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Functional and evolutionary characterisation of the neuroendocrine and reproductive system of molluscs using the great pond snail (*Lymnaea stagnalis*)

PhD thesis

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1. Introduction

The major sex steroids in humans, progesterone, testosterone, and 17β-estradiol, were discovered in the 1920-30s (Corner and Allen, 1929; David et al., 1935; Huffman et al., 1940). Not long afterwards, the presence of vertebrate sex steroids in marine molluscan tissues was first reported (Hagerman and Wellington, 1957). At the time, it was reasonable to assume that these steroids were of endogenous origin in molluscs (and in invertebrates in general) and were possibly used as hormones in the same way as they are in vertebrates. This led people to investigate the effects of sex steroids in molluscs, the first study was published as early as 1959 (Aubry, 1959). All but a handful of the 300+ scientific papers and reviews, mainly using analytical, immunological, molecular, and behavioural measurements, have since concluded either that 1) the gonadotropin-releasing hormone is the same in both invertebrates and vertebrates; 2) molluscs are able to biosynthesize vertebrate steroids *de novo*; 3) molluscs appear to contain steroid receptor-like binding activity; and 4) molluscs respond in one way or another when exposed to vertebrate steroids.

Starting from 2010, critical reviews called into doubt the claims that reproductively-related peptides, sex steroids, and their receptors that are present in vertebrates, and that can also be found in molluscs, necessarily have the same function (Balbi et al., 2019; Fernandes et al., 2011; Fodor et al., 2020; Fodor and Pirger, 2022; Horiguchi and Ohta, 2020; Minakata and Tsutsui, 2016; Pirger et al., 2018; Scott, 2012, 2013, 2018).

2. Aims

- To identify the coding region of ly-GnRH/CRZ peptide
- To analyse the evolutionary relationships of the GnRH neuropeptide superfamily
- To investigate the distribution of the transcript and the peptide in the CNS and periphery
- To identify the active peptide and describe its physiological role
- To identify the homologs to vertebrate genes involved in sex steroid synthesis and receptor-mediation
- To investigate the uptake, metabolism, and depuration of sex steroids
- To investigate whether the expression of reproduction-mediating neuropeptides changes due to chronic progestogen exposure

3. Materials and methods

3.1. Experimental animals

L. stagnalis specimens were obtained from our laboratory-bred stocks (BLRI). Snails were kept in large holding tanks containing 10L oxygenated artificial snail water with low copper content at a constant

temperature of 20 °C (±1 °C) on a light:dark regime of 12 h:12 h. Specimens were fed on lettuce ad libitum three times a week. All procedures were performed according to the protocols approved by the Scientific Committee of Animal Experimentation of the Balaton Limnological Research Institute (VE-I-001/01890-10/2013).

3.2. Neural transcriptome sequencing

The whole CNS was dissected, homogenized, and RNA was isolated with the Direct-zol™ RNA MiniPrep kit. The RNA was quantified by a Qubit 3.0 device using BR RNA Kit and the quality was checked on Agilent Bioanalyzer 2100 using RNA 6000 Nano Kit. Nanopore sequencing was carried out for identifying the coding regions. The library was prepared using the cDNA-PCR Kit. For homolog searching, relevant invertebrate and vertebrate sequences were used as search queries from the NCBI database. For verification and sequence correction, the findings were compared with virtual cDNA sequences extracted from our unannotated genomic data to which we have access as part of the genome consortium. The identified sequences were submitted to the NCBI Nucleotide database. Conserved domain search using NCBI CDD/SPARCLE was performed to check if the key regions were present in the deduced protein sequences.

3.3. Phylogenetic analysis

We applied the Maximum Likelihood to investigate the molecular phylogenetics of the ly-GnRH/CRZ neuropeptide. The alignment used to generate the maximum likelihood tree consisted of 56 amino acid sequences of GnRH superfamily preprohormones retrieved from NCBI and the deduced amino acid sequence of ly-GnRH/CRZ preprohormone. The alignment was performed in the Molecular Evolutionary Genetics Analysis v7 software. Bootstrapping support for the tree was conducted with 1000 bootstrap replicates.

3.4. Peptide extraction and mass spectrometry analysis

To confirm the presence of the deduced active ly-GnRH/CRZ peptide, the whole CNS was dissected from the animals, extracted, and investigated with HPLC-MS and MALDI-TOF.

3.5. RT-PCR analysis of ly-GnRH/CRZ expression in central and peripheral tissues

The expression of ly-GnRH/CRZ was investigated in the CNS, heart, ovotestis, and seminal vesicles. RNS was isolated from the dissected samples, then cDNA was made with the RevertAid H Minus First Strand cDNA Synthesis Kit.

3.6. Immunohistochemistry

Immunohistochemistry was performed in the whole CNS (ly-GnRH/CRZ; GnRH) CYP19A; nPR), heart (ly-GnRH/CRZ; 5-HT), and reproductive system (ly-GnRH/CRZ; 5-HT). The samples were fixed and cryoprotected in 20% glucose solution. After the incubation, series of 12–14 μm-thick cryostat sections were cut, thaw-mounted onto gelatin-aluminium-coated slides. After blocking, the samples were incubated with the appropriate primary and secondary antibodies. The stained tissues were analysed with a TCS SP8

DMI laser confocal scanning microscope equipped with appropriate wavelength-filter configuration settings and a transmitted light detector.

3.7. Retrograde labelling by nickel‑lysine backfill

For nickel-lysine backfill of the ventral- and anterior lobe of the right cerebral ganglion, the whole CNS with the penis nerve was dissected from snails and placed in a chamber containing a nickel–lysine. After the incubation, nickel was precipitated by adding 5–10 drops of a saturated alcohol solution of rubeanic acid. Afterward, samples were processed for IHC.

3.8. Peptide synthesis and injection

The synthesis of the ly-GnRH/CRZ active peptide was performed with Fmoc chemistry. Peptide chains were elongated on a Tentagel S-Ram resin and the synthesis was performed using a CEM Liberty Blue machine. The resulted crude peptide was purified by reverse-phase HPLC. For testing the possible effects of ly-GnRH/CRZ peptide on feeding, locomotion, and egg-laying, 10 μg synthetic peptide was injected into the hemolymph of the animals.

3.9. Behavioural tests

For behavioural assays, specimens were divided into control and ly-GnRH/CRZ injected experimental groups. Feeding rate (rasping number/2min), locomotion (distance/5min), and egg-laying (laid egg masses and eggs) were investigated.

3.10. Laboratory exposures of L. stagnalis to labelled steroids

Radiolabelled 17β-[^{3}H]-E_2, [^{3}H]-T, [^{3}H]-P and 17-[^{3}H(N)]-EE_2 standards were used to investigate uptake, metabolism, and depuration of steroids. The disappearance of radioactivity was calculated with a scintillation counter. For each steroid exposure, five animals were immediately frozen after exposure (day 0 of depuration) and the remaining five were placed in freshwater for depuration under semi-static conditions. The animals were sampled and frozen on day 10. Calculation of clearance rates was carried out for each steroid.

3.11. Steroid extraction and separation methods

For extraction of radioactive steroids from snail tissues, the animals sampled after the 8 h of exposure (i.e. day 0) and depuration (i.e. day 10) were defrosted at room temperature, shucked, blotted dry, and weighed. The tissues were extracted with methanol and ethyl acetate. To separate the free, water-soluble, and esterified metabolites, a portion of each extract was evaporated and partitioned.

3.12. Western blot analysis and mass spectrometry identification

To identify the molecules marked by the vertebrate antibodies, the whole CNS was prepared, homogenised, run on 10% SDS-PAGE and blotted onto a nitrocellulose membrane following the standard semi-dry blotting protocol. The membrane was blocked and incubated with the appropriate primary and secondary antibodies. The chemiluminescence was visualized with Western Blotting Luminol Reagent. For MS

identification, the respective bands were excised from the parallel SDS-PAGE gel, destained, cleaned, and in-gel digested. The peptides were cleaned with solid phase extraction. The proteomics analysis was performed with Bruker EASY-nLC equipment coupled with a nano-ESI MS instrument.

3.13. Chronic progestogen exposure

Progesterone, drospirenone, gestodene, and levonorgestrel were used for the chronic progestogen exposure. The animals were exposed to the combination of the 4 compounds at 10 ng/L equi-concentration (representing the environmental situation) for 21 days.

3.14. Comparative neural transcriptome sequencing

After the 21-day exposure, the whole CNS was dissected, RNA was isolated, and quality and quantity check was performed. The library was prepared using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina. mRNA was isolated from 400 ng total RNA using NEBNext Poly(A) mRNA Magnetic Isolation Module. The mRNA was fragmented, end prepped, adapter-ligated, and the library was amplified. The quality of the libraries was checked on 4200 TapeSation System using D1000 Screen Tape, the quantity was measured on Qubit 3.0. Illumina sequencing was performed on the NovaSeq6000 instrument. The reads was mapped to the sequences of the 13 reproduction-mediating neuropeptide precursors.

4. Results

4.1. The nucleotid and peptide sequence of ly-GnRH/CRZ

After the neural transcriptome sequencing, we were able to determine the full-length coding region (1225 bp). We identified the signal peptide, the mature peptide with the signature tribasic cleavage site and an alpha amidation signal, and the ly-GnRH/CRZ-associated peptide. As expected, the mature peptide sequence is highly conserved, while the signal peptide and GnRH/CRZ-associated peptide show high variability. The mass spectrometry analysis confirmed the presence of the predicted active peptide in the central nervous system. These findings and our results suggest that utilization of the amidated undecapeptide as the functional invGnRH/CRZ molecule is a common feature in bivalves and gastropods.

4.2. Phylogenetic analysis

To make the evolutionary relationships of the GnRH neuropeptide superfamily up-to-date, we made a phylogenetic analysis with the ly-GnRH/CRZ peptide and other identified peptides of the superfamily. The constructed phylogenetic tree correlated well with previous studies, the GnRH/CRZ branching supported the notion that sequences originally termed molluscan GnRHs are no more related to vertebrate GnRH than to AKH, ACP or CRZ.

4.3. Presence of ly-GnRH/CRZ transcript and peptide in the CNS and periphery

Our RT-PCR investigation revealed the widespread presence of the transcript. The IHC investigations with a specific antibody showed the presence of the peptide in all CNS ganglia. Both specific and non-specific antibodies against GnRH yielded a positive signal in the CNS, however, with a highly different distribution. Among peripheral tissues, we found abundant ly-GnRH/CRZ-ir fibers on the surface of the heart atria running perpendicularly to the longitudinal axis of the muscle fibers; however, the heart muscle fibers themselves were not immunopositive. In ovotestis, immunoreactivity was not observed. In contrast, we were able to detect immunpositivity in the seminal vesicles.

4.4. Role of ly-GnRH/CRZ in different behaviours

Based on our results, ly-GnRH/CRZ peptide did not change the feeding rate. In contrast, it caused a significant change in the locomotor activity. Similar to this, the reproductive animals injected with ly-GnRH/CRZ significantly changed. Our results support that neuropeptides originally termed invGnRH/CRZs are multifunctional and the peptide family should be classified as CRZ.

4.5. *L. stagnalis* homologs to vertebrate genes involved in sex steroid synthesis and receptor-mediation

Regarding enzymes involved in the vertebrate sex steroidogenesis pathway, we did not find any homologous sequence to vertebrate genes coding for CYP11A, CYP19A, and 3α-HSD. However, we did find homologs to StAR, 3β-HSD, CYP17, 17β–HSD, and 5α-reductase. In contrast, we could not identify any homologs to sex steroid receptor genes nPR and nAR of vertebrates. Moreover, we did not find any homologs to SBGs. However, we did find homologs to nER and PGRMC1.

4.6. Uptake of radioactive steroids

There was a marked decrease in radioactivity in all the vessels containing snails with the uptake rate of P being marginally fastest. Our results confirm that although vertebrate sex steroids can be measured in molluscan tissues, they are so readily absorbed from the environment and can be stored for such a long time, that their presence is very poor evidence of endogenous synthesis.

4.7. Composition of radioactive steroids in snail tissues before and after depuration

Extraction and phase separation of the metabolites in the snail tissues showed the presence of a high proportion of radioactivity for P, E_2, and T, respectively, in the lipid-rich heptane fraction where esterified steroids would be expected to accumulate. The proportion of EE_2 that had been potentially esterified was, as expected, much lower. Most of the rest of the radioactivity was recovered in the 80% ethanol fraction which contains free steroids. Only a small proportion of radioactivity was found in the water-soluble fraction. After ten days of depuration, approximately 70% of the radioactivity was still present in the animals.

4.8. Identification of proteins in the CNS marked by the vertebrate antibodies

Despite the lack of CYP19A and nPR genes in *L. stagnalis*, our IHC investigations with the anti-human CYP19A and anti-human nPR antibodies yielded a positive signal in the CNS. To determine which

molecules were labelled by the human antibodies, we performed PAGE and WB analysis on the CNS homogenate. The anti-human CYP19A antibody yielded one discrete and two amorphous bands. Interestingly, it also marked the 140 kDa marker protein. The anti-human GnRH antibody gave two discrete bands with much higher mass value. The anti-human nPR antibody resulted in three discrete bands. The ~30 kDa band was marked by both the anti-human CYP19A and anti-human nPR antibodies, while the ~50 kDa band was marked by both anti-human GnRH and anti-human nPR antibodies. The subsequent MS identification gave no significant match between the fragments obtained from the bands and the three human protein sequences (or their in silico predicted fragments). We were able to identify some proteins that may have been marked by the antibodies. However, there was no evidence that any of these proteins were the ones that cross-reacted with the relevant antibodies.

4.9. Expression of reproduction-mediating neuropeptides

Our investigations revealed no significant changes in the expression of the reproduction-mediating neuropeptides between the control and progestogens treated snails.

5. Summary

Although molluscs have been used extensively as model species to investigate neuroendocrine processes regulating behaviours such as reproduction, there is a long-standing debate about their (neuro)endocrine system including the role of the GnRH peptide and the function of the sex steroid hormones. Keeping this in mind, my PhD work aimed to investigate the functionality and evolution of the neuroendocrine system of molluscs by investigating the well-established invertebrate model species, the great pond snail (*L. stagnalis*). My results are summarized in the points below with the relevant publications being highlighted.

1) We identified the coding sequence of ly-GnRH/CRZ and confirmed the presence of the active peptide in the CNS. Our findings suggest that utilization of the amidated undecapeptide as the functional invGnRH/CRZ molecule is a common feature in bivalves and gastropods. The molecular phylogenetic results supported the notion that sequences originally termed molluscan GnRHs are more related to CRZs

2) Our *in vitro* and *in vivo* experiments demonstrated that ly-GnRH/CRZ is involved in the regulation of locomotion and reproduction and may play a role in the modulation of heart control. Interestingly, we did not find any link to the feeding. It should be noted that we used only one concentration (10 μg peptide/each animal) during the injection so it remains possible that this concentration was too low to cause any effect on feeding. Therefore, future physiological works should aim to use further peptide concentrations. Although there is still

only a limited number of functional studies with invGnRH/CRZs, our findings add to a growing body of literature supporting invGnRH/CRZ peptides as being responsible for the regulation of both reproductive and non-reproductive functions. Moreover, our results support the proposal that the originally named invGnRHs should be classified as CRZs.

3) Our neural transcriptome and genome data revealed the absence of several key sequences/genes that are essential to accomplish the classical vertebrate-type sex steroid synthesis, as well as the receptor-mediation of sex steroids. Given the presence of the identified genes and the phenomenon of enzyme promiscuity, we do not rule out that *L. stagnalis* (and molluscs in general) is able to perform all steps of the vertebrate sex steroid synthesis pathway, but the yields are likely to be far too low to maintain a sex steroid-based hormonal system. Clearly, one must question whether natural selection in molluscs would have likely favoured the evolution of a steroid-based endocrine system.

4) We confirmed that molluscs can absorb, metabolize, and accumulate vertebrate steroids. This questions how much (if any at all) of the vertebrate sex steroids found in molluscan tissues are of endogenous origin.

5) Using antisera generated against the vertebrate CYP19A and nPR, our IHC revealed immunopositive signal in the CNS. Since neither genes can be found in *L. stagnalis* (and in invertebrates in general), this supports the idea that immunostaining using (especially polyclonal) antibodies generated against mammalian proteins is a highly unreliable procedure for identifying or localizing specific proteins in tissues when applied to invertebrates. The WB and MS results obtained also confirmed this.

6) Although no functional nuclear sex steroid receptor can be found in *L. stagnalis* or in molluscs in general, we do not rule out that molluscs do seem respond (i.e. their physiology is potentially affected) in one way or another when exposed to vertebrate sex steroids. This can happen via the identified membrane sex steroid receptors, or via non-specific receptors (i.e. receptors for other compounds), or simply as a result of source re-allocation caused by the metabolism of sex steroids.

7) Our research group previously demonstrated that *L. stagnalis*'s reproduction alters when the specimens are chronically exposed to progestogens. However, we did not detect any change in

the expression of neuropeptides responsible for the regulation of the reproduction. We suppose that the reproductive allocation of the hermaphroditic specimen changes due to the hormone exposure and this is not manifested at the level of the expression of reproduction-related neuropeptides. The mechanisms underlying the reproductive allocation change requires further investigations.

In summary, our findings support the idea that molluscan endocrinology differs from the well-characterized vertebrate endocrine system.

6. References

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10. **Fodor I**, Zrínyi Z, Maász G, Urbán P, Koene JM, Pirger Zs: Progesztogén indukálta hatások a nagy mocsári csiga (*Lymnaea stagnalis*) neuroendokrin rendszerében. *IX. Ökotoxikológiai konferencia, Budapest, Magyarország, 2019.11.22.* (poszter)
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12. **Fodor I**, Zrinyi Z, Urbán P, Tsai PS, Koene JM, Pirger Zs: Gonadotropin releasing hormone (GnRH) or corazonin (CRZ) - two faces of the same neuropeptide. *1st Symposium on Invertebrate Neuroscience, Tihany, Hungary, 2019.08.13-17.* (poszter)
13. **Fodor I**: Gonadotropin-felszabadító hormon (GnRH) expresszió vizsgálata progesztogén kezelt nagy mocsári csiga (*Lymnaea stagnalis*) központi idegrendszerében. *I. Régiós Környezettoxikológiai PhD Konferencia, Veszprém, Magyarország, 2018.12.12.* (előadás)
14. **Fodor I**, Svigruha R, Zrínyi Z, Büki G, Urbán P, Pirger Zs: Gonadotropin-felszabadító hormon (GnRH) expresszió vizsgálata progesztogén kezelt nagy mocsári csiga (*Lymnaea stagnalis*) központi idegrendszerében. *VIII. Ökotoxikológiai konferencia, Budapest, Magyarország, 2018.11.23.* (poszter)

7.4. Presentations out of the PhD topic

1. **Fodor I**, Svigruha R, Urbán P, Gálik B, Kemenes G, Kemenes I, Pirger Zs: Cellular and molecular mechanisms of age-related changes in a defined neuronal network encoding associative memory. *25. Tavaszi Szél konferencia, Pécs, Magyarország, 2022.05.06-08.* (poszter)
2. Svigruha R, **Fodor I**, Schmidt J, Győri J, Padisák J, Pirger Zs: A progesztogén hatóanyagok hosszú távú terhelése során megfigyelhető egyed- és molekuláris szintű változások a nagy vízibolha (*Daphnia magna*) egyedeiben. *25. Tavaszi Szél konferencia, Pécs, Magyarország, 2022.05.06-08.* (poszter)
3. **Fodor I**, Svigruha R, Urbán P, Gálik B, Kemenes G, Kemenes I, Pirger Zs: Cellular and molecular mechanisms of age-related changes in a defined neuronal network encoding associative memory. *IBRO2022 Workshop, Budapest, Magyarország, 2022.01.27-28* (poszter)

4. Svigruha R, **Fodor I**, Schmidt J, Győri J, Padisák J, Pirger Zs: A progesztogén hatóanyagok hosszú távú terhelése során megfigyelhető egyed- és molekuláris szintű változások a nagy vízibolha (*Daphnia magna*) egyedeiben. *XI. Ökotoxikológiai konferencia, 2021.11.26.* (online előadás)
5. Farkas A, Svigruha R, **Fodor I**, Somogyvári D, Győri J: Neonikotinoidok és emelkedő hőmérséklet interaktív hatásainak vizsgálata a nagy vízibolha (*Daphnia magna*) modellen. *XI. Ökotoxikológiai konferencia, 2021.11.26.* (online előadás)
6. Svigruha R, **Fodor I**, Padisák J, Pirger Zs: Progesztogének okozta változások és azok ökológiai vonatkozásai egy vízi gerinctelen modellállat (*Lymnaea stagnalis*) embrióiban és felnőtt egyedeiben. *X. Ökotoxikológiai konferencia, 2020.12.04.* (online előadás)
7. Maász G, **Fodor I**, Molnár É, Zrínyi Z, Svigruha R, Kiss T, Pirger Zs: A környezetből kimutatható pszichoaktív hatóanyag-szennyezések felmérése és az általuk indukált változások vizsgálata a nagy mocsári csiga (*Lymnaea stagnalis*) központi idegrendszerében. *IX. Ökotoxikológiai konferencia, Budapest, Magyarország, 2019.11.22.* (poszter)
8. Pirger Zs, **Fodor I**, Svigruha R, Molnár É, Zrinyi Z, Kiss T, Maász G: Környezeti kockázatbecslés alapján beállított gyógyszerszennyezések élettani hatásai nagy mocsári csigán (*Lymnaea stagnalis*). *IX. Ökotoxikológiai konferencia, Budapest, Magyarország, 2019.11.22.* (előadás)
9. Molnár É, **Fodor I**, Pirger Zs, Maász G: Gyógyszerhatóanyag-koncentrációk környezeti kockázati elemzése a Balatonban. *IX. Ökotoxikológiai konferencia, Budapest, Magyarország, 2019.11.22.* (előadás)
10. Svigruha R, **Fodor I**, Maász G, Szoboszlay S, Bordós G, Pirger Zs: Jelölt mikroműanyag-partikulumok mozgása a nagy vízibolha nemzedékei között. *IX. Ökotoxikológiai konferencia, Budapest, Magyarország, 2019.11.22.* (poszter)
11. Maász G, **Fodor I**, Molnar E, Zrínyi Z, Svigruha R, Udvardi R, Laszlo Z, Kiss T, Pirger Zs: Monitoring of environmental psychoactive drug contaminations and investigation of the induced neuronal changes. *1st Symposium on Invertebrate Neuroscience, Tihany, Hungary, 2019.08.13-17.* (előadás)
12. Svigruha R, Zrínyi Z, **Fodor I**, Maasz G, Pirger Zs: Impact of progestogen contaminations on the general physiological state of *Lymnaea stagnalis*.*1st Symposium on Invertebrate Neuroscience, Tihany, Hungary, 2019.08.13-17.* (poszter)
13. Molnar E, Hahn J, **Fodor I**, Zrinyi Z, Szoboszlay S, Pirger Zs, Maasz G: Environmental risk assessment of pharmaceuticals in the largest shallow lake in Central Europe. *17th International Conference on Chemistry and Environment, Thessaloniki, Greece, 2019.06.16-20.* (poszter)

14. Maász G, Máyer M, Zrínyi Z, Molnár É, Kuzma M, **Fodor I**, Pirger Zs, Takács P: Gyógyszerhatóanyag maradványok vízminőségi és ökológiai kockázatának vizsgálata. *20. Kolozsvári Biológus Napok, Kolozsvár, Románia, 2019.04.12-14.* (előadás)
15. Molnár É, **Fodor I**, Takács P, Zrínyi Z, Kuzma M, Mayer M, Pirger Zs, Maász G: Gyógyszerhatóanyag-koncentrációk felmérése a Balatonban és annak vízgyűjtő területén a szezonális hatások figyelembevételével. *VIII. Ökotoxikológiai konferencia, Budapest, Magyarország, 2018.11.23.* (előadás)
16. Svigruha R, Zrínyi Z, **Fodor I**, Kardos V, G-Tóth L, Pirger Zs: Gerinctelen vízi modellállatokban megfigyelhető változások progesztogén hatóanyagok hosszú távú terhelése során. *VIII. Ökotoxikológiai konferencia, Budapest, Magyarország, 2018.11.23.* (poszter)
17. Maász G, Molnár É, Kuzma M, Mayer M, Zrínyi Z, **Fodor I**, Takács P, Pirger Z: „Tisztább, mint az átlag" - folytatódnak a gyógyszermaradvány felmérések a Balatonban. *Fiatal Analitikusok XXVI. Előadóülése, Budapest, Magyarország, 2018.11.12.* (előadás)
18. Molnár E, **Fodor I**, Takács P, Zrinyi Z, Kuzma M, Mayer M, Pirger Zs, Maász G: The environmental impact of summer social events on the largest shallow lake in Central. *40th International Conference on Environmental & Food Monitoring, Santiago de Compostela, Spain, 2018.06.19-22.* (poszter)
19. Molnár É, **Fodor I**, Takács P, Zrínyi Z, Mayer M, Pirger Zs, Maász G: Analytical measurement of active pharmaceutical ingredients in Lake Balaton and its catchment area. *Környezettoxikológiai Munkabizottság előadóülése, MTA VEAB székház (Veszprém), 2018.05.30.* (előadás)
20. **Fodor I**, Valasek A, Urbán P, Pirger Zs, Fekete Cs, Kerepesi I: A comparative study on optimisation of protein extraction method for Saccharomonospora azurea. *10th Central and Eastern European Proteomic Conference, Budapest, Hungary, 2016.10.11-14.* (poszter)
21. Maász G, Zrínyi Z, Takács P, Lovas S, **Fodor I**, Pirger Zs: Krónikus progesztogén terhelés indukálta molekuláris, szövettani és szomatikus index változások az őshonos búzaszemű keszegen (*Rutilus rutilus*). *Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája (FAMÉ), Pécs, Magyarország, 2016.06.01-04.* (poszter)
22. Maasz G, Lovas S, Zrinyi Z, **Fodor I**, Kiss T, Pirger Zs: Electrophysiology-LC-MS online system. *34th IMMS, Fiera di Primiero, Italy, 2016.05.15-18.* (poszter)
23. Lovas S, **Fodor I**, Kiss T, Pirger Zs: Changes of electrical characteristics induces by piority pollutant hormones in *Lymnaea* identified neurons. *IBRO2016 Workshop, Budapest, Magyarország, 2016.01.21-22.* (poszter)
24. Valasek A, **Fodor I**, Kiss I, Kovacs M, Toth Zs, Urbán P, Jambor E, Laszlo M, Fekete Cs, Kerepesi I: From genomics to proteomics in the field on antibiotic research. *Hungarian Molecular Life Sciences, Eger, Hungary, 2015.03.27-29.* (poszter)