEWICZ (ed.), Geobotanical identification of the development tendencies in forest associations in the regions of Poland, pp: 433-449. [In Polish with English summary].

LIS, Z. 1979. Natural limits of *Fagus sylvatica* and *Abies alba* in the Lublin area. Folia Soc. Scient. Lublin. Biol. 21: 119-128. [In Polish with English summary].

WILGAT, T. (ed.) 2004. The Roztocze National Park – nature and humans. Roztoczański Park Narodowy, Zwierzyniec. [In Polish with English summary].

Appendix (Maps and forest management plans)

Mapa drzewostanowa prowizorycznego urządzenia lasów Nadleśnictwa Państwowego Kosobudy, Dyrekcja Lasów Państwowych Okręgu Lubelskiego w Lublinie, według stanu na 1.01.1946r., skala 1:20000.

Mapa drzewostanowa prowizorycznego urządzenia lasu Nadleśnictwa Państwowego Zwierzyniec, Dyrekcja Lasów Państwowych Okręgu Lubelskiego w Lublinie, według stanu na 1.01.1946r., skala 1:20000.

Mapa przeglądowa faz rozwojowych drzewostanu, Roztoczański Park Narodowy, obręb ochronny Kosobudy, Województwo Lubelskie, stan na 1.01.2001r., skala 1:25000. Wykonano w Biurze Urządzania Lasu i Geodezji Leśnej Odział w Lublinie, wykonała Jolanta Smyk.

Mapa przeglądowa faz rozwojowych drzewostanu, Roztoczański Park Narodowy, obręb ochronny Zwierzyniec, Województwo Lubelskie, stan na 1.01.2001r., skala 1:25000. Wykonano w Biurze Urządzania Lasu i Geodezji Leśnej Odział w Lublinie, wykonała Jolanta Smyk.

Opis taksacyjny wraz z tabelą klas wieku, Nadleśnictwo Zwierzyniec, Dyrekcja Lasów Państwowych Okręgu Lubelskiego, stan na 1.10.1946r.

Operat ochronny ekosystemów leśnych rewizji nadzwyczajnej Roztoczańskiego Parku Narodowego, obręb Kosobudy, tom I, szczegółowe dane inwentaryzacji lasu, wg stanu na dzień 01.01.2001r.

Operat ochronny ekosystemów leśnych rewizji nadzwyczajnej Roztoczańskiego Parku Narodowego, obręb Zwierzyniec, tom II, szczegółowe dane inwentaryzacji lasu, wg stanu na dzień 01.01.2001r.

Acta Universitatis Lodziensis
———— 2010 ————
Folia Biologica et Oecologica 6: 145–150
(Acta Univ. Lodz, Folia Biol. Oecol.)

KATARZYNA ZIELIŃSKA, JANINA JAKUBOWSKA-GABARA, JEREMI KOŁODZIEJEK

Department of Geobotany and Plant Ecology, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland, kziel@biol.uni.lodz.pl

ADAPTATION OF THE "HERBARIUM" COMPUTER DATABASE TO ARCHIVING AND ANALYSIS OF FLORISTIC DATA

Abstract: The aim of the study was to present "Herbarium" computer database. The basic goal of construction of the database was gathering and analysing of archival and contemporary floristic data referring to the area of Central Poland. Registered information considers the stands and characteristic biological and ecological traits of particular taxa. Life forms, indices of ecological requirements, the phytogeographical elements, protection and threat status are among them. The database enables the presentation of geographical distribution of taxa in the area of Central Poland with the cartogram method based on the grid of 2 km^2 according to the Distribution Atlas of Vascular Plants in Poland (ATPOL).

Key words: database, floristic data, cartogram method, Central Poland

1. INTRODUCTION

Since the first floristic research conducted in Central Poland in the 19th and at the beginning of the 20th century, particular plant species have been identified in that area by next generations of botanists. The data thus collected, varying in the degree of detail and accuracy, has been included in both published and unpublished works, as well as in scientific notes from field work. The term Central Poland refers to the area situated in the central part of the country, as earlier specified by: OLACZEK (1974), MOWSZOWICZ (1978), KUROWSKI (1986), JAKUBOWSKA-GABARA, PISAREK (1997), JAKUBOWSKA-GABARA (2005) and JAKUBOWSKA-GABARA *et al*.

DOI: 10.2478/v10107-009-0013-2

(2009). A great deal of floristic data has been documented in herbarium collections compiled in *Herbarium Universitatis Lodziensis* (LOD). Further information, regarding approximately 1600 species of vascular plants found in that area, has been provided owing to long-term research, rich literature and herbarium collections (JAKUBOWSKA-GABARA 2005; JAKUBOWSKA-GABARA *et al.* 2009). Yet, what is clearly missing is any data compilation specifying the geographical distribution of the plant species in that area and changes in the distribution over time. Such compilation is a key element in dealing with a vast number of phytogeographical problems and is also a valuable source of information on biodiversity protection.

A great deal of the scattered data requires unification and compilation into one data collection. Contemporary computer techniques allow such data compilation and processing. What is more, computerized information systems that can store and quickly retrieve even large amounts of data are becoming more widespread, especially in the field of floristic cartography (FALIŃSKI 1990). In 2003, by a special order of the Department of Geobotany and Plant Ecology of the University of Łódź a floristic database called “Herbarium” was designed and constructed. The database provides a wide spectrum of possibilities of collecting data on plant stands, together with any information regarding the biological and ecological characteristics of particular species. To a large extent, all the strong points of the database result from a good co-operation and numerous consultations between the botanists of the Sub-Department of Plant Systematics and Geography and computer programmers. The “Herbarium” database has been constructed in the Linux System, characterized by low-failure frequency and wide accessibility. The system also enables adjusting the current database to its new, improved version, without losing any previously entered data.

2. DATABASE AIMS AND USE

The main aim of a database construction is the archiving of data. It has been assumed that the “Herbarium” database will store both the archival and contemporary data regarding particular plant species in the area of Central Poland. Two methods have been employed to collect the data: descriptive research and the

cartogram method. A grid of basic squares 2 km by 2 km based on the ATPOL grid (ZAJĄC 1978) has been applied. Therefore, the floristic data presented in the cartogram can be easily and thoroughly incorporated into ATPOL (Distribution Atlas of Vascular Plants in Poland). The cartogram thus prepared enables establishing precise locations of particular plant species. The cartogram presented in this paper displays the main rivers in the area of Central Poland: the Bzura, the Pilica and the Warta (Fig. 1).

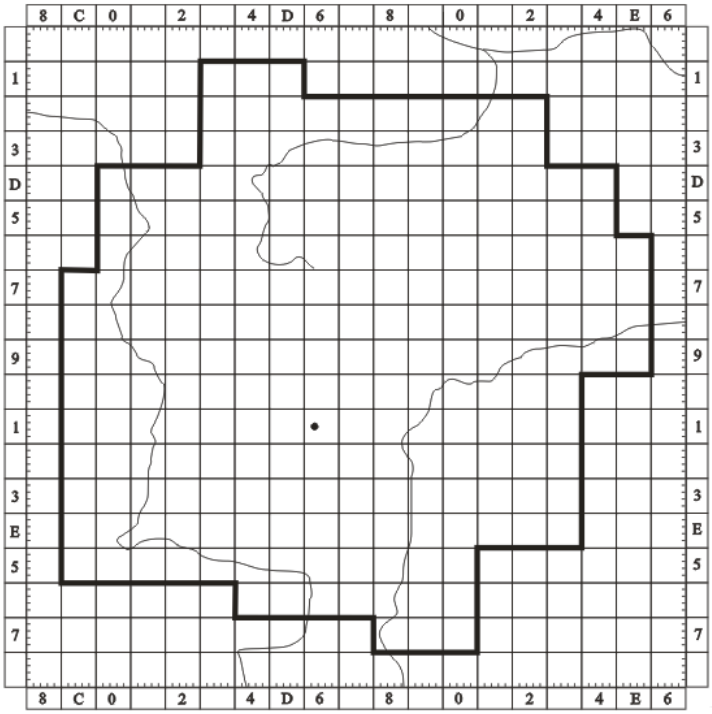

Fig. 1. Area of Central Poland in the ATPOL grid (according to ZAJĄC 1978) and an example of a locality (dot) in square DE1621: DE – symbol of a 100 km^2 square, 16 – symbol of a 10 km^2 square, 21 – symbol of a 2 km^2 square.

The "Herbarium" database enables both graphical and tabular representation of data. The latter proves useful while specifying the source data of particular plant species.

One of the key characteristics of the database is the possibility of entering data from different sources. The floristic data is incorporated into particular data

category based on herbarium collections, as well as published and unpublished data. As for the latter, the author, the title and the year of publication (or the year it was written) are noted. With herbarium collections the catalogue number of a given collection is also specified.

As mentioned before, the “Herbarium” database offers information on the location of particular species, but it archives data on species richness and habitats as well. It also makes it possible to specify by whom the individual species have been registered, labeled and their taxonomic group defined. Thus, an archive offering the most comprehensive information possible on herbarium collections and the species composition of Central Poland flora is being constructed. The data collected in the “Herbarium” database is not only available for contemporary research, but can also prove crucial and adequate to future researchers.

Another aim behind constructing such a database is to analize the collected floristic data regarding the characteristics of particular species. Each species is characterized by a specified set of traits, defined with the use of available species classifications, such as Raunkiaer’s life-form classification (RAUNKIAER 1934), species division into geographical-historical groups by KORNAŚ (1968) and a set of ecological indicator values (ELLENBERG *et al*. 1992; ZARZYCKI *et al.* 2002). Legal protection status of individual species and their category of threat have also been determined. Moreover, the database is equipped with a report designer function that allows of any given combination of data that needs to be collected. To give an example, it’s possible to retrieve data on the list of species found in the wetlands, under strict protection and registered in DE 3444 square in the 90s of the 19th century. The database has been constructed in such a manner that all possible ways of analysis of the data collected therein are possible.

The “Herbarium” database may also prove useful in evaluating projects that could affect the environment, or in opinionating special development plans. Besides, it can be used to help prepare proposals for enforcing protection laws in areas of valuable nature, to make reports on nature condition and red lists of threatened species and habitats of a given region. At present, the database consists of approximately 140 000 data items regarding vascular plant species, including those

registered in the Distribution Atlas of Vascular Plants in Poland (ZAJĄC A., ZAJĄC M. 2001).

3. REFERENCES

ELLENBERG, H., WEBER, H.E., DÜLL, R., WIRTH, V., WERNER, W., PAULISSEN, D. 1992. Indicator values of plants in Central Europe. Scripta Geobotanica 18: 1–258.

FALIŃSKI, J.B. 1990. Kartografia geobotaniczna. Cz. 1. Zagadnienia ogólne, kartografia florystyczna i fitogeograficzna. PPWK im. E. Romera, Wrocław-Warszawa, pp. 1–284.

JAKUBOWSKA-GABARA, J. 2005. Vascular Flora of Central Poland – Diversity, Changes, Threats. Biodiversity in Relation to Vegetation Zones in Europe, In: K. CZYŻEWSKA, J. HEREŹNIAK (eds), University of Łódź Press, Łódź, pp. 19–30.

JAKUBOWSKA-GABARA, J., PISAREK, W. 1997. Materiały do flory naczyniowej Polski Środkowej. Fragm. Flor. Geobot., ser. Polonica 4: 9–15.

JAKUBOWSKA-GABARA, J., WITOSŁAWSKI, P., ZIELIŃSKA, K. 2009. Flora naczyniowa – różnorodność, zmiany, zagrożenia. In: J.K. Kurowski (ed.), Szata roślinna Polski środkowej. Towarzystwo Ochrony Krajobrazu, Wydawnictwo EKO-GRAF, Łódź, pp. 57–80.

KORNAŚ, J. 1968. Geograficzno-historyczna klasyfikacja roślin synantropijnych. Mat. Zakł. Fitosocj. Stosow. UW, Warszawa-Białowieża 25: 33–41.

KUROWSKI, J.K. 1986. Ocena stanu ochrony flory w rezerwatach przyrody Polski Środkowej. Acta Univ. Lodz., Folia Sozol. 3: 205–224.

MOWSZOWICZ, J. 1978. Conspectus florae Poloniae Medianae (plantae vasculare). Przegląd flory Polski Środkowej (rośliny naczyniowe). Wydawnictwo Uniwersytetu Łódzkiego, p. 395.

OLACZEK, R. 1974. Materiały do flory Polski Środkowej. Zesz. Nauk. UŁ, Ser. II, 54: 27–39.

RAUNKIAER, C. 1934. The Life Forms of Plants and Statistical Plant Geography. Oxford University Press, Oxford, p. 632.

ZAJĄC, A. 1978. Założenia metodyczne Atlasu rozmieszczenia roślin naczyniowych w Polsce. Wiad. Bot. 22: 145–155.

ZAJĄC, A., ZAJĄC, M. (eds) 2001. Distribution Atlas of Vascular Plants in Poland. Atlas rozmieszczenia roślin naczyniowych w Polsce. Instytut Botaniki UJ, Kraków, pp. 1–715.

ZARZYCKI, K., TRZCIŃSKA-TACIK, H., RÓŻAŃSKI, W., SZELĄG, Z., WOŁEK, J., KORZENIAK, U. 2002. Ecological indicator values of vascular plants of Poland. Ekologiczne liczby wskaźnikowe roślin naczyniowych Polski. Instytut Botaniki im. W. Szafera PAN, pp. 1–183.