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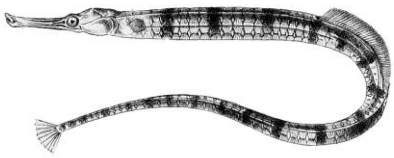
SUMMARY OF THE

PhD THESIS

STUDIES ON THE INFLUENCE OF POLLUTANTS ON SOME FISH SPECIES OF THE FAMILY SYNGNATHIDAE

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CONTENTS

STUDIES ON THE INFLUENCE OF POLLUTANTS ON SOME FISH SPECIES OF THE FAMILY SYNGNATHIDAE

OBJECTIVES AND AIM OF THE STUDY

The Black Sea is an ecosystem with unique peculiarities, which has undergone dramatic changes during the past sixty years, both in what concerns abiotic parameters, but especially in biodiversity. Pollution has become worldwide and, implicitly, in the Black Sea, one of the major threats aquatic species are facing, due to anthropogenic discharges reaching the marine environment. It is already well known that some species are threatened by these human influences, while others are insufficiently studied. In addition, some species sensitive to environmental changes are used as bioindicators of the contamination with chemicals. Given the capacity of marine organisms to accumulate via various pathways the chemicals occurring in their living environment (water, sediments, food), their use as bioindicators is supported by various examples. Bioindicator species are ideal for biomonitoring, and the most suitable for the marine environment are considered mollusks, particularly common mussels, which have been the focus of expanded studies worldwide, as well as at the Romanian Black Sea coast (Butler, 1971; Haug, 1974; Phillips, 1980; Sericano et al., 1990; Oros et al., 2003; Oros, 2009; Oros & Gomoiu, 2012; Roşioru et al., 2012; Roşioru et al., 2014).

However, other species inhabiting shallow coastal areas, undergoing anthropogenic influences, could be used as bioindicators, all the more so if we are considering species sensitive to environmental parameters (*Hippocampus guttulatus*, Cuvier 1829) (Woodall, 2012) or species with a broad distribution (*Syngnathus* sp.). Syngnathids are small marine fishes, inhabiting seagrass meadows and areas covered by macrophyte algae, where their shape and coloring offers them camouflage. In the Black Sea, literature mentions several Syngnathid species (pipefish, genus *Syngnathus: Syngnathus typhle, Syngnathus schmidti, Syngnathus variegatus, Syngnathus tenuirostris (acus), Syngnathus abaster,* genus *Nerophis*: *Nerophis ophidion;* seahorses, genus *Hippocampus: Hippocampus guttulatus*, potentially *Hippocampus hippocampus* and *Hippocampus fuscus*). It is this controversy on the existence of one or several species of the genus *Hippocampus* at the Romanian Black Sea coast that triggered this research.

The **aim** of this thesis is the thorough investigation of Syngnathids occurring at the Romanian Black Sea coast and the paper is structured on three major categories of objectives, as follows:

1) ***Objectives of enhancing the knowledge on the biology and ecology of the target species:***
   Characterization of the Syngnathid species identified at the Romanian Black Sea coast; Calculation of the length-weight relationship for the species *H. guttulatus*; Determination of the effects of various live feed diets on the long-snouted seahorse *H. guttulatus*, with the aim of establishing the best diet for captive rearing.
2) ***Objectives of performing genetic analyses for species determination:***
   Karyotype display of *H. guttulatus* of the Romanian Black Sea coast and mtDNA amplification and dendrogram construction.
3) ***Objectives of quantifying the contamination with anthropogenic origin xenobiotics:***
   Pollutant bioaccumulation in the wholse tissue of the long-snouted seahorse *H. guttulatus*; Heavy metal bioaccumulation in the whole tissue of the long-snouted seahorse *H. guttulatus* and the greater pipefish *S. acus* of the Romanian Black Sea coast, expressed by the Bioconcentration Factor (BCF) and the Biota-Sediment Accumulation Factor (BSAF); Contamination with polycyclic aromatic hydrocarbons (PAHs) of Syngnathids in Romanian coastal waters.

The novelty of this thesis is rendered by approaching some species that have not been studied to date at the Romanian Black Sea coast and the results obtained will definitely contribute to enhancing the scientific knowledge on the family Syngnathidae.

It is this novelty of approach that raised some drawbacks in finding information for documentation, the only data for comparison purposes being available from the south-eastern Black Sea area (Turkey: Başusta et al., 2014; Kasapoglu & Duzgunes, 2014). Furthermore, for the species *H. guttulatus* no data were available in FishBase (Froese & Pauly, 2004). This lack of data, correlated with the fact that the species is rated by IUCN as Data Deficient (DD) (Woodall, 2012), confirms the need to perform further research, aiming at obtaining new data for the Black Sea.

Moreover, throughout the research programme, it was found that non-selective fishing is another threat for these species at the Romanian coast, whereas many Syngnathid specimens resulting from by-catches from the fishing points along the Romanian coastline were identified and used for contaminant analysis. These by-catches, which would have been otherwise dried and sold as souvenirs or simply discarded, can be used for performing further tests and analyses (for example, determining trends of xenobiotic contamination without collecting any live individuals from the marine environment).

Key-words: contamination, heavy metals, hydrocarbons, growth parameters, Syngnathids, karyotype, mtDNA, food

PART I. STATE-OF-THE-ART

1. BLACK SEA AND ANTHROPOGENIC POLLUTION

The Black Sea ecosystem, similarly to other European countries (the Baltic Sea, the Mediterranean Sea, the North-Eastern Atlantic), has undergone dramatic changes in all its subsystems, immediately after 1970, as a follow-up of industrial sprawl and intensive agriculture, marked by eutrophication (the massive overfertilisation of marine waters with nitrogen and phosphorous compounds, mainly generated by agriculture, domestic and industrial sources) (Mamaev et al., 1995).

By all means, other forms of pollution have also been documented, such as discharging insufficiently treated waste waters (which resulted in microbiological contamination), oil pollution from accidental or operational spills from vessels, pollution with other toxicants, such as pesticides (most of them coming from farming and reaching the sea by tributary rivers), heavy metal contamination (E.g. cadmium, copper, chrome, lead) from industry, contamination with radioactive substances from nuclear generators or traces of the Chernobil nuclear accident of 1986 (INCDM, 2012a).

The increasing anthropogenic origin contaminant inputs and habitat degradation and loss have caused major changes in aquatic ecosystems, raising, at the same time, the scientific interest for two cutting-edge areas of research: on the one hand, the accumulation and toxic effects of contaminants on aquatic organisms and, on the other hand, the processing and accumulation of such contaminants by marine living resources (mainly those for human consumption, but not exclusively) (Oros, 2009).

Apart from the species targeted by commercial fisheries, the Black Sea is also inhabited by species with no economic value for human consumption, but with a special conservative interest, enriching the Black Sea biodiversity, such as the fish belonging to the family Syngnathidae. This paper aims at exploring the characteristics of this bony fish family of the Romanian Black Sea coast (*Part II, Subchapters 3.1-3.4*), as well as the influence of anthropogenic pollutants on these species (*Part II, Subchapters 3.4-3.7*).

Seeing that worldwide the main threat for the species belonging to the family Syngnathidae is represented by anthropogenic pollution and destruction of typical habitats (Woodall, 2012) and that these species inhabit shallow coastal areas, exposed to pollutant influence, the investigation of concentrations of heavy metals and organic contaminants (hydrocarbons and pesticides) can surely contribute to acquiring new knowledge on the levels recorded by pollutants in marine biota.

PART II. OWN CONTRIBUTIONS

The species of the family Syngnathidae are exposed to the influence of these pollutants particularly due to the fact that they inhabit shallow areas close to the coast, where typical habitats are found (seagrass meadows and rocky substrates covered by macrophyte algae), the more so at the Romanian Black Sea coast, which is strongly anthropogenized. Under such circumstances, the study aimed at analyzing the contamination with three of the most significant toxicants of the marine environment - organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs) and heavy metals - in the whole tissue of seahorses/pipefish collected along the southern Romanian Black Sea coast. Additionally, the characteristics of the studied species were described and some biochemical parameters were determined (dry weight, moisture, raw protein, raw lipid, ash).

While it is acknowledged and demonstrated by scientific analyses that mussels or the gastropod *Rapana venosa* have the capacity of bioaccumulating xenobiotics, data are scarce on how the seahorse and pipefish organisms intake contaminants, particularly in the Black Sea.

The target species for analysis were the pipefish and seahorses collected from the southern part of the Romanian Black Sea coast. All individuals were measured and weighed, determining the length-weight relationship. Additionally, various Syngnathid specimens from by-catches from the fishing points along the coast were used, their occurrence proving that non-selective fisheries can also be a serious threat to these species.

In order to settle the controversy regarding the occurrence of one or several seahorses species in Romanian Black Sea waters, genetic analyses were performed, aiming at detecting potential polymorphisms.

2. MATERIAL AND METHODS

Sample collection was made manually by autonomous divers from three sampling stations in 2013 (south to north): Mangalia, Saturn-Venus and Cazino Constanța, and two sampling stations in 2014 (south to north): Costinești and Cazino Constanța. In order to reduce to the minimum the mortalities caused, the number of pipefish and seahorses collected from the environment was low, yet the samples were sufficient to provide for three replicates of the determinations made. Additionally, a large number of individuals resulting from by-catches of the fishing points located along the Romanian Black Sea coast, as well as from survey trawling made by NIMRD ”Grigore Antipa” during scientific expeditions were used, particularly for performing measurements and calculating the length-weight relationship. This capitalization of individuals resulting from by-catches is non-invasive, not putting any additional pressure on the stocks of the target species. The biological material was frozen and subsequently subjected to specific laboratory analyses (Nenciu-Zaharia et al., 2013; Nenciu et al., 2014a; Nenciu et al., 2014b) (*Subchap. 2.1*).

For Syngnathid ***species identification***, the ”Determinator of the Main Black Sea Fish” (Radu & Radu, 2008) and ”A Guide to the Identification of Seahorses“ (Lourie et al., 2004) (*Subchap. 2.2*) were used. For the ***calculation of the length-weight relationship***, the equation $W = a \cdot Lt^b$ (Carlander, 1977) was used, where: W = fish weight; Lt = fish total length; a and b = regression constants (*Subchap. 2.3*).

For ***genetic analyses*** (karyotype display, mtDNA amplification), the *H. guttulatus* individuals were manually collected by autonomous divers and transported alive to the laboratory, and subsequently subjected to the specific analysis protocol. In order to display the seahorse karyotype and identify chromosomes, an adapted version of the Hafez method was used (Hafez et al., 1981) (*Subchap. 2.4*).

For ***genetic variability determination***, the working method comprised the following stages:
- ***collecting tissue samples*** from *H. guttulatus* fin clips (preserved in pure ethanol until analyzed);
- ***extracting DNA*** from fin clips by proteinase K digestion and phenol : chloroform purification (Taggart et al., 1992);
- ***determining polymorphisms by PCR-RFPL*** (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism): the cytochrome b region was amplified by Polymerase Chain Reaction (PCR). Primers according to Ludwig et al., 2002 were used. The estimated size of the amplified fragment covering the whole cytochrome b region was 1.2 kb. DNA amplification by Polymerase Chain Reaction (PCR) was made with a GeneAmp PCR System 9700 (Applied Biosystems) thermocycler. For polymorphism analysis (RFLP), eight restriction enzymes were used, in order to detect potential polymorphisms. The samples subjected to digestion were then separated by electrophoresis in agarose gel 2.5% 0.5% x buffer solution / boric acid / ethylenediaminetetraacetic acid (EDTA). The DNA was then visualized under ultraviolet light (wave length 302 nm), after the reaction with ethidium bromide (Onără et al., 2012);
- ***analyzing nucleotide sequences***: for this study were considered the cytochrome b nucleotide sequences of *H. guttulatus* and other species of the genus *Hippocampus* available in GenBank. In order to verify the similitude with other sequences of the *Hippocampus* species in GenBank, the nucleotide fragments of cytochrome b from *H. guttulatus* were analyzed using the BioEdit software. The nucleotide sequences were aligned using the Clustal W 1.4 software, and the dendrogram was constructed using the MEGA5 software, by the UPGMA method (Unweighted Pair-Group Method using Arithmetic Averages) (Sneath & Sokal, 1973) and the Kimura 2-parameter model, based on genetic distances (Onără et al., 2012).

In order to set-up the ***experiment on the best diet for feeding seahorses in captivity***, the *H. guttulatus* individuals were collected by divers from the Olimp area (southern Romanian coast, rocky area, covered by seagrass and perennial algae). They were transported alive to the NIMRD aquaculture laboratory and acclimated for 24 hours in a 80 l tank, fitted with aeration. No food was provided during the acclimation period (Nenciu et al., 2015). The witness batch was represented by adult *H. guttulatus* individuals collected from the same sampling area. They were also transported to the NIMRD premises and euthanized using MS 222 (Tricaine methanesulfonate, 250 mg/l concentration). Subsequently, they were sexed, measured and weighed, then frozen to be later subjected to biochemical composition analysis. The duration of the experiment was 10 days (15-24 July 2014). Photoperiod period 16 h L : 8 h D, similar to normal summer conditions at the Romanian Black Sea. Three experimental tanks were set in the laboratory, with water parameters similar to the natural environment in terms of temperature, salinity and light, and filled with 10 l of seawater (1 l/seahorse). All three tanks were properly aerated and provided with artificial plants as holdfasts for the seahorses. Water was changed daily, so as to provide a constant density of prey in the experimental tanks, thus temperature and salinity were consistent with values in the natural environment. Tank A contained 10 *H. guttulatus* individuals (6 males, 4 females) fed exclusively on the rotifer *Brachionus plicatilis*. Tank B contained 10 *H. guttulatus* seahorse individuals (3 males, 7 females) fed exclusively on the brine shrimp *Artemia salina*. Tank C contained 10 *H. guttulatus* individuals (5 males, 5 females) fed on a 50-50% *B. plicatilis* and *A. salina* diet. Food was given daily, at a density of 70 ind./ml for rotifers and 15 ind./ml for brine shrimp. The rotifer *B. plicatilis* was obtained from a strain provided by the courtesy of the Central Fisheries Institute in Trabzon (CFRI). It was subsequently reared in the NIMRD laboratories. The brine shrimp *A. salina* was obtained from dormant eggs (cysts) purchased from the market (JBL Artemio Pur) and then incubated in seawater with aeration. After completion of the experimental interval, the biochemical composition of the three *H. guttulatus* batches and of the control batch was analyzed using standard methods (Roșioru et al., 2014). The results were statistically interpreted using IBM SPSS Statistics v. 20, applying the One-Way Anova (Analysis of Variance) parametric test (*Subchap. 2.5*).

For the ***determination of the concentrations of the five heavy metals*** (copper - Cu; cadmium - Cd; lead - Pb; nickel - Ni; chrome - Cr) both in biota samples and water and sediment samples, the sampling, conservation and preliminary processing of samples, as well as the analysis methodology were compliant with reference methods recommended in the study of marine pollution (IAEA-MEL, 1999; Grashoff et al., 1999). In order to establish the correlations between concentrations in biota, water and/or sediments, the **Bioconcentration Factor (BCF)** was calculated using the formula: $BCF = C_B/C_W$, where C_B = concentration of chemical per kg of dry weight of the analyzed tissue and C_w = amount of chemical dissolved in water/l, and the **Biota-Sediment Accumulation Factor** using the formula: $BSAF = C_B/C_S$, where C_B = concentration of chemical per kg of dry weight of the analyzed tissue and C_S = concentration in sediments (weight of chemical per kg of dry sediment).

Polycyclic aromatic hydrocarbons (PAHs) were determined using a method developed by NIMRD based on the "Training Manual for Measuring Organochlorine Pesticides and Hydrocarbons in Environmental Samples"(IAEA-MEL, 1995).

The ***organochlorine pollutants***' content in seawater, sediments and animal tissue was made by gas-chromatography, using a Perkin Elmer CLARUS 500 gas-chromatograph equipped with electron capture detector (Coatu et al., 2013).

With the aim of establishing statistical correlations between the contaminant concentrations in the animal tissue and the environment and for calculating certain indices (E.g. BCF - Bioconcentration Factor or BSAF - Biota Sediment Accummulation Factor), water and sediment samples were collected from the same locations. Water samples were collected using Nansen bottles and sediments using the Van Veen grab (Nenciu et al., 2013; Nenciu et al., 2014a).

In order to determine the ***biochemical composition*** of marine organisms, standard analysis methods were used, refined within NIMRD "Grigore Antipa" (Roșioru, 2008). Moisture and dry weight were determined by drying in a furnace at 105°C for 24 h, while ash was determined by burning at 550°C for 6 h. The protein content (% of dry weight) of *H. guttulatus* individuals was analyzed using the Lowry method (Lowry et al., 1951), while lipid content was determined using the Soxhlet method.

All data were ***statistically processed*** using Microsoft Excel 2007, IBM SPSS Statistics v. 20 (T test, One-Way Anova - Analysis of Variance) (Petcu, 2011) and Statistica v. 10 softwares.

3. RESULTS AND DISCUSSIONS

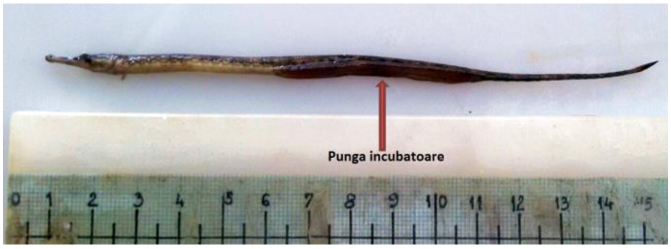
3.1. Characterization of species in the family Syngnathidae

The family Syngnathidae is part of the Order Syngnathiformes and comprises seahorses (*Hippocampus* sp.), pipefish and seadragons. As general description, the body of Syngnathids is elongated (either straight - genus *Syngnathus*, or with a 90° angle between the head and torso - genus *Hippocampus*), covered with regular bony metameric plates, closely fitted together, thus forming a very rigid exoskeleton, as the plates of one level form a full ring. The number of rings usually corresponds to the vertebrae. The head is elongated, the snout is long and tube-shaped and the mouth is small and located at the tip of the tube. The jaws lack teeth and are fused together. They have a single well-developed dorsal fin (the second), with no spiny rays, the anal fin is scant, the caudal fin is very small or absent, the two pectoral fins are either small or lacking, and ventral fins are usually absent. The gills are generally reduced to a small opening on the upper side of the operculum (Radu & Radu, 2008). With reference to their breeding, the eggs are laid by the female on the ventral side of the male, either in the thoracic or in the caudal area. In most genera, some lateral folds of the male body cover the incubating region, thus forming a real incubating broodsack/pouch. During development, the eggs are fed by the male's body, the nutrients and oxygen diffusing through the broodsack walls (Radu & Radu, 2008).

The oldest Syngnathid fossils date back from the Middle Miocene (Lower Sarmatian, Coprolithic Horizon) and were discovered in Slovenia (Zalohar et al., 2009). The evolutionary processes led to the transformation of a conventionally shaped fish into one that does not look like a fish anymore (Zalohar et al., 2012). One of the morphologic characteristics used for the taxonomic classification of Syngnathids is the fusion of cranial and feeding structures.

The separation from the evolutionary point of view between seahorses and pipefish, occurring during the late Oligocene, was determined used a lax "molecular clock", setting older dates as the upper limits (Teske & Beheregaray, 2009). During that age seahorses got their vertical position, a distinctive feature which offered them a high potentail for camouflage in the fully expanding seagrass meadows, and subsequently the specialization of their feeding system occurred. The vertical position is the only morphological difference between seahorses and pipefish (Zalohar & Hitij, 2012), which was confirmed by the discovery of a seahorse fossil in the hills of Tunijce (Slovenia), in the Coprolithic Horizon, dated from the Middle Miocene. Researchers suggested that this was an intermediary group between seahorses and pipefish.

The family Syngnathidae is one of the largest famlies whose members use one of the rarest reproductive strategies in the animal world: male pregnancy/gestation, defining gestation as the process of incubating embryos inside the body after the merger between the egg and the spermatozoon (Stolting & Wilson, 2007). Many families have granted males with parental responsibilities, yet Syngnathids went event further, the males being the ones fertilizing and then incubating the eggs previously laid by the female inside the broodsack.



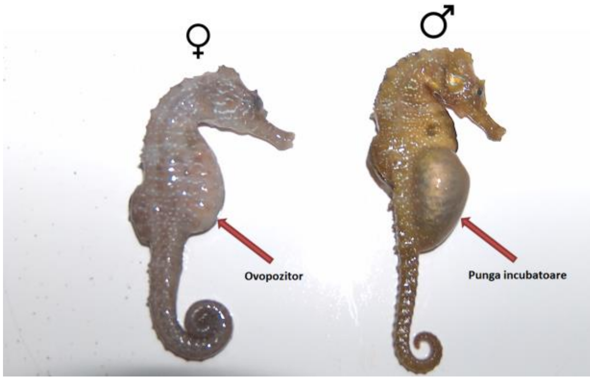


Fig. 3.1.1. ***S. acus*** **male** ***(original photo).*** **Fig. 3.1.2.** ***H. guttulatus*** **female (left) and male (right)** ***(original photo).***

Pipefish (subfamily Syngnathinae) are a subfamily of small fish, which, along with seahorses, form the family Syngnathidae. Most pipefish are marine dwellers and only some species live in fresh water. They are abundant in coastal waters of tropical and temperate zones. Most pipefish species are usually 35-40 cm long and live in areas sheltered by coral reefs or seagrass beds. There are around 200 pipefish species in the world, of different sizes and shapes; thus, the largest species reaches more than 65 cm, and the smallest species around 3 cm. Similarly to their close relatives, seahorses, pipefish breed in a particular way, as females lay the eggs in a pouch located on the male's abdomen, where they are incubated and subsequently released by the male in a labor-like process (Kleiber et al., 2011).

In the Black Sea, several species of the subfamily Syngnathinae are accounted for (*Syngnathus typhle* Linnaeus, 1758*, Syngnathus schmidti* Popov, 1927*, Syngnathus variegatus* Pallas, 1814*, Syngnathus tenuirostris (acus)* Rathke, 1837*, Syngnathus abaster* Risso, 1827*, Nerophis ophidion* Linnaeus, 1758) (Radu & Radu, 2008). Individuals belonging to all these species were identified during the performance of this research in by-catches of the fishing points located along the Romanian Black Sea coast (Fig. 3.1.1).

The seahorse (Fig. 3.1.2) is one of the most charismatic species in the Black Sea and, despite that apparently it has no economic significance (it is not a species usable as food source for people), it raises interest for use as bioresource for traditional medicine, either for the developing aquarium business, or it is simply sold illegally as souvenir or curio. In Romanian Black Sea waters, literature sources (Lourie et al., 2004; Foster & Vincent, 2004) indicate the occurrence of three species, namely *Hippocampus guttulatus* (Cuvier, 1829), *Hippocampus hippocampus* (Linnaeus, 1758) and *Hippocampus fuscus* (Rüppel 1838). At the Romanian coast, more recent papers (Radu & Radu, 2008) report a single species, while older references mention several species (Bănărescu, 1964). Nevertheless, only a genetic study can confirm the occurrence of the three different species, which has not been yet undergone in the Black Sea. Consequently, one of the objectives of this thesis is to genetically analyze the specimens collected from the environment, in order to confirm or reject the occurrence of the three species or potential subspecies (*Subchapter 3.3. Genetic analyses for the species Hippocampus guttulatus of the Romanian Black Sea coast*).

3.2. Calculation of the length-weight relationshiop for *Hippocampus guttulatus* of the Romanian Black Sea coast

In order to determine this correlation, a total number of 74 *H. guttulatus* individuals were collected (33 females and 41 males). The mean length of the whole batch was 8.03±0.91 cm and the mean weight 2.07±0.79 cm. Concerning the differences between the two sexes, the mean length of females was 7.79±0.90 cm, while males measured 8.21±0.90 cm on average; the mean weight of females was 1.88±0.84 g, while for males 2.22±0.73 g. Statistically, these differences are not significant, whereas the values of $p > 0.05$ (weight M vs. F $p = 0.0669$, length M vs. F $p = 0.0509$) (Fig. 3.2.1).

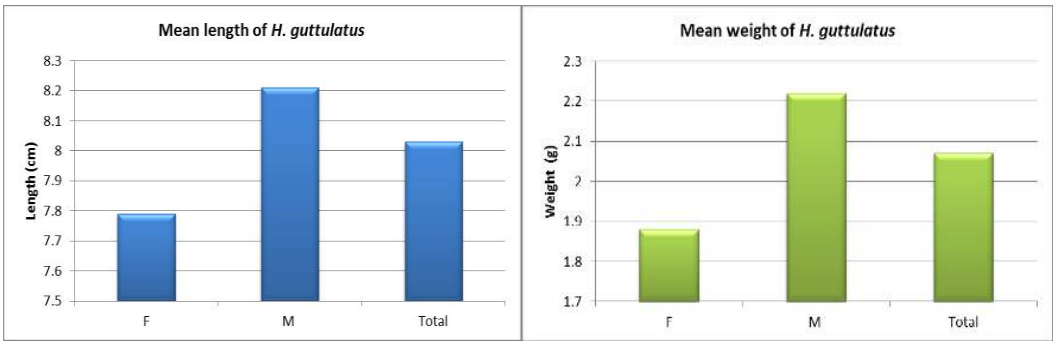


Fig. 3.2.1. Mean length and weight of *H. guttulatus* individuals measured and weighed.

The calculated parameters of the length-weight relationship and the biometric characteristics obtained as a follow-up of measurements for the species *H. guttulatus* (number of samples, length and weight ranges, mean lengths and weights, a and b coefficients, determination coefficient R^2) are detailed in Table 3.2.1. The values of R^2 were determined as follows: total $R^2 = 0.64$, males $R^2 = 0.55$, females $R^2 = 0.71$, being comparable with the values recorded in the same species in the south-eastern Black Sea and the Mediterranean Sea ($p > 0.05$), but significantly smaller than the values recorded in individuals sampled from the Atlantic Coasts ($p < 0.05$) (Table 3.2.1).

Table 3.2.1. Length-weight relationship parameters for the species *H. guttulatus* (comparison between North-Western Black Sea - Romania, South-Eastern Black Sea - Turkey, Aegean Sea - Turkey and Atlantic Ocean - Southern Portugal).

Location	Sex	N	Length range (cm)	Weight range (g)	*a* *Intercept*	*b* *Slope*	R^2	References
North-Western Black Sea - Romania	F	33	6.10-9.90 Mean 7.79	0.52-4.30 Mean 1.88	0.002	3.23	0.71	Own results, 2015
	M	41	7.00-10.30 Mean 8.21	1.54-4.41 Mean 2.22	0.023	2.14	0.55	Own results, 2015
	Total	**74**	**6.10-10.30 Mean 8.03**	**0.52-4.41 Mean2.07**	**0.006**	**2.72**	**0.64**	**Own results, 2015**
South-Eastern Black Sea - Turkey	F	59	5.70-8.60 Mean 7.15	0.85-2.60 Mean 1.72	0.008	2.64	0.89	Başusta et al., 2014
	M	79	6.30-9.00 Mean 7.65	1.06-2.90 Mean 1.98	0.004	2.97	0.92	Başusta et al., 2014
	Total	138	5.70-9.00 Mean 7.35	0.85-2.90 Mean 1.87	-	-	-	Başusta et al., 2014
South-Eastern Black Sea - Turkey	Total	272	Mean 8.31	Mean 2.14	0.007	2.64	0.69	Kasapoglu & Duzgunes, 2014
Aegean Sea - Turkey	Total	200	10.00-16.50 Mean 13.25	2.54-11.8 Mean 7.17	0.010	2.47	0.64	Gürkan & Taşkavak, 2007
Arade Estuary/ Atlantic Ocean S Portugal	Total	84	3.6-18.50 Mean 11.05	-	0.007	2.71	0.99	Veiga et al., 2009

The length-weight relationships for the species *H. guttulatus* are shown for the total batch, as well as differentiated by sexes (Fig. 3.2.2). For the overall sample, the resulting length-weight equation is $W = 0.00668 \cdot Lt^{2.727096}$. For males, the equation is $W = 0.02318 \cdot Lt^{2.14758}$, while for females $W = 0.00227 \cdot Lt^{3.23741}$.

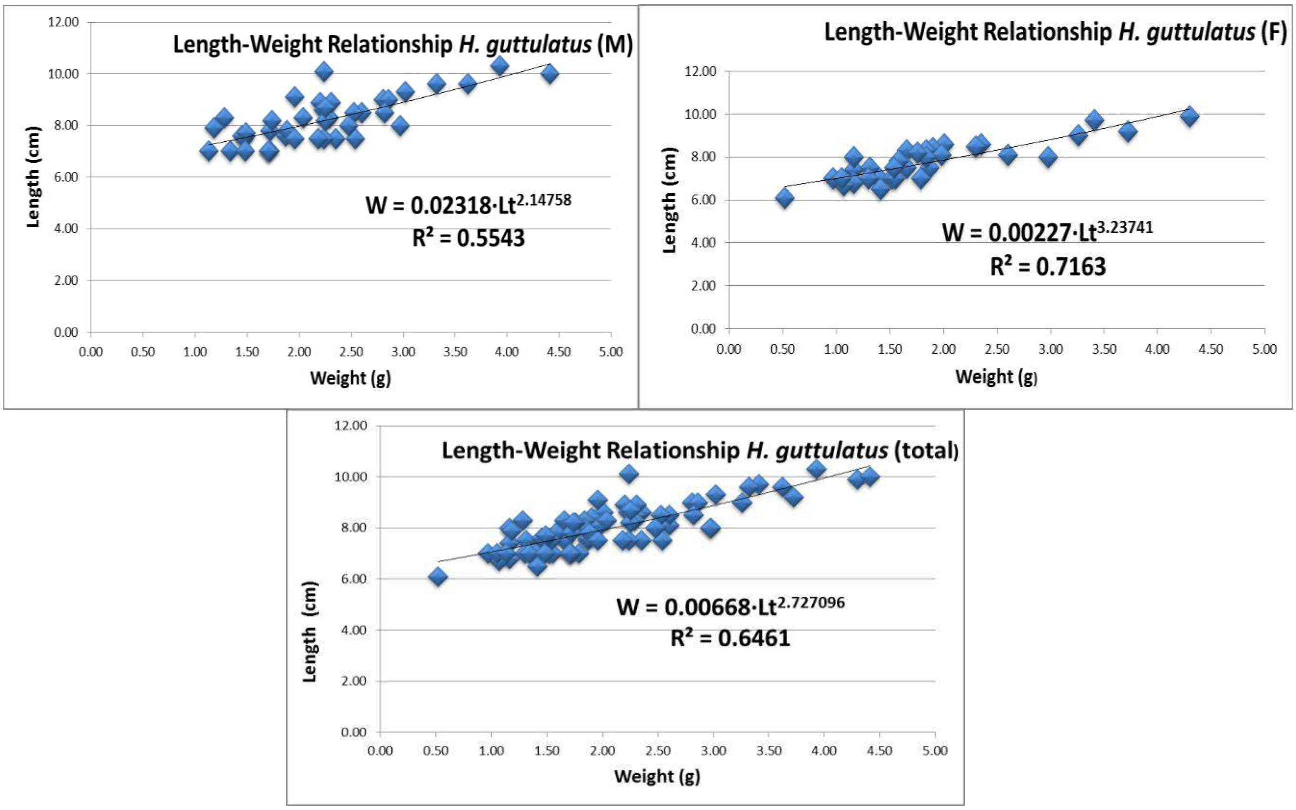


Fig. 3.2.2. Length-weight relationships for *H. guttulatus* (males, females and total).

When performing a comparison of the mean lengths of *H. guttulatus* individuals of different marine areas, it was found that the lengths of seahorses from the Romanian Black Sea coasts are comparable with the ones from the south-eastern corner of the Black Sea ($p > 0.05$), while specimens from the Aegean Sea (Izmir Bay) are significantly larger ($p < 0.05$), with sizes comparable to those measured on the Atlantic coast (Arade Estuary, Portugal).

With reference to the weight of *H. guttulatus* specimens, Fig. 3.2.3 shows clearly that individuals from the Aegean Sea - Izmir Bay are much heavier, the difference between the latter and specimens from the Black Sea (both from the north-west and the south-east) being statistically significant ($p < 0.05$). It was noted that individuals from the Black Sea, regardless of the collection area, recorded similar weights ($p > 0.05$).

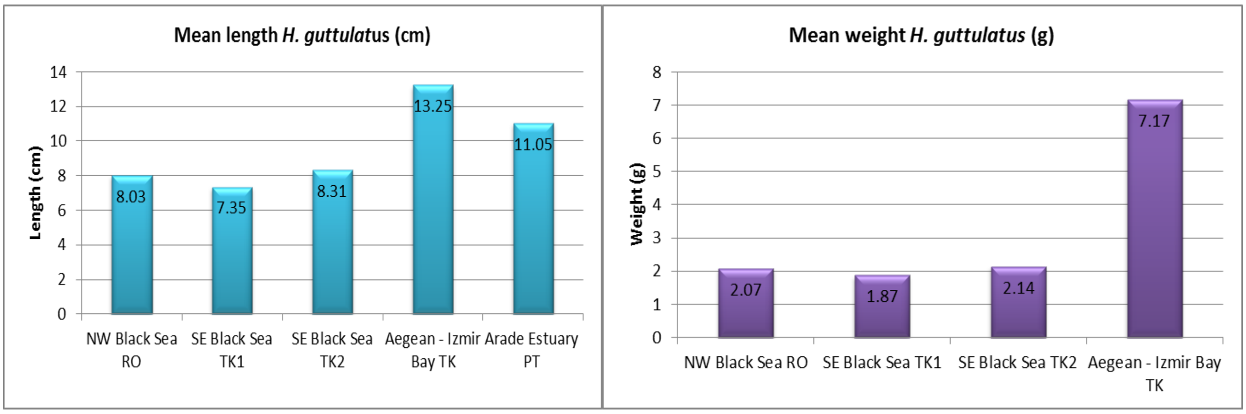


Fig. 3.2.3. Comparison of mean lengths and weights recorded by *H. guttulatus* in various marine areas.

The condition factor (K) was determined using the equation $K= (W/L^3) \times 100$, where W = fish weight and L = total length of the fish (Başusta et al., 2014). Compared to seahorse specimens in the south-eastern Black Sea (mean 0.4183, Başusta et al., 2014), the values obtained for the Romanian coast (K total = 0.3997, K females = 0,3976, K males = 0.4011) indicate a slightly better condition factor of the individuals collected from the Turkish Black Sea coast, yet not statistically significant ($p > 0.05$).

The length-weight relationship is used to determine the condition factor of fish and the type of growth (isometric or allometric). A coefficient b = 3 means a linear isometric growth of the fish (length and weight increases proportionally) (Ricker, 1975). An over-growth in length as compared to weight translates into a coefficient $b < 2.5$, and an over-growth in weight as compared to length is expresses by a value of $b > 3.5$ (Froese, 2006).

The values of coefficient b obtained for the H. *guttulatu*s specimens measured and weighed during this study indicated an overall negative allometric growth (b = 2.72), which means that the length of individuals increases faster compared to their weight. On sexes, males also recorded a negative allometric growth (b = 2.14), while females recorded a positive allometric growth (b = 3.23), unlike in other studies, where the growth reported was allometric negative both for females and males (Başusta et al., 2014).

For the species *H. guttulatus* no available data were found in FishBase (Froese & Pauly, 2004). This aspect, along with IUCN's classification of *H. guttulatus* as Data Deficient (DD), accounts for the need of future research, aiming at obtaining new information for the Black Sea.

3.3. Genetic analyses for the species *Hippocampus guttulatus* of the Romanian Black Sea coast

Previous research on the karyotype of the species *H. guttulatus* in the Mediterranean Sea revealed diploid values 2n = 44 chromosomes (Vitturi, 1988; Vitturi et al., 1998).

The results of this investigation led to the conclusion that *H. guttulatus* individuals sampled from the Romanian Black Sea coast also have a katyotype with diploid values 2n = 44 chromosomes, 2 sm-m+42 a; FN = 46 (Fig. 3.3.1-3.3.2).

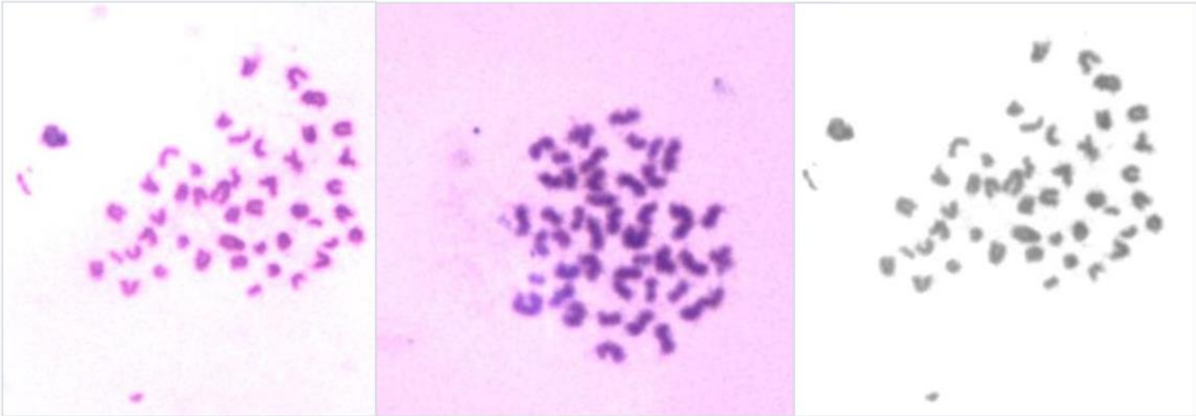


Fig. 3.3.1. Metaphase plates in *H. guttulatus* photographed with an immersed lens microscope *(photo Elena Taflan, DDNI Tulcea)*.

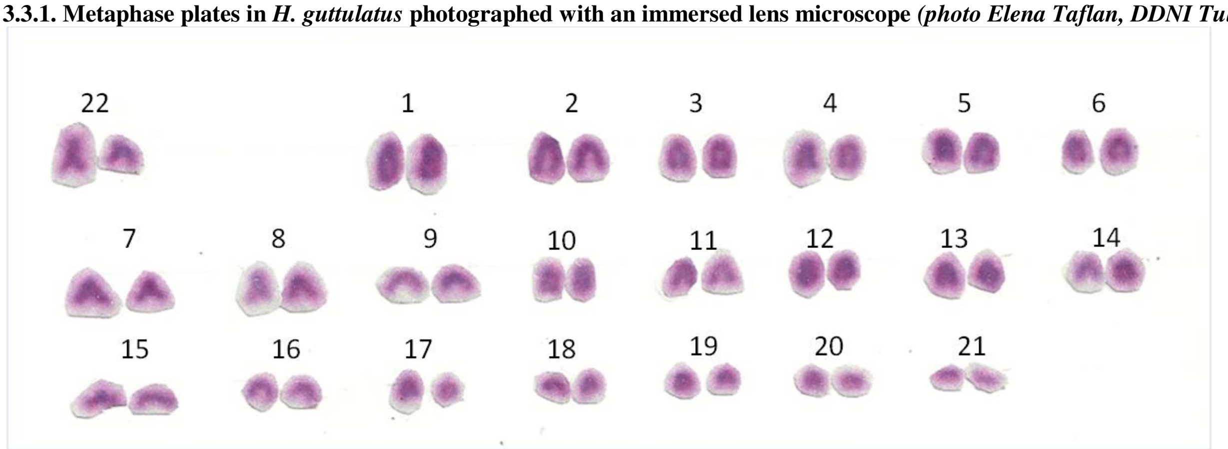


Fig. 3.3.2. Karyotype of *H. guttulatus* of the Romanian Black Sea coast *(photo Elena Taflan, DDNI Tulcea)*.

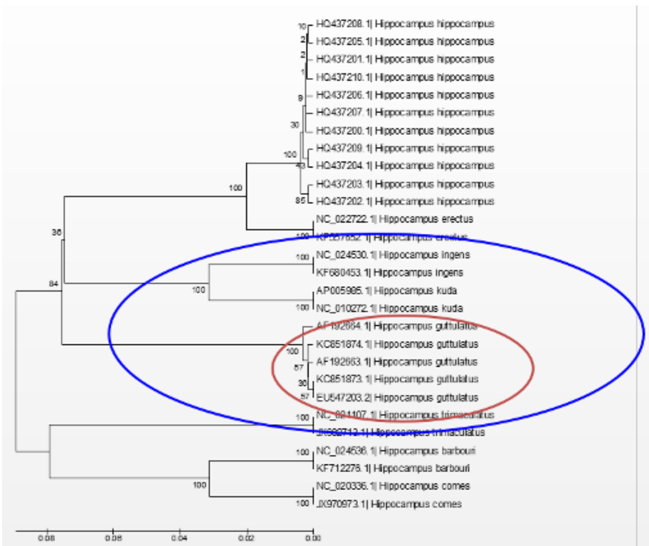


Fig. 3.3.3. Dendrogram constructed based on the Kimura-2 parameter of the cytochrome b region from 7 Hippocampus sp. specimens collected from the Romanian Black Sea coast.

Thus, there is a karyotype with 22 pairs of chromosomes, of which 21 pairs comprise acrocentric chromosomes (pairs 1 - 21), with decreasing sizes, and the last pair (22) is made of 2 medium sized meta-submetacentric chromosomes, thus resulting in a fundamental number of 46 chromosome arms, 2 sm-m+42 a; FN = 46 (Fig. 3.3.2). Whereas no heterotypical elements were detected in male and female karyotypes, it was concluded that the species *H. guttulatus* does not have sexually differentiated chromosomes.

For the construction of the phylogenetic dendrogram, molecular phylogeny was used, which involves the use of biological macromolecules to obtain information on the evolutionary history of living beings and their phylogeny. For this study, the nucleotide sequences of cytochrome b of *H. guttulatus* and other species of the genus *Hippocampus* available in GenBank were included (*Subchapter 2.4. Genetic analyses for species determination*). The dendrogram of the cytochrome b region resulting after using the UPGMA method and the Kimura 2 parameter model, based on genetic distances, showed two major clusters (Fig. 3.3.3). The species *H. guttulatus* was grouped as a distinct branch, yet phylogenetically closer to the species *H. hippocampus*, *H. erectus*, *H. ingens* and *H. kuda* (with whom it also showed phenotypic resemblances).

mtDNA amplification for the seven specimens collected from the Romanian Black Sea was successfully completed (Fig.

3.3.4). No polymorphisms were identified in the seven specimens, using eight restriction enzymes [*Tru*9I (Promega R7011), *Hph*I (NEB R0158), *Rsa*I (Promega R6371), *Dde*I (Promega R6291), *Hae*III (Promega R6171), *Bsl*I (NEB R0555), *Bam*HI (Promega R6021) and *Bsa*JI (NEB R0536)], which confirms the hypothesis that all individuals analyzed belong to the same species, namely *H. guttulatus*, rejecting the theory claiming that two other seahorse species (*H. hippocampus* and *H. fuscus*) also inhabit the shallow areas of the Romanian Black Sea coast.

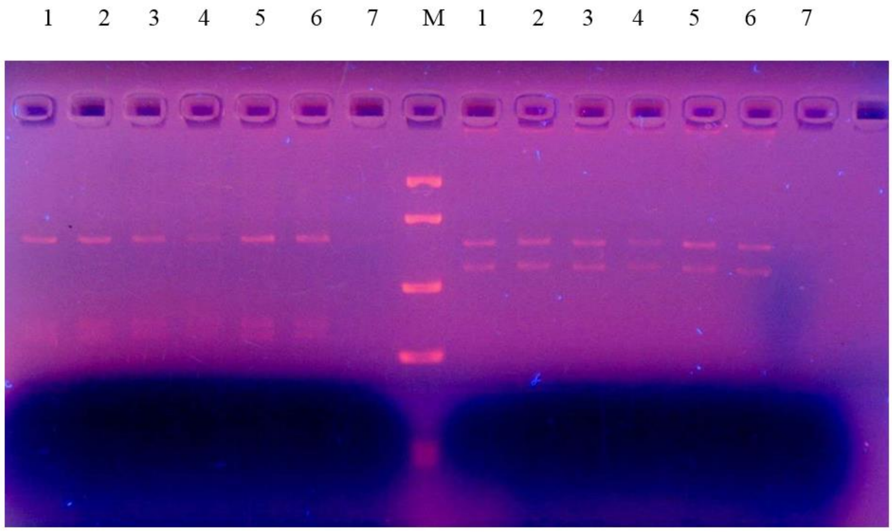


Fig. 3.3.4. Image of the agarose gel where enzymatic digestion products were separated using the *HphI* and *Bsrl* enzymes. The figures 1-7 indicate the *H. guttulatus* individuals *(photo Dalia Onără, INCDD Tulcea).*

3.4. Determination of the effects of different live feed diets on the seahorse *Hippocampus guttulatus*

In Romania, the first experiments conducted on the breeding and rearing in captivity of seahorses were carried out by the National Institute for Marine Research and Development (NIMRD) “Grigore Antipa” Constanța, in 2008, in the frame of a research project. The concrete results of the experiments conducted, expressed as a percentage of animal survival and animals released into the sea, have shown that the breeding and subsequent rearing of these fish in captivity is feasible. However, the major drawback in rearing *H. guttulatus* was supplying the most appropriate diet for the fry, as many individuals died of starvation before reaching maturity due to the lack of a small-sized food alternative. Under these circumstances, the current research was an attempt to determine which live diet is better accepted by seahorses in a controlled environment, for future rearing in captivity. The methodology for setting-up the experiment is detailed in în *Subchapter 2.5 Live feed experiment set-up* of this thesis.

As a follow-up of the experiment performed during the doctoral research, concerning length measurements it was noted that length increase in *H. guttulatus* individuals was linear in all three feeding regimes, the final length (+10 days) being higher than the +5 days length and initial length, respectively (Fig. 3.4.1). The maximum increase in length was reported for the combined diet (Tank C, combined *Brachionus plicatilis* + *Artemia salina* diet), followed closely by the *A. salina* diet (Tank B). The minimum increase was registered by seahorses fed with *B. plicatilis* (Tank A). With reference to weight variation, the maximum increase was reported for the combined diet (Tank C), followed by the brine shrimp diet (Tank B) and the rotifer diet (Tank A) (Figure 3.4.1). This can be explained by the higher lipid ratio contained by *A. salina* (18.9% dry weight) (Léger et al., 1987) as compared to *B. plicatilis* (12% dry weight) (Onciu, 1998).

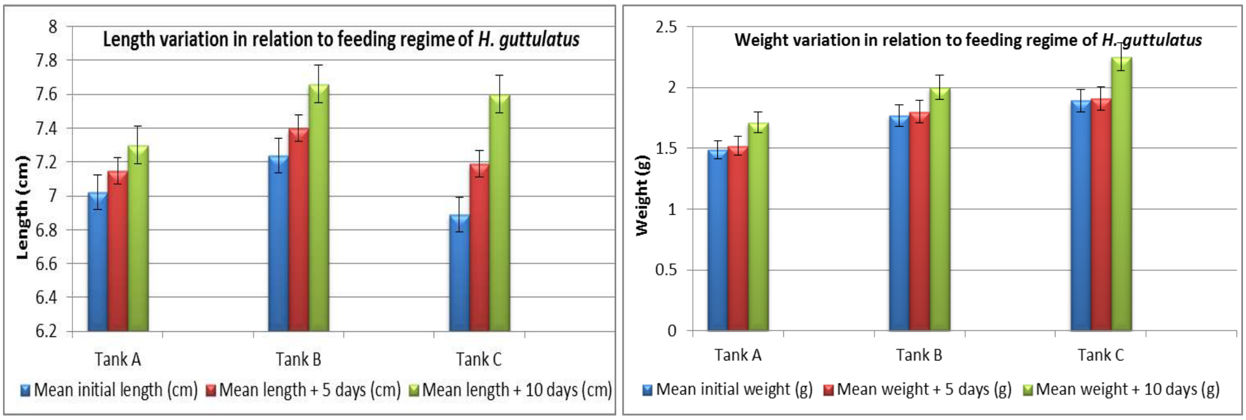


Fig. 3.4.1. Length and weight increase of *H. guttulatus* according to the diet *(Nenciu et al., 2015).*

However, the differences in length and weight reported after 5 and 10 days, respectively, between the three experimental tanks were not statistically significant. After applying the One-Way Anova (Analysis of Variance) parametric test, it resulted that for the variable “mean length” after 5 days the means of the three groups (*H. guttulatus,* Tank A = 7.15 cm , *H. guttulatus,* Tank B = 7.40 cm, *H. guttulatus,* Tank C = 7.19 cm) did not differ significantly among each other ($F = 0.545$; $p = 0.586 > \alpha = 0.05$) (Figure 3.4.2.a). Similarly, after 10 days, the means of the three groups (*H. guttulatus,* Tank A = 7.30 cm, *H. guttulatus,* Tank B = 7.66 cm, *H. guttulatus,* Tank C = 7.60 cm) did not differ significantly among each other ($F = 0.823$; $p = 0.462 > \alpha = 0.05$) (Figure 3.4.2.b). For the variable “mean weight”, the values recorded after 5 days did not differ significantly among the three batches: *H. guttulatus,* Tank A =

1.52 g, *H. guttulatus,* Tank B = 1.80 g, *H. guttulatus,* Tank C = 1.91 g (F = 1.149; p = 0.332 > α = 0.05) (Figure 3.4.2.c). In a similar manner, the mean weights measured after 10 days of controlled feeding did not record statistically significant differences among the three diets administered: *H. guttulatus,* Tank A = 1.71 g, *H. guttulatus,* Tank B = 2.07 g, *H. guttulatus,* Tank C = 2.25 g (F = 0.927; p = 0.442 > α = 0.05) (Figure 3.4.2.d).

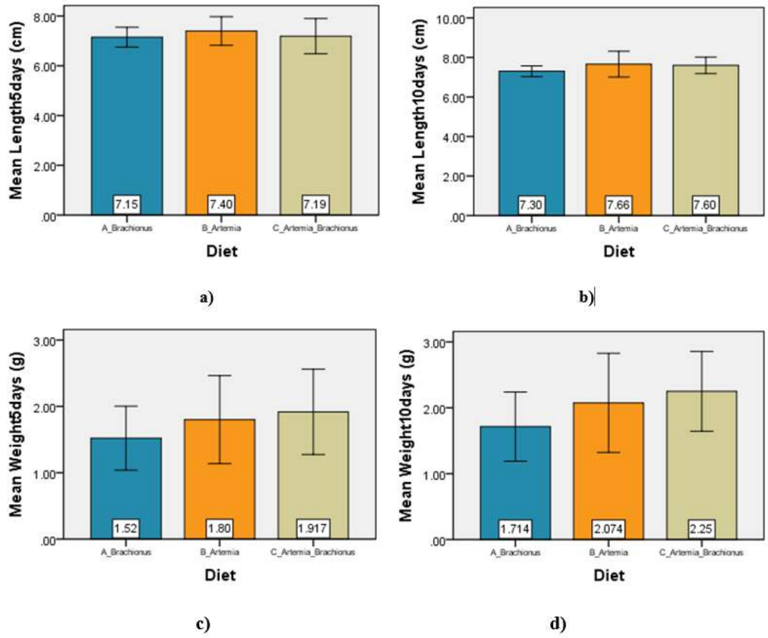


Fig. 3.4.2. Differences between the three experimental tanks: a) mean length +5 days; b) mean length +10 days; c) mean weight +5 days; d) mean weight +10 days *(Nenciu et al., 2015).*

Concerning the biochemical composition, the mean values obtained after 5 and, respectively, 10 days of differentiated feeding were referred to the mean value recorded by the control batch, as *H. guttulatus* individuals were similar in length and weight (statistically confirmed, F = 0.896; p = 0.420 > α = 0.05 for initial length, and F = 1.118; p = 0.342 > α = 0.05, for initial weight). Dry weight (DW) and moisture (WW%) recorded some differences between tanks and moments of the experiment (DW = dry weight, WW = wet weight). The highest dry weight value was recorded in *H. guttulatus* individuals from Tank A (fed with *B. plicatilis*) after 5 days (24.85%), while the lowest in Tank B (fed with *A. salina*) after 5 days (22.34%) (Fig. 3.4.3). In a complementary manner, the lowest moisture value was recorded in *H. guttulatus* individuals from Tank A (fed with *B. plicatilis*) after 5 days (75.15%), while the highest water content was recorded in samples from Tank B (fed with *A. salina*) after 5 days (77.66%) (Fig. 3.4.3).

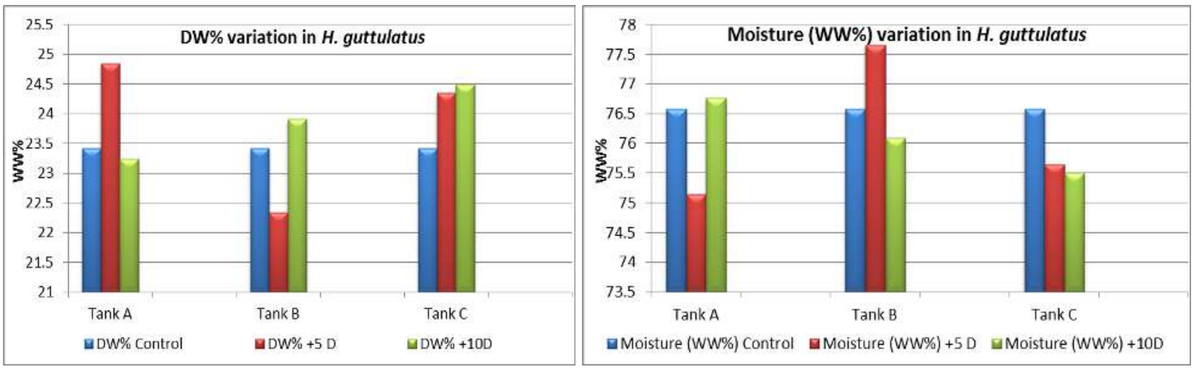


Fig. 3.4.3. Dry weight (wet weight - WW%) and moisture (WW%) recorded by the *H. guttulatus* individuals in the three experimental tanks *(Nenciu et al., 2015).*

Ash (DW%) values were the highest in the *B. plicatilis* diet (Tank A), while brine shrimp (Tank B) and combined (Tank C) diets recorded similar values.

Protein content varied differently after 5 days and 10 days. Thus, after 5 days of feeding, the highest protein content was recorded in *H. guttulatus* individuals from Tank C (combined), while the lowest was recorded in seahorses from Tank A (rotifer diet). However, after 10 days, these values shifted: the highest protein content was recorded in *H. guttulatus* individuals from Tank A (rotifer diet), while the lowest in seahorses in Tank C (combined) (Fig. 3.4.4). Lipid content was the highest in the *A. salina* diet (Tank B) after 10 days, as well as in the combined diet (Tank C) after 5 days (Fig. 3.4.4).

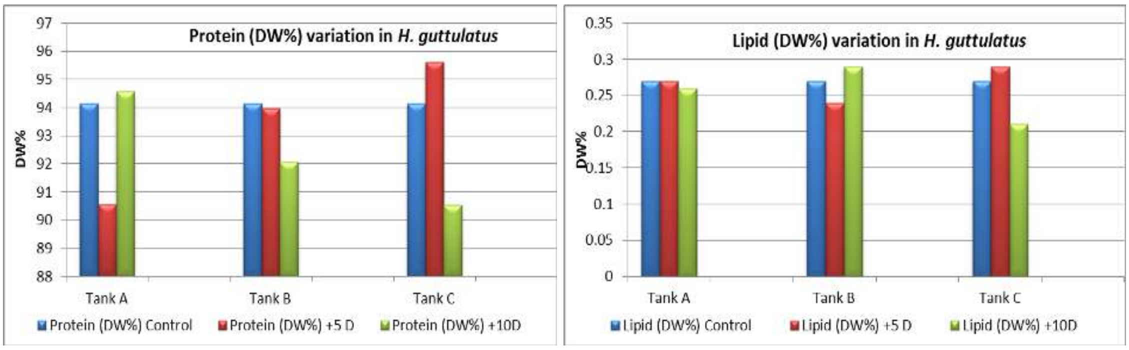


Fig. 3.4.4. Protein (DW%) and lipid (DW%) content in *H. guttulatus* individuals from the three experimental tanks *(Nenciu et al., 2015).*

However, the biochemical composition resulted in a higher protein content of the batch fed with *B. plicatilis*, somehow unexpected after observing the feeding behavior of seahorses, which seemed to prefer larger-sized prey (*A. salina*). These values can be explained by the higher protein content in *B. plicatilis* (63% dry weight) (Léger et al., 1987) as compared to *A. salina* (54.3% dry weight) (Léger et al., 1987). Due to its biochemical composition, the rotifer *B. plicatilis* is a quality source of food (total lipid content 12% DW, protein 63% DW) when reared with *Nannochloropsis* sp. (Onciu, 1998).

On the other hand, a proximate analysis of *A. salina* revealed an equilibrated high-protein diet indicating that macronutrient requirements are probably satisfied for most predators, seahorses included. The brine shrimp's (fed with *Tetraselmis* sp.) biochemical composition is the following: total lipid content 18.9% DW, protein 52.2% DW in *A. salina* nauplii (Léger et al., 1987). Lipid content in *H. guttulatus* individuals was the highest in the *A. salina* diet (Tank B) after 10 days, as well as in the combined diet (Tank C) after 5 days, explainable by the higher lipid ratio contained by *A. salina* as compared to *B. plicatilis* (12% DW) (Onciu, 1998).

The influence of environmental factors must not be overlooked, as the evolution of temperature and salinity during the 10 days of the experiment may have influenced the feeding behavior of *H. guttulatus* individuals and consequently their metabolic activity. The evolution of the protein content in the three batches stands out: apart from Tank A (*H. guttulatus* fed exclusively with the rotifer *B. plicatilis*), in Tank B (*H. guttulatus* fed with *A. salina*) and C (combined diet) the protein content dropped significantly after 10 days of controlled feeding, from 93.98% DW to 92.07% DW and from 95.62% DW to 90.54% DW, respectively. When correlating this drop in protein content with temperature and salinity, it can be noted that exactly after 5 days of experiment temperature started to rise (from 23.5°C to 25.22°C), while salinity dropped (from 14 PSU‰ to 12.92 PSU‰), suggesting that the metabolism of *H. guttulatus* may have been slowed down.

The preliminary conclusions of this research are that a combined diet of rotifers (*B. plicatilis*) and brine shrimp (*A. salina*) is the most recommended for rearing seahorses (*H. guttulatus*) in captivity, due to the advantages that the two invertebrates have separately. On the one hand, rotifers develop greater densities and have a higher protein content (reflected in the protein content of the batches analyzed), while brine shrimps have a higher lipid content and are easier to prey on, being larger and more visible in the tanks. Other diets should also be tested in captive conditions. Future research will focus on analyzing the stomach content of *H. guttulatus* individuals obtained from by-catches of the fishing points along the Romanian Black Sea coast, aimed at accurately determining the type of prey preferred by this species.

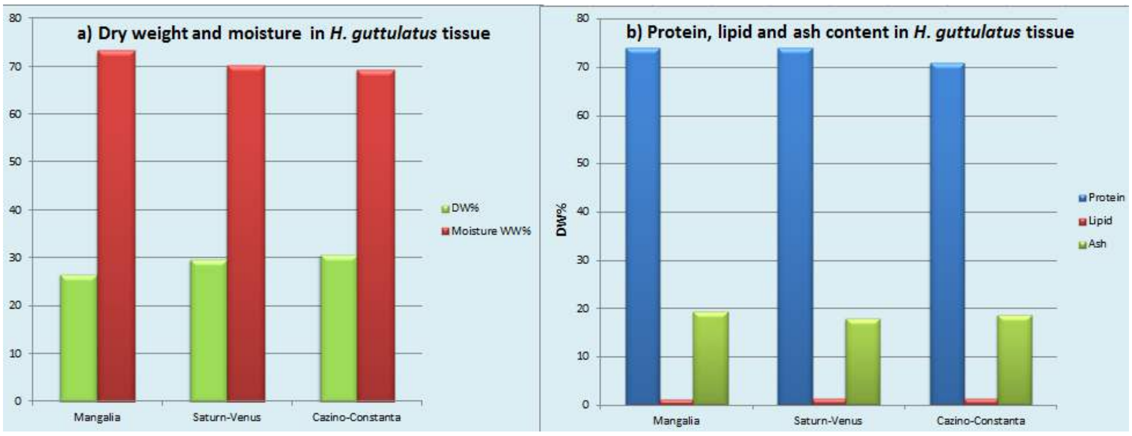
3.5. Investigation of pollutant accumulation in the seahorse *Hippocampus guttulatus* whole tissue

H. guttulatus (as other seahorse species) inhabits shallow areas close to the coast, where it finds proper habitats in seagrass meadows and hard substrate areas covered with macrophyte alage. Yet, this proximity with the coastal zone makes is extremely vulnerable to anthropogenic discharges (Nenciu et al., 2013). Consequently, in this study three of the most hazardous types of contaminants of the marine environment were analyzed, namely organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Cu, Cd, Pb, Ni, Cr), in the whole tissue of *H. guttulatus*.

The sampling methodology (samples were collected from three stations located in the south of the Romanian Black Sea coast, Cazino-Constanța, Saturn-Venus and Mangalia), as well as the specific laboratory analyses methods are detailed in *Subchapters 2.1. Sample collection, 2.6. Determination of heavy metal concentrations, 2.7. Determination of polycyclic aromatic hydrocarbons (PAHs), 2.8. Determination of organochlorine pesticide concentrations* and *2.9. Determination of proximate biochemical composition* of this thesis.

The analysis of the proximate biochemical composition of *H. guttulatus* (dry weight, moisture, ash, crude protein, crude lipid) was performed (WW = wet weight; DW = dry weight). Dry weight (%) ranged from 26.64±0.50 (Mangalia) to 30.69±0.27 (Cazino-Constanța). Moisture (WW%) ranged from 69.31±0.27(Cazino-Constanța) to 73.36±0.5 (Mangalia). Ash (DW%) ranged from 17.90±0.95 (Saturn-Venus) to 19.38±0.62 (Mangalia). Protein content (DW%) ranged from 70.82±5.15 (Cazino-Constanța) to 74.07±3.55 (Mangalia). Lipid content (DW%) ranged from 1.17±0.13 (Mangalia) to 1.28±0.21 (Cazino-Constanța) (Fig. 3.5.1.a).

Dry weight and lipid content recorded the minimum values in seahorses collected in Mangalia and maximum values at Cazino-Constanța. Moisture and protein content registered minimum values in seahorses collected at Cazino-Constanța and maximum values at Mangalia (Fig. 3.5.1.b).



a) **b)**

Fig. 3.5.1. a) Dry weight/moisture ratio b) protein, lipid and ash content (%DW) in *H. guttulatus* tissue *(Nenciu et al., 2014b).*

The analysis of the **organochlorine pesticide (OCPs)** levels in seahorse tissue revealed certain differences between the sampling stations and between the analyzed pesticides themselves. The maximum value recorded was for aldrin in Mangalia (0.208173 µg/g WW), while lindane was almost below the detection limit (0.0004 µg/g WW) in all three sampling locations. The values recorded for hexachlorobenzene (HCB) were quite low in all three sampling sites (mean 0.016189 µg/g WW) and for heptachlor the value recorded in Cazino-Constanța (0.083625 µg/g WW) was significantly higher compared to the other two stations (0.0003 µg/g WW). p,p'DDT values in Cazino-Constanța (0.051445 µg/g WW) were also significantly higher than those recorded in Saturn-Venus (0.026322 µg/g WW) and Mangalia (0.012507 µg/g WW). For the remaining pesticides analyzed (dieldrin - mean 0.068848 µg/g WW, endrin - mean 0.072671 µg/g WW, p.p'DDE - mean 0.082492 µg/g WW, p.p'DDD - 0.084484 µg/g WW), the values recorded were quite similar, with slight differences between stations (Fig. 3.5.2).

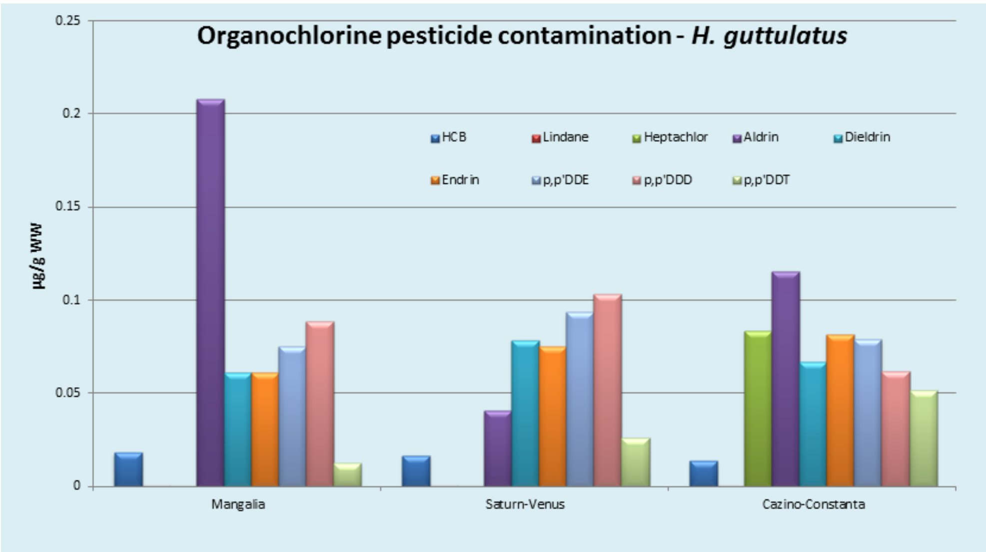


Fig. 3.5.2. OCP contamination (µg/g m.p.) of *H. guttulatus* tissue on compounds *(Nenciu et al., 2014b).*

The total organochlorine pesticide content in seahorse whole tissue ranged between 0.43502 µg/g WW in Saturn-Venus and 0.55348 µg/g WW in Cazino Constanța (mean 0.5047), thus pointing out an overall higher contamination with organochlorine pesticides in the station close to the Constanța Port.

This overview of the OCP values in seahorse tissue revealed that, despite its low lipid content compared to other marine organisms (mussels, for instance), seahorses still bioaccumulate organochlorine compounds, which are usually absorbed by fatty tissues. However, the extent to which their metabolism and enzymatic activity is affected by OCPs is yet to be investigated during further research.

Similarly to organochlorine pesticides, **polycyclic aromatic hydrocarbons (PAHs)** were also identified in the seahorse tissue, with differences between the 16 analyzed compounds (Fig. 3.5.3).

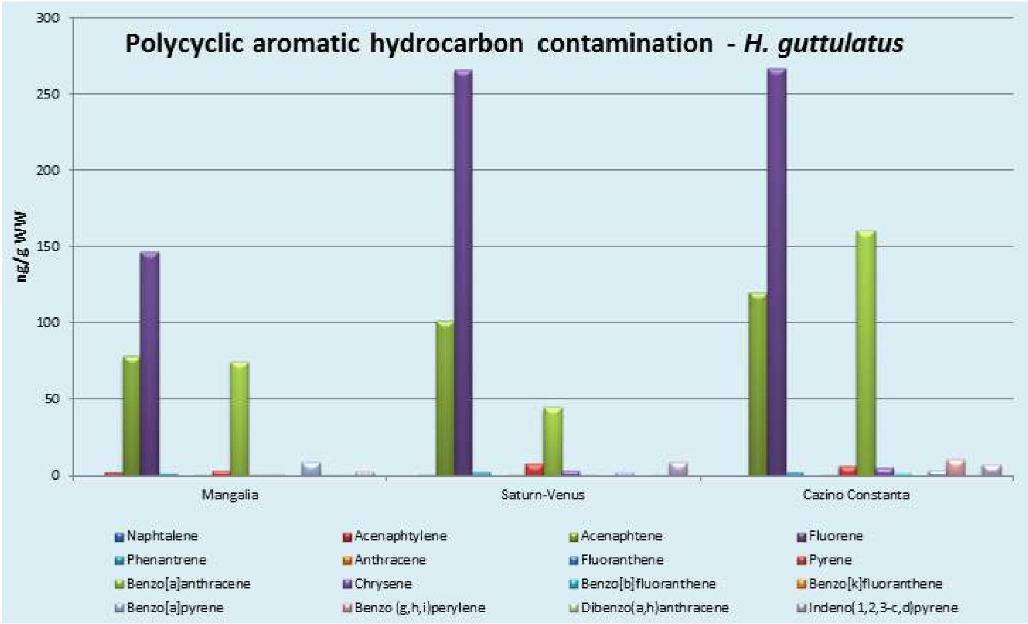


Fig. 3.5.3. Polycyclic aromatic hydrocarbon contamination (ng/g WW) of *H. guttulatus* on compounds *(Nenciu et al., 2014b).*

The highest level was recorded by fluorene both in Saturn-Venus and Cazino-Constanța (265.7 ng/g WW and 267.3 ng/g WW, respectively), while naphtalene, anthracene, benzo[k]fluoranthene values were below the detection limit. Fluorene is a low mollecular weight PAH, with 2-3-aromatic rings, characteristic for oil discharghes and oil product spills (Țigănuș et al., 2013). This high fluorene value recorded in seahorse tissue in all stations may indicate this type of pollution of water in the sampling areas, resulting in a rapid intake of this PAH by the seahorse body. Three of the polycyclic aromatic compounds, namely acenaphtene, fluorene and benzo[a]anthracene recorded higher values (means 100.0 ng/g WW, 226.4 ng/g WW and 93.5 ng/g WW, respectively), while the other remaining PAHs did not show an alarming contamination. However, the total polycyclic aromatic hydrocarbon content in seahorse tissue samples showed a higher degree of contamination in the Cazino-Constanța Station (587.8 ng/g WW), decreasing southwards to 439.5 ng/g WW in Saturn-Venus and 322.5 ng/g WW in Mangalia.

No data were available to compare the levels of organochlorine pesticides and polycyclic aromatic hydrocarbons in seahorshes in the Black Sea to seahorse species in other marine areas worldwide, to see whether these are normal or exceptional values. It may be possible that these high values are the results of some interference compounds, as pigments still had remained in the extract, after the clean-up step (Nenciu et al., 2014b). Consequently, the analyses must be repeated, using the same sampling stations.

For **heavy metals**, however, some information for comparison with other seahorse species were available (Qiang et al., 2008). As such, the heavy metal content in *H. guttulatus* tissue analyzed was similar and comparable for copper, cadmium and lead, as follows: Cu - 5.56 µg/g DW in *H. guttulatus* and 6.03 µg/g in *Hipppocampus kuda* (Bleeker, 1852), Cd - 1.51 µg/g in *H. guttulatus* and 1.54 µg/g DW in *Hippocampus spinosissimus* (Roule, 1916), Pb - 2.50 µg/g DW in *H. guttulatus* and 2.07 µg/g DW in *H. histrix* (Kaup, 1856). These comparable values confirm the accuracy of the analysis perfomed on the Black Sea samples and indicate that seahorses along the Romanian Black Sea coast do bioaccumulate heavy metals (Nenciu et al., 2014b).

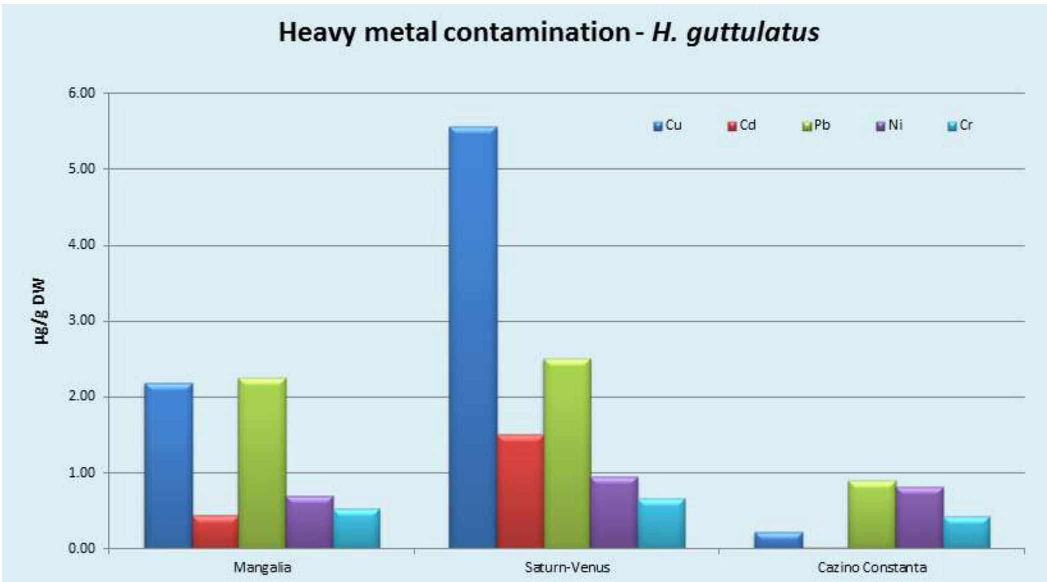


Fig. 3.5.4. Heavy metal contamination (µg/g DW) in the species *H. guttulatus (Nenciu et al., 2014b).*

The highest value recorded in the samples was for copper in Saturn-Mangalia (5.56 µg/g DW), while the lowest contamination level was for cadmium in the Cazino-Constanța Station (0.02 µg/g DW) (mean 2.66 µg/g DW). Lead contamination was also significant, with a mean value reaching 1.88 µg/g DW. Cadmium, nickel and chrome recorded similar mean values, ranging from 0.54 µg/g DW (Cr) to 0.82 µg/g DW (Ni) (Fig. 3.5.4). Due to the high copper and lead levels in Saturn-Venus, this station resulted in being the most contaminated with heavy metals (total 11.19 µg/g DW), followed by Mangalia (6.10 µg/g DW) and Cazino-Constanța (2.39 µg/g DW). This high level of copper in *H. guttulatus* is probably a result of copper contamination of the surrounding water, which may be caused by vessel traffic (it is used as antifouling agent on hulls) (Nenciu et al., 2014b).

The overall conclusion of this first research attempt on the bioaccumulation level in the Black Sea *Hippocampus guttulatus* confirms the hypothesis that seahorses, like most of marine species, absorb the xenobiotics reaching the aquatic environment (Nenciu et al., 2014b). All these compounds are highly toxic, even in low concentrations, especially if they accumulate in the metabolically active sites, and they could significantly influence the survival of a sensitive species such as the long-snouted seahorse. It is, thus, essential to perform future research aimed at analyzing the extent to which OCPs, PAHs and heavy metals influence the metabolic and enzymatic activity of *H. guttulatus*.

3.6. Studies on heavy metal bioaccumulation in Black Sea coast seahorse *Hippocampus guttulatus* and pipefish *Syngnathus acus* whole tissue expressed by the Bioconcentration Factor (BCF) and Biota-Sediment Accumulation Factor (BSAF)

This research aimed at analyzing the heavy metal bioaccumulation (Cu, Cd, Pb, Ni, Cr) in two Syngnathid species populating shallow waters of the Romanian Black Sea coast: the long-snouted seahorse (*Hippocampus guttulatus*, Cuvier, 1829) and the greater pipefish (*Syngnathus acus*, Linnaeus, 1758), referenced to the concentrations of the same five elements in the surrounding water and sediments, expressed by the Bioconcentration Factor (BCF) and Biota-Sediment Accumulation Factor (BSAF).

The sampling and laboratory analyses methodology by absorption spectrometry using the graphite furnace atomic absorption spectrometers model ATI-UNICAM 939Z and SOLAAR M6 Dual Thermo Electron-UNICAM of NIMRD NIMRD ”Grigore Antipa” Constanța are detailed in *Subchapters 2.1. Sample collection* and *2.6. Determination of heavy metal concentrations* of this thesis.

BCF and BSAF in Hippocampus guttulatus and Syngnathus acus (2014)

The analyses performed revealed that the five heavy metals (Cu, Cd, Pb, Ni, Cr) recorded variable concentrations in the two sampling stations, in all three environments studied [biota µg/kg dry weight (DW), water µg/l, sediment µg/kg DW], with statistically significant differences ($p < 0.05$, T test). In order to uniformize the value for the BCF and BSAF calculation, the measurement units were transformed into ppb (parts per billion). The results are given in Table 3.6.1.

Table 3.6.1. Heavy metal contamination values in biota, water and sediments (2014).

Station	Cazino Constanța					
		Cu	Cd	Pb	Ni	Cr
ppb µg/kg	*H. guttulatus*	14000	220	280	3220	120
ppb µg/kg	*S. acus*	7890	90	1220	1330	730
ppb µg/l	Water	1.64	0.84	3.05	1.15	1.09
ppb µg/kg	Sediments	18850.00	210.00	5280.00	73090.00	48930.00
Stația	**Costinești**					
		Cu	Cd	Pb	Ni	Cr
ppb µg/kg	*H. guttulatus*	17270	120	260	6640	11790
ppb µg/kg	*S. acus*	7120	110	130	1510	480
ppb µg/l	Water	0.84	0.86	2.95	1.64	1.81
ppb µg/kg	Sediments	15460.00	170.00	3210.00	50530.00	79120.00

Thus, in the **biota samples**, in the Cazino Constanța station, the highest values were recorded by copper both in *H. guttulatus* (14000 µg/kg DW) and in *S. acus* (7890 µg/kg DW) tissue, followed by nickel in *H. guttulatus* (3220 µg/kg DW) and *S. acus* (1330 µg/kg DW) tissue. In the same station, lead recorded the peak value in *S. acus* tissue (1220 µg/kg DW), similarly to chrome (730 µg/kg DW). Overall, in the Cazino Constanța Station, in the species *H. guttulatus*, heavy metals recorded decreasing values ordered Cu > Ni > Pb > Cd > Cr, while *S. acus* - Cu > Ni > Pb > Cr > Cd (Fig. 3.6.1).

In the southern station, Costinești, again copper recorded the peak values in both species: 17270 µg/kg DW in *H. guttulatus* and 7120 µg/kg DW in *S. acus.* Chrome and nickel also recorded high values in *H. guttulatus* tissue: 11790 µg/kg DW and 6640 µg/kg DW, respectively. The order of heavy metal concentrations was the following: Cu > Cr > Ni > Pb > Cd in *H. guttulatus* and Cu >Ni > Cr > Pb > Cd in *S. acus* (Fig. 3.6.1).

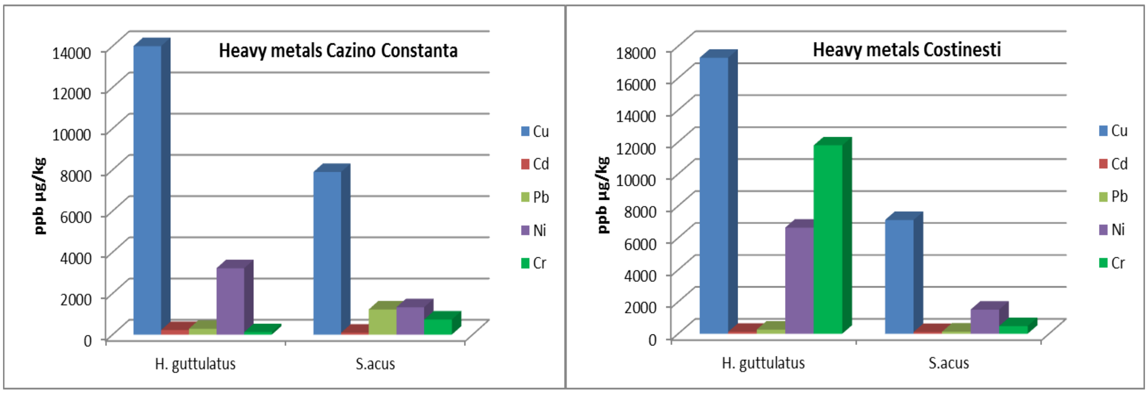


Fig. 3.6.1. Heavy metal contamination in biota in the Cazino Constanța and Costinești Stations (2014).

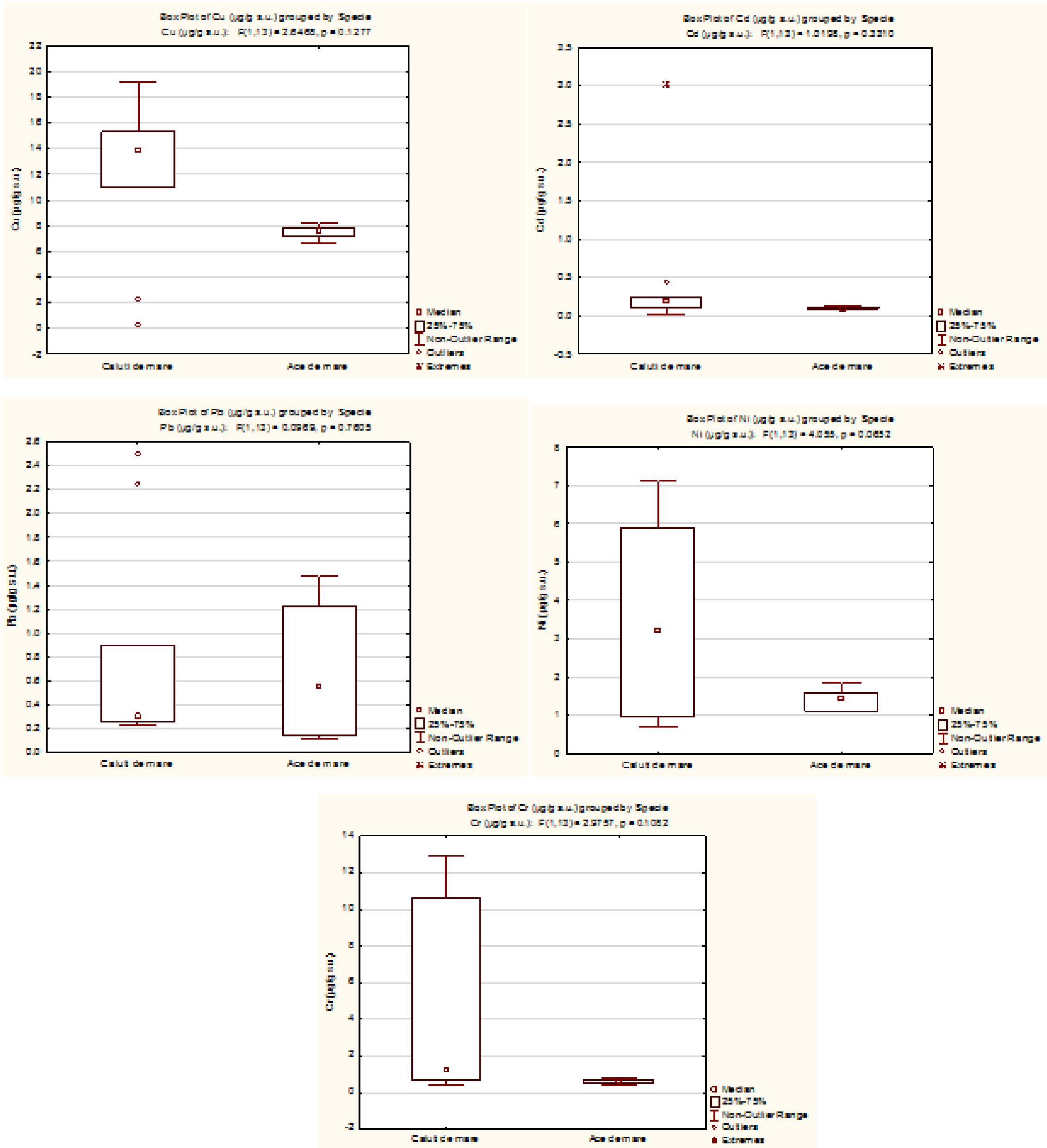


Fig. 3.6.2. Comparison between the Cu, Cd, Pb, Ni and Cr concentrations in *H. guttulatus* vs. *S. acus* tissue.

In **water samples**, due to dilution processes and natural variability, heavy metal concentrations were low in both sampling stations. However, the higher values recorded by lead in both locations stand out (1.09 µg/l in Cazino Constanța and 1.81 µg/l in Costinești). Rather high concentrations were also recorded by copper in Cazino Constanța (1.64 µg/l) and chrome in Costinești (1.81 µg/l). Cadmium recorded the lowest values in both sampling stations: 0.84 µg/l in Cazino Constanța and 0.86 µg/l in Costinești (Fig. 3.6.3). The order of concentrations of the five heavy metals in water was the following: in Cazino Constanța Pb > Cu > Ni > Cr > Cd, and in Costinești Pb > Cr >Ni > Cd > Cu.

In **marine sediments**, the order of concentrations of the five heavy metals analyzed was the following: in Cazino Constanța Ni > Cr > Cu > Pb > Cd, and in Costinești Cr > Ni > Cu > Pb > Cd. The high values of nickel accumulated in sediments in Cazino Constanța (73090 µg/kg DW) and Costinești (50530 µg/kg DW) stand out. Chrome also recorded high values, with the peak being documented in Costinești (79120 µg/kg DW) and 48930 µg/kg DW in Cazino Constanța. The lowest values were recorded by cadmium: 210 µg/kg DW in Cazino Constanța and 170 µg/kg DW in Costinești (Fig. 3.6.3).

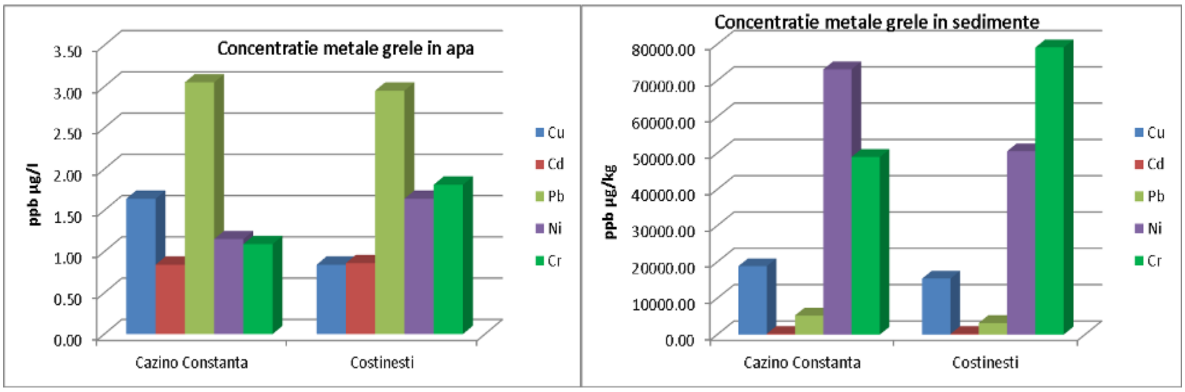


Fig. 3.6.3. Heavy metal concentrations in water and sediment samples (2014).

Overall, what stands out is the high concentration of copper in both sampling stations, in both target species, which raised the need to identify the potential source of contamination.

Applying the above mentioned calculation formulae, the following values were obtained for the Bioconcentration Factor (BCF) and Biota-Sediment Accumulation Factor (BSAF) (in red exceedings of threshold values) (Table 3.6.2):

Table 3.6.2. BCF and BSAF values recorded for *H. guttulatus* and *S. acus* in the two sampling locations (2014).

Cazino Constanța Station	Cu	Cd	Pb	Ni	Cr
BCF *H. guttulatus*	**8536.58[c]**	261.9	91.8	**2800[a,b]**	110.09
BSAF *H. guttulatus*	0.74	**1.04[d]**	0.05	0.04	0.002
BCF *S. acus*	**4819.97[a,b]**	107.14	400	**1156.52[a]**	669.72
BSAF *S. acus*	0.41	0.42	0.23	0.018	0.014
Costinești Station	**Cu**	**Cd**	**Pb**	**Ni**	**Cr**
BCF *H. guttulatus*	**20599.52[c]**	139.53	88.13	**4048.78[a,b]**	**6513.81[c]**
BSAF *H. guttulatus*	**1.11[d]**	0.7	0.08	0.13	0.14
BCF *S. acus*	**8476.19[c]**	127.9	44.06	920.73	265.19
BSAF *S. acus*	0.46	0.64	0.04	0.02	0.006

[a] BCF ≥ 1000 (bioaccumulative) (REACH)
[b] BCF ≥ 2000 (bioaccumulative) (TSCA)
[c] BCF ≥ 5000 (very bioaccumulative) (REACH & TSCA)
[d] BSAF > 1 (microconcentrator) (Dallinger, 1993)
[e] BSAF > 2 (macroconcentrator) (Dallinger, 1993)
BSAF < 1 (deconcentrator) (Dallinger, 1993)

According to the high concentrations recorded by copper in both species studies, the peak value of the Bioconcentration Factor (BCF) was recorded for this element in both sampling stations, in both species analyzed (Fig. 3.6.4 a și b). Thus, the values recorded in *H. guttulatus* rate copper as *very bioaccumulative*, BCF >5000, while in *S. acus* copper is *very bioaccumulative* in Costinești (BCF >5000) and *bioaccumulative* (BCF >2000) in Cazino Constanța.

Another bioaccumulative metal is, according to the results obtained, nickel, which recorded exceedings of threshold values in Cazino Constanța both in *H. guttulatus* and in *S. acus* tissue (BCF >2000). In the Costinești Station, the value of nickel also led to the conclusion that this element is *bioaccumulative* (BCF >2000), but only for the species *H. guttulatus*. Another high value of the Bioconcentration Factor in the Costinești Station was also recorded by chrome (BCF >5000), in seahorse tissue.

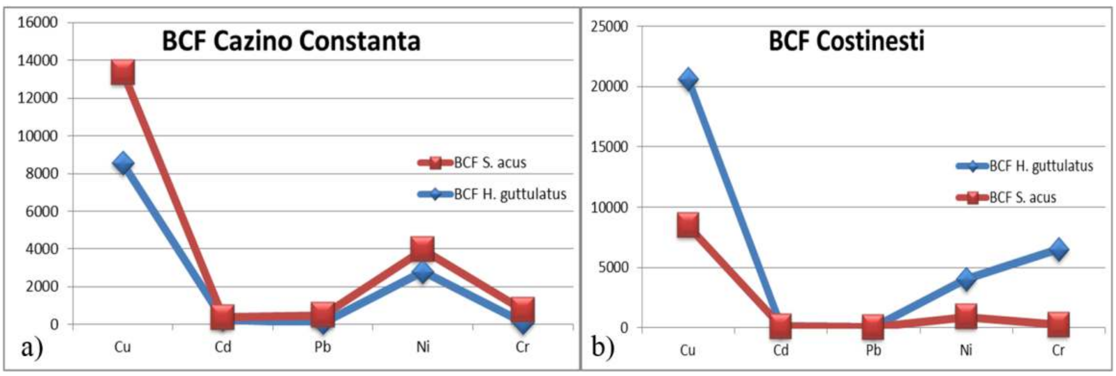


Fig. 3.6.4. Bioconcentration Factor values (BCF) in the two sampling stations (2014).

The values of the Biota-Sediment Accumulation Factor (BSAF) recorded by pipefish were low (<1) while for the seahorse tissue exceedings were recorded only for cadmium (Constanța) and (Costinești) (BSAF>1). The other elements recorded values that rate them as deconcentrators (BSAF < 1) (Fig. 3.6.5 a and b).

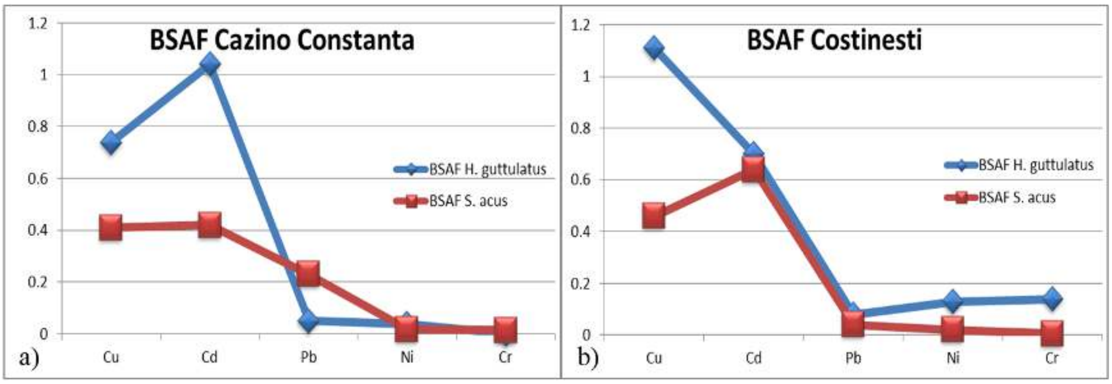


Fig. 3.6.5. Biota-Sediment Accumulation Factor (BSAF) in the two sampling stations (2014).

BCF and BSAF in Hippocampus guttulatus (2013)

The formulae for calculating the bioconcentration and biota-sediment accumulation factor were also applied for heavy metal contamination data of 2013 (the levels recorded in 2013 by the five heavy metals analyzed in *H. guttulatus* tissue samples, Cu, Cd, Cr, Ni și Pb, are detailed in *Subchapter 3.5. Investigation of pollutant accumulation in the seahorse Hippocampus guttulatus whole tissue* of this thesis).

The heavy metal concentrations recorded in the **water** samples showed that the highest value was recorded for chrome in the Cazino-Constanța Station (8.4 ppb). Going southwards, in the Saturn-Venus Station, chrome concentrations reached 4.3 ppb, while in the Mangalia Station - 1 ppb, with great differences between stations. Copper concentrations in seawater, however, decreased from south to north, with 4.1 ppb in Mangalia, 2.3 ppb in Saturn-Venus and 1.3 ppb in Cazino-Constanța. Cadmium also recorded the highest value in Mangalia (1 ppb), followed by 0.6 ppb in Cazino-Constanța and 0.3 ppb in Saturn-Venus. For lead, the peak value was measured in seawater from Saturn-Venus (2.4 ppb), with lower concentrations in Cazino-Constanța (0.7 ppb) and Mangalia (0.5 ppb). Nickel recorded the highest value in Cazino-Constanța (1.3 ppb), followed by very similar concentrations in Saturn-Venus (0.9 ppb) and Mangalia (0.8 ppb). Overall, the highest contamination level of seawater with heavy metals was recorded in the Cazino-Constanța Station (12.5 ppb), followed closely by Saturn-Venus (10.3 ppb) and then by Mangalia (7.5 ppb).

The highest value for heavy metals in **sediments** was recorded for copper in the Cazino-Constanța Station (43540 ppb DW), followed by Mangalia (12460 ppb DW) and a lower value in Saturn-Venus (5010 ppb DW). Cadmium also recorded the highest value in Cazino-Constanța (1910 ppb DW), while in Saturn-Venus (120 ppb DW) and Mangalia (60 ppb) much lower values were recorded (Fig. 3.6.6).

The highest value for heavy metals recorded in the **biota** (*H. guttulatus* tissue) samples was for copper in the Saturn-Venus Station (5560 ppb DW). Copper also recorded a quite high value in Mangalia (2190 ppb DW), while in Cazino-Constanța the copper concentration was only 220 ppb DW. Cadmium also recorded the peak value in Saturn-Venus (1510 ppb DW), followed by Mangalia (440 ppb DW) and Cazino-Constanța (20 ppb DW) (Fig. 3.6.7).

Lead concentrations were also similar in seahorse tissue collected from Saturn-Venus (2500 ppb DW) and Mangalia (2250 ppb DW), while in Cazino-Constanța the value was lower (900 ppb DW). Nickel recorded comparable values between the three stations (820 ppb DW - Cazino Constanța, 950 ppb DW - Saturn-Venus and 690 ppb DW - Mangalia), similarly to chrome (430 ppb DW - Cazino Constanța, 670 ppb DW - Saturn-Venus and 530 ppb DW - Mangalia).

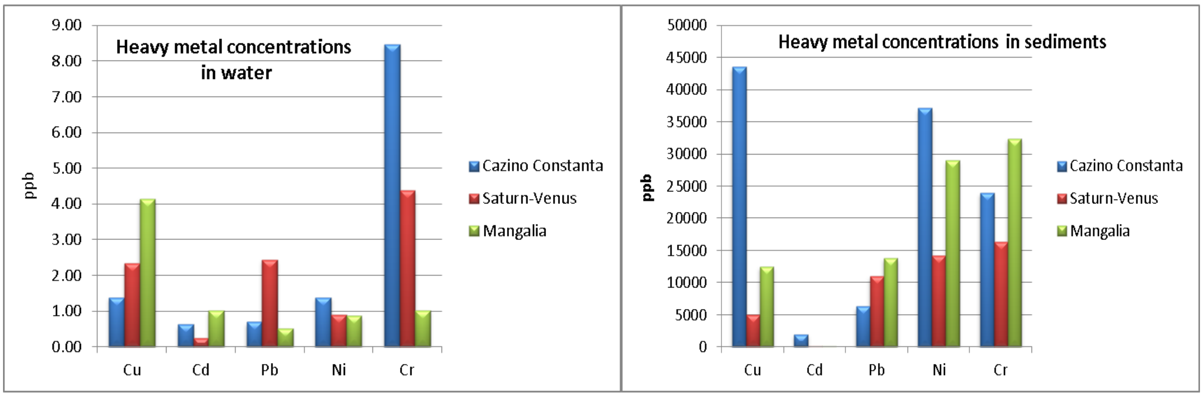


Fig. 3.6.6. Heavy metal concentrations in water and sediments (2013).

Due to the high copper and lead levels in Saturn-Venus, this station resulted in being the most contaminated with heavy metals (total 11190 ppb DW), followed by Mangalia (6100 ppb DW) and Cazino-Constanța (2390 ppb DW).

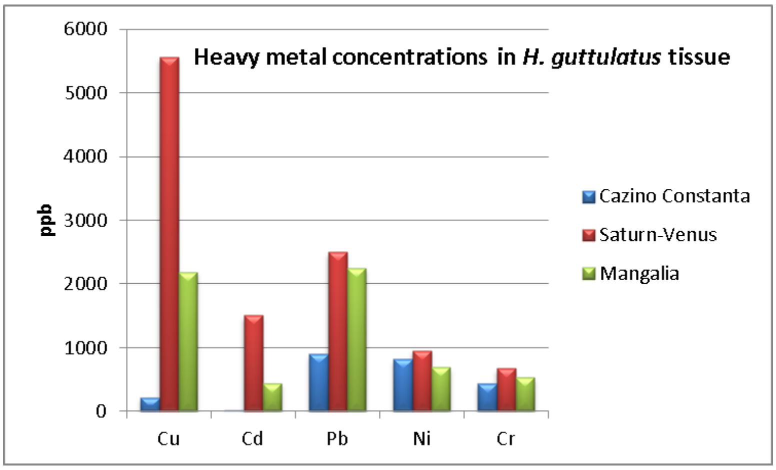


Fig. 3.6.7. Heavy metal concentrations in *H. guttulatus* tissue (2013).

Table 3.6.3. shows the values of BCF and BSAF recorded for *H. guttulatus* in the three sampling stations in 2013 and threshold value exceedings are marked in red. The yellow highlight indicates a very high Bioconcentration Value, recorded for cadmium.

The highest BCF (6291 ≥ 5000) was calculated for cadmium in the Saturn-Venus Station, followed by lead in Mangalia (4326 ≥ 1000), which rate cadmium as *very bioaccumulative* and lead as *bioaccumulative*. Other significant BCF overtakings were recorded for copper in the Saturn-Venus Station (2376 ≥ 1000), lead in the Cazino-Constanța Station (1285 ≥ 1000), nickel (1055 ≥ 1000) and lead (1024 ≥ 1000) in the Saturn-Venus Station, which can all be rated as *bioaccumulative*.

Table 3.6.3. BCF and BSAF values calculated for the three sampling locations (2013) *(Nenciu et al., 2014a)*.

Heavy metal / Factor	Cu	Cd	Pb	Ni	Cr
Cazino Constanta Station					
BCF	159	31	**1285[a]**	594	50
BSAF	< 1	< 1	< 1	< 1	< 1
Saturn-Venus Station					
BCF	**2376[b]**	**6291[c]**	**1024[a]**	**1055[a]**	152
BSAF	**1.1[d]**	**12.5[e]**	< 1	< 1	< 1
Mangalia Station					
BCF	528	435	**4326[b]**	793	519
BSAF	< 1	**7.3[e]**	< 1	< 1	< 1

[a] BCF ≥ 1000 (bioaccumulative) (REACH)
[b] BCF ≥ 2000 (bioaccumulative) (TSCA)
[c] BCF ≥ 5000 (very bioaccumulative) (REACH & TSCA)
[d] BSAF > 1 (microconcentrator) (Dallinger, 1993)
[e] BSAF > 2 (macroconcentrator) (Dallinger, 1993)

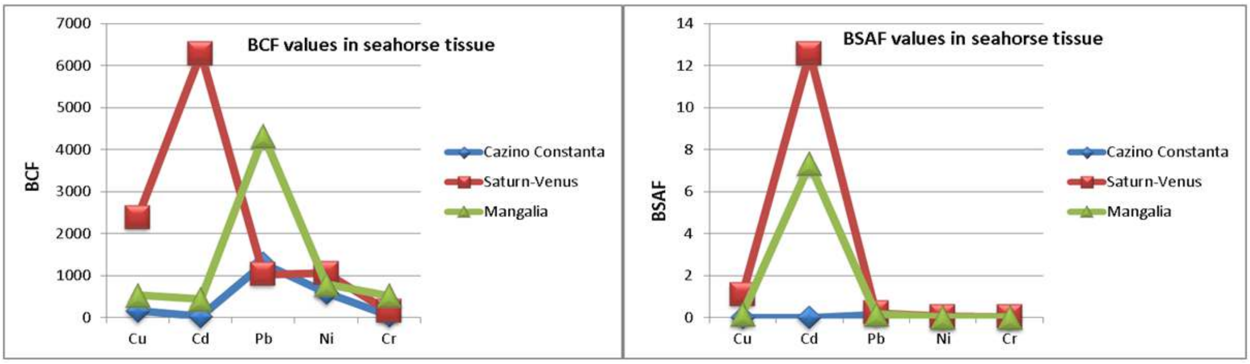


Fig. 3.6.8. BCF and BSAF values calculated for the three sampling stations (2013) *(Nenciu et al., 2014a)*.

The Biota-Sediment Accumulation Factor (BSAF) values were also calculated for *H. guttulatus*, in spite of the species not being a purely benthic one. Yet, long-snounted seahorses live close to the seabed, clung on macrophyte algae thalli, which makes them susceptible the pollutant uptake from a contaminated sediment. With reference to the values recorded by the BSAF (Fig. 5 b), only three values overcame the threshold values pointed-out in literature, namely cadmium (12.5 > 2) and copper (1.1 > 1) in Saturn-Venus, and again cadmium (7.3 > 2) in the Mangalia Station. These values rate cadmium as *macroconcentrator*, and copper as a *microconcentrator* (Fig. 3.6.8).

Statistical correlations between heavy metal values recorded in water, sediments and biota (2013, 2014)

In order to determine the statistical correlations established between the whole tissue of *H. guttulatus*, *S. acus* and other fish species, as well as with those in water and sediment, the data from 2013 and 2014 were analyzed using the Statistica v. 10 software. The graphic representation of the recorded value allows summarizing the concentrations of the five heavy metals in the two target species throughout the study period.

The results for *H. guttulatus* are given in Fig. 3.6.9-3.6.10. It is very clear that, in 2014, in the Costinești Station high levels of Cu, Ni and Cr were recorded, while Cd and Pb concentrations were lower. It is interesting to point out that, apart for Pb, in the Cazino-Constanța Station, in 2014 higher values were recorded compared to 2013.

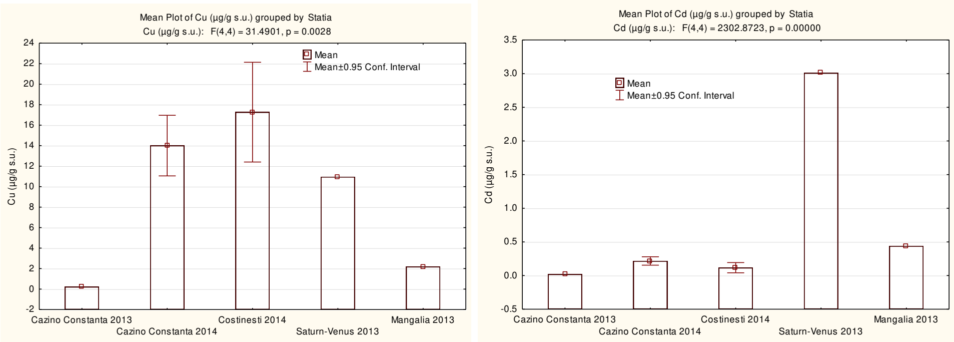


Fig. 3.6.9. Cu and Cd concentrations recorded in *H. guttulatus* tissue (2013, 2014).

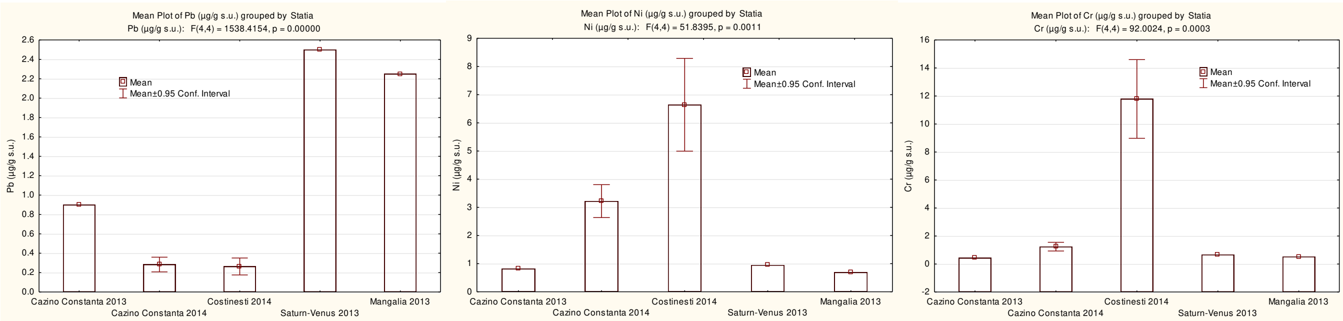


Fig. 3.6.10. Pb, Ni and Cr concentrations recorded in *H. guttulatus* tissue (2013, 2014).

The results for *S. acus* are given in Fig. 3.6.11-3.6.12. With reference to heavy metal concentrations recorded in *S. acus* tissue, for three of the elements (Cu, Pb și Cr), higher values were recorded in the Cazino-Constanța Station, while Cd and Ni recorded higher values in the southern station - Costinești.

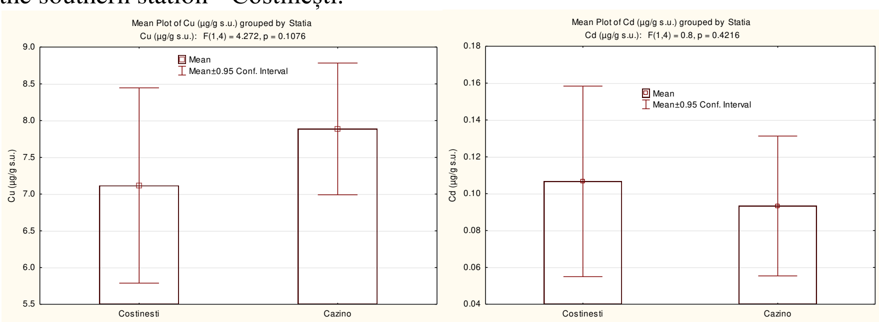


Fig. 3.6.11. Cu and Cd concentrations recorded in *S. acus* tissue (2014).

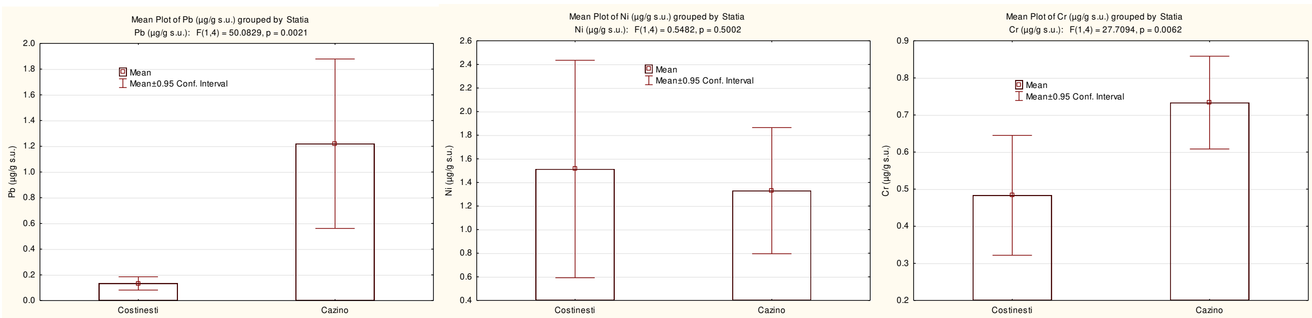


Fig. 3.6.12. Pb, Ni and Cr concentrations recorded in *S. acus* tissue (2014).

Concerning the correlation between concentrations recorded in biota (*H. guttulatus* and *S. acus*) and those in seawater, a positive correlation was reported only for Cu ($p<0.05$) and Pb ($p > 0.05$, statistically non-significant (Fig. 3.6.13-3.6.14).

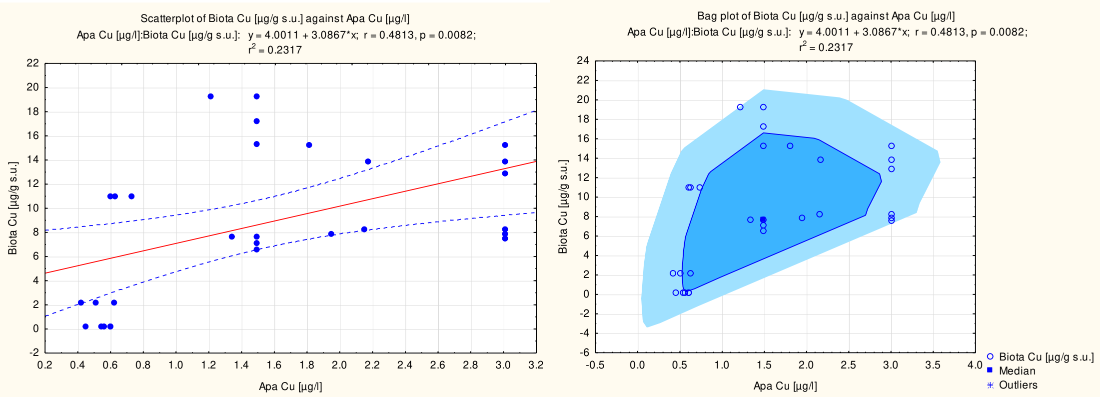


Fig. 3.6.13. Correlation between Cu concentrations in biota and seawater (2013-2014).

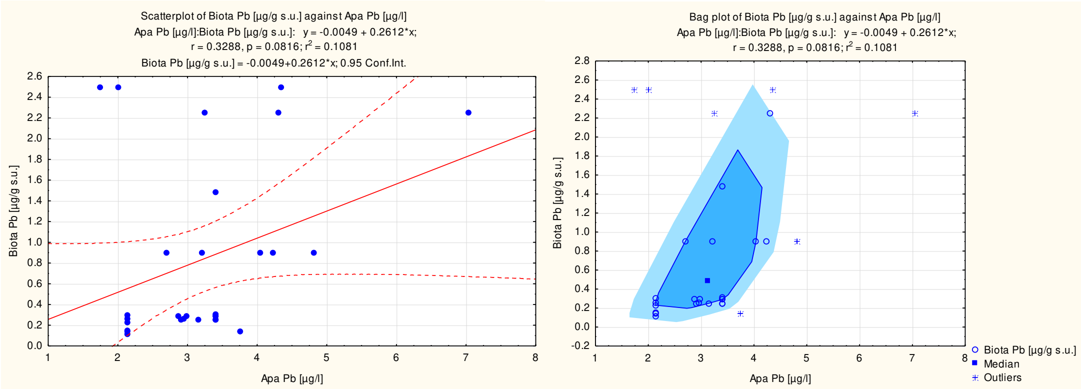


Fig. 3.6.14. Correlation between Pb concentrations in biota and seawater (2013-2014).

For sediments, the only positive correlation set for concentrations in the biota was for Pb, yet statistically non-significant (p>0.05) (Fig. 3.6.15).

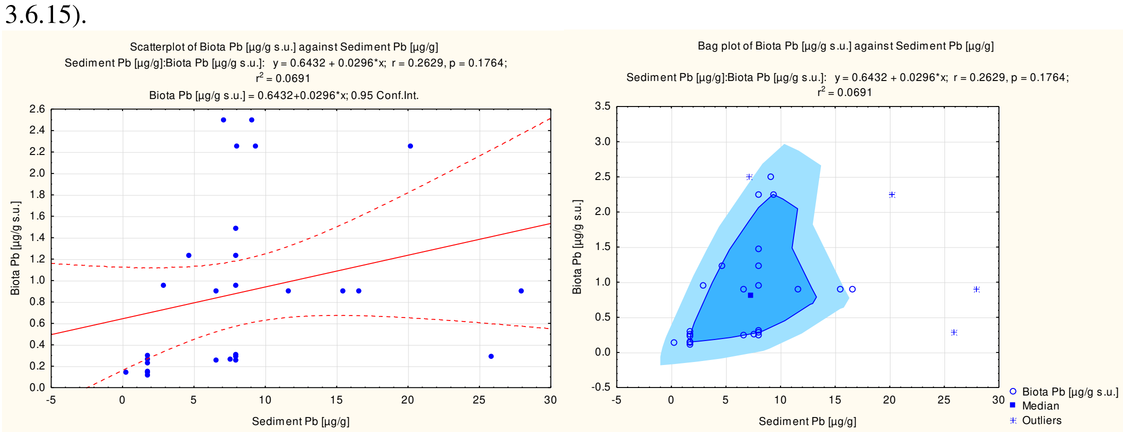
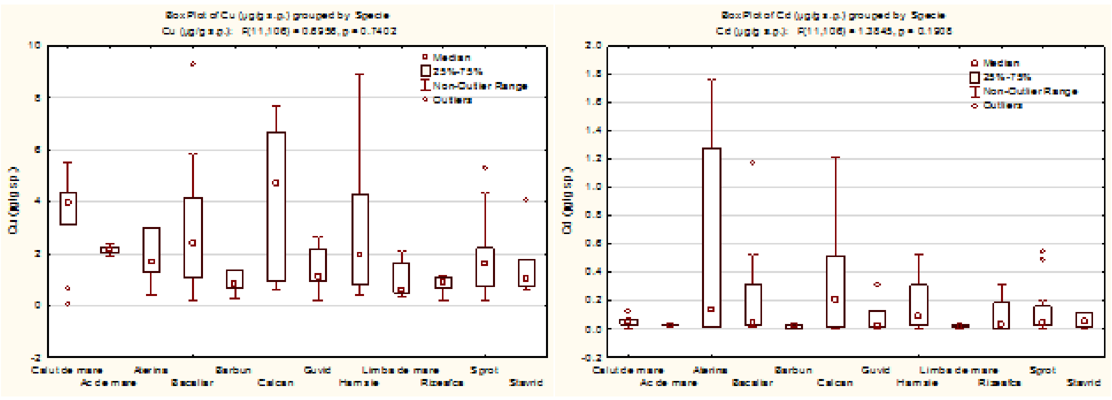


Fig. 3.6.15. Correlation between Pb concentrations in biota and sediments (2013-2014).

Given the bioaccumualtion levels of heavy metals in the two species studied, it was interesting to compare these values with the ones recorded by the five elements in other fish (Fig. 3.6.16) and mollusk (Fig. 3.6.17) species from the Romanian Black Sea coast.



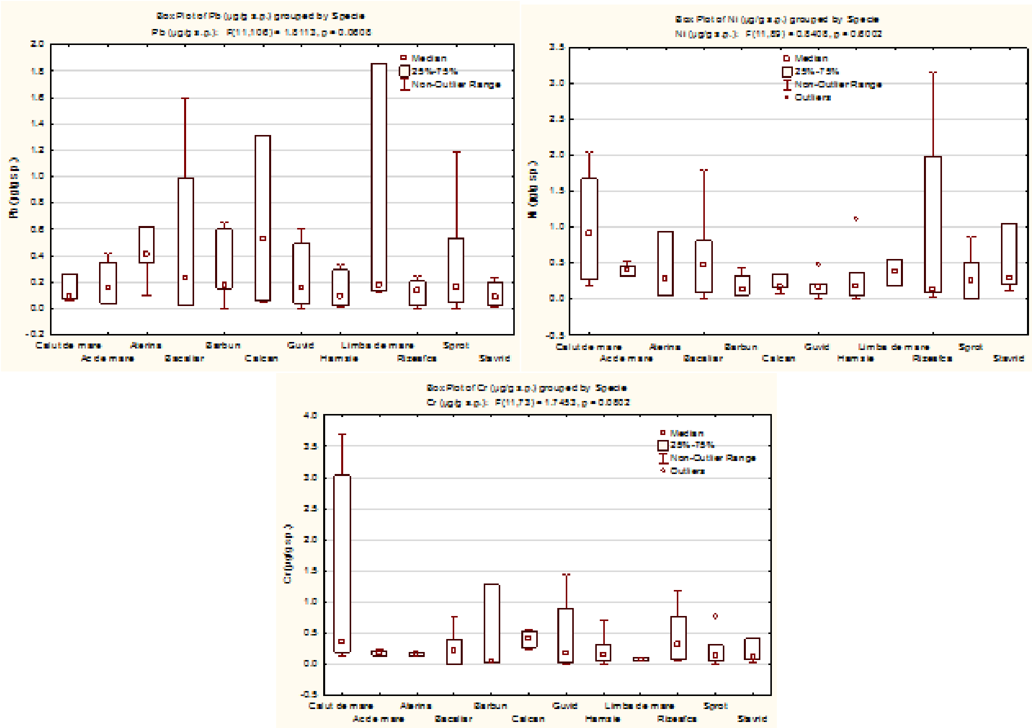


Fig. 3.6.16. Heavy metal contamination levels of Syngnathids compared to other fish species of the Romanian Black Sea coast.

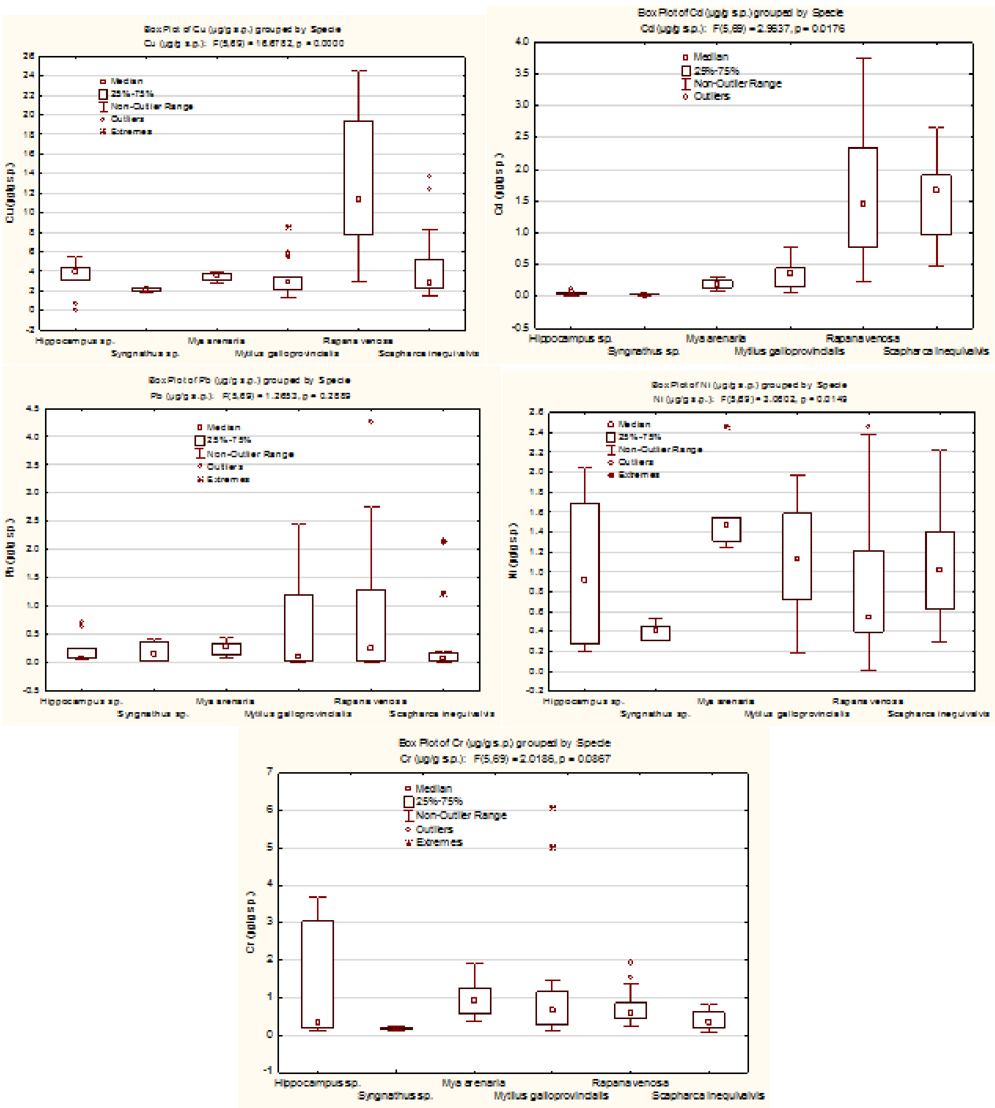


Fig. 3.6.17. Heavy metal contamination levels of Syngnathids compared to mollusk species of the Romanian Black Sea coast.

The extremely high values recorded by copper in some marine organisms may be explained by the presence of haemocyanin, a respiratory protein in the form of metalloproteins containing two copper atoms, irreversibly bound by an oxygen molecule. Deoxygenated haemocyanin is colorless, while the presence of oxygen turns it blue ("blue blood "). It carries oxygen in the haemolymph (invertebrate blood) of some molluks and in most arthropods. This could explain the high copper values recorded in *Rapana venosa* and the other analyzed mollusks. However, it is not the case of Syngnathids and the other fish species in the Black Sea, which, as vertebrates, have their blood based on the haemoglobin respiratory pigment (Rainbow et al., 1989). Consequently, the copper source is probably external and, most certainly, of anthropogenic origin.

The results obtained proved that both seahorses and pipefish collected from shallow coastal waters do bioaccumulate heavy metals at different rates, significant differences ($p < 0.05$, T test) being recorded also between sampling stations. For instance, in 2014, copper recorded the peak values in both species. High values were also recorded in seahorse tissue for nickel and chrome (Costinești), and in pipefish tissue for lead (Constanța).

3.7. Research on polycyclic aromatic hydrocarbons (PAH) contamination of Syngnathids in Romanian coastal waters

Polycyclic aromatic hydrocarbons are the result of incomplete combustion of coal, gas, wood etc., and other anthropogenic sources of PAHs can be oil spills, industrial wastes or discharges from waste water treatment plants (Țigănuș et al., 2013). The main pathways for introducing PAHs in the marine environment are atmosphere deposits, wastes and industrial or oil spills reaching directly the marine environment (Țigănuș, 2015). The contamination of marine waters and particularly sediments affects the marine benthos, due to the fact that these compounds are deposited and accumulate in the substrate, becoming a major pool of toxicants. Syngnathids are fish living close to the seabed, depending on the vegeration developing on the substrate, thus they can be affected by the PAHs accumulated in sediments. The analyses collected in 2013 confirmed the fact that the species *H. guttulatus* does bioaccumulate this type of compounds, regardless of its low lipid content.

This research aimed at performing a comparison of the PAH bioaccumulation levels in the two species of Syngnathids studied: *H. guttulatus* and *S. acus*. The sampling methodology (the samples were collected from two stations located in the southern sector of the Romanian coast, Cazino-Constanța și Costinești), as well as the laboratory analysis methods are detailed in *Subchapters 2.1. Sample collection* and *2.7. Determination of polycyclic aromatic hydrocarbons (PAHs)*.

As a follow-up of analyzing the tissue samples, it was found that the highest concentration in *H. guttulatus* was recorded by benzo[a]anthracene in the Costinești Station (876.2 ng/g WW), while the other polycyclic compounds recorded low values (Fig. 3.7.1). In *S. acus* tissue, the peak value was also recorded by benzo[a]anthracene, but this time in the Cazino Constanța Station (59.2 ng/g WW). A relatively close value was recorded by benzo(g.h.i)perylene in the Costinești Station (58.6 ng/g WW (Fig. 3.7.1).

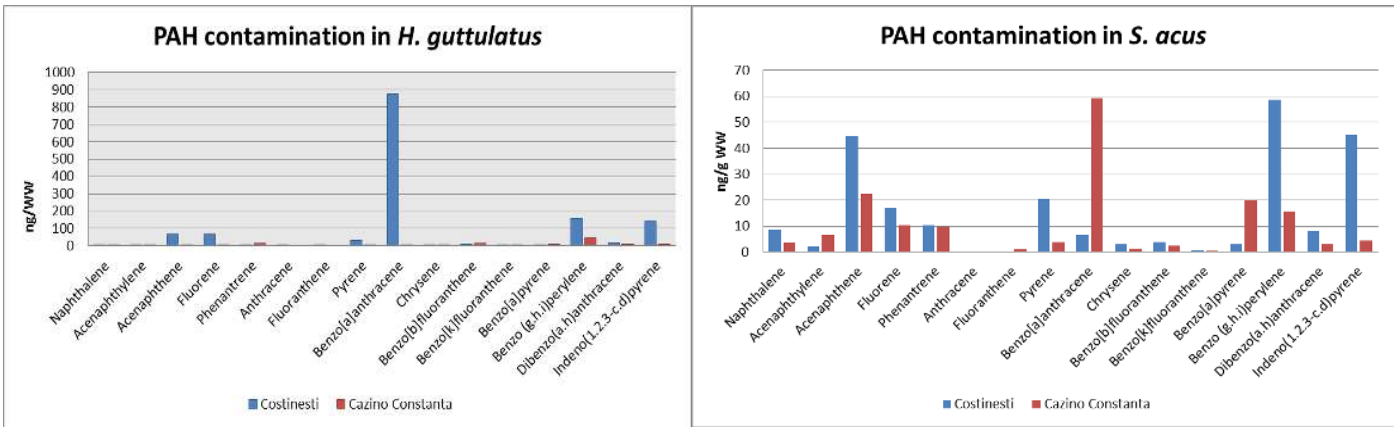


Fig. 3.7.1. Polycyclic aromatic hydrocarbon contamination of *H. guttulatus* (left) and *S. acus* (right) on compounds (2014).

The benzo(g.h.i)perylene sources in the marine environment are exhaust gas emissions and smoke resulting from the combustion of wood and coal, industrial effluents, waste water treatment plant discharges, discharges from waste incinerators and aluminium foundries. Trace amounts are also found in cigarette smoke. Benzo(g.h.i)perylene is toxic and suspected to be carcinogenic. In pipefish tissue, anthracene and fluorathene recorded very low values (below the limit of detection) in both sampling stations. While the values recorded by the sum of the 16 polycyclic compounds were significantly higher in *H. guttulatus* tissue, it stands out that in *S. acus* the concentrations of compounds were close as value among many compounds. In *H. guttulatus*, the high value of Σ_{16}PAH is the result of the very high concentration of benzo[a]anthracene: $\sum_{16}$PAH = 737 ng/g WW in *H. guttulatus* and $\sum_{16}$PAH = 197.2 ng/g in *S. acus*, respectively.

Overall, biota samples resulted more contaminated in the Costinești Station, for both species (Fig. 3.7.2).

On species, seahorses accumulated polycyclic compounds to a greater extent, on the one hand due to their higher concentration of lipids compared to pipefish and on the other hand to their lower mobility.

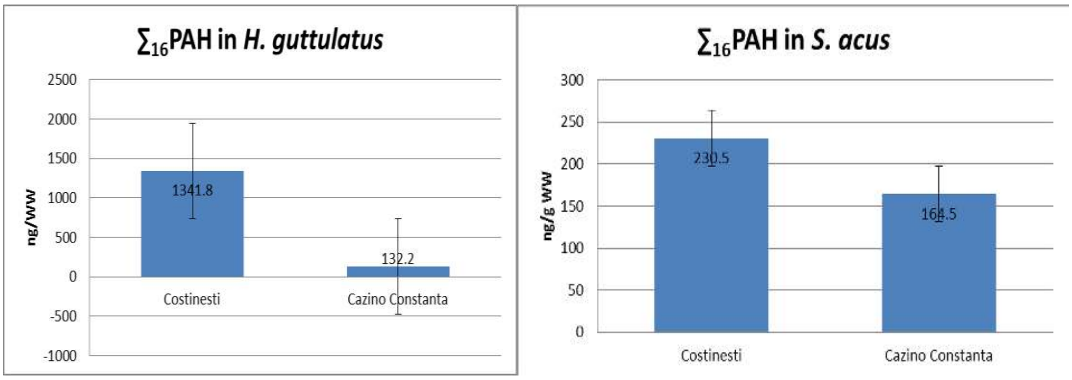


Fig. 3.7.2. Σ_{16}PAH contamination in *H. guttulatus* and *S. acus* on stations (2014).

With reference to values recorded in the environmental components, sediments were more contaminated with the sum of the 16 hydrocarbons (Σ_{16}PAH) in Constanţa (value in sediments Constanţa 406.5 ng/g DW, sediments Costineşti 220.5 ng/g DW.). In water, higher concentrations were also recorded in Constanţa (5.71 µg/l), while in Costineşti the values were low (0.61 µg/l). However, as previously demonstrated, in biota samples of both studied species, the highest Σ_{16}PAH concentrations were recorded in Costineşti (Fig. 3.7.3).

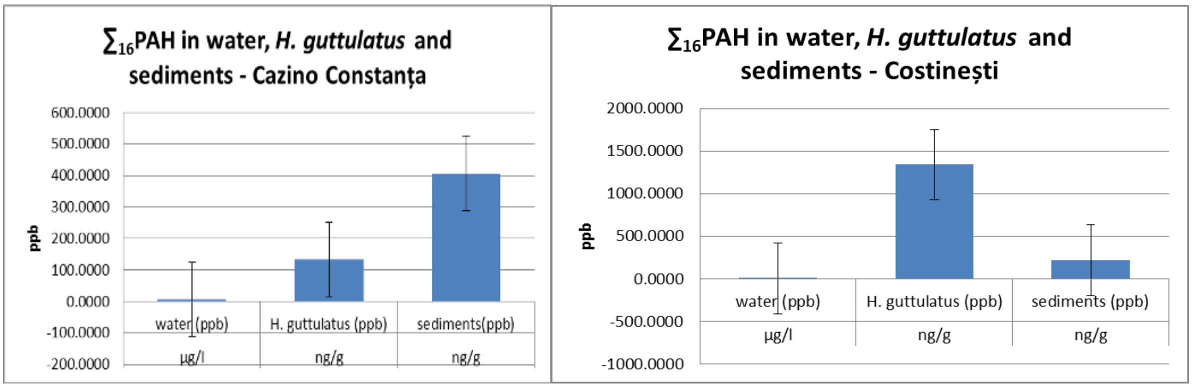
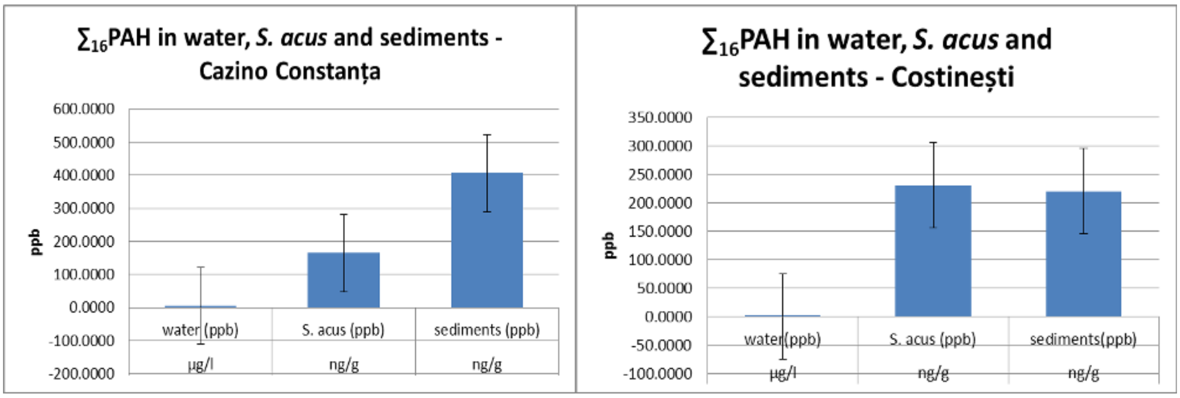


Fig. 3.7.3. Σ_{16}PAH contamination in *H. guttulatus* compared to water and sediments - Cazino Constanța and Costineşti (2014).



Σ_{16}PAH contamination in *S. acus* compared to water and sediments - Cazino Constanța and Costineşti (2014).

According to Varanasi (Varanasi et. al., 1993), marine organisms can be classified into four categories, depending on the total content of PAHs: *not contaminated*, *minimally contaminated*, *moderately contaminated* and *highly contaminated* (Table 3.7.1.)

Tabel 3.7.1. Classification of the *H. guttulatus* and *S. acus* samples according to the PAH contamination extent.

Classification		Cazino *H. guttulatus*	Costinesti *H. guttulatus*	Cazino *S. acus*	Costineşti *S. acus*
	Σ_{16}PAHs (ng g $^{-1}$ WW)	Σ_{16}PAHs (ng g $^{-1}$ ww)			
		mean	mean	mean	mean
not contaminated	<10	-	-	-	-
minimally contaminated	10–99	-	-	-	-
moderately contaminated	100–1000	132.2 ± 14.1		164.4 ± 22.7	230.5 ± 14.5
highly contaminated	>1000		1341.8 ± 84.1		

The results obtained as a follow-up of the analyses performed on the tissue of the two Syngnathid species (*H. guttulatus* and *S. acus*) confirmed the fact that they accumulate polycyclic aromatic compounds from the marine environment, all samples analyzed being rated as contaminated to a certain extent (a *highly contaminated* sample - seahorse tissue in Costinești 1341.8 ± 84.1 ng/g WW) and the other three *moderately contaminated*, with values over 100 ng/g WW.

It stands out that of the biota samples analyzed, only one recorded a $\sum_{16}$PAHs value greater than 1000 (seahorse tissue in Costinești: 1341.8 ± 84.1 ng/g WW), which rates this sample as ***highly contaminated***. All the other samples recorded values much lower values (pipefish Costinești: 230.5 ± 14.5 ng/g WW; pipefish Constanța: 164.4 ± 22.7 ng/g WW; seahorse Constanța: 132.2 ± 14.1 ng/g WW), as they were *moderately contaminated*.

OVERALL CONCLUSIONS

This research was a first attempt to perform a thorough study of Syngnathids of the Romanian Black Sea coast and was directed towards three major categories of objectives, namely ***objectives of enhancing the knowledge on the biology and ecology of the target species, of performing genetic analyses for species determination*** and ***of quantifying the contamination with anthropogenic origin xenobiotics.*** The objectives were reached by a series of investigations which resulted in some relevant conclusions.

Characterization of species in the family Syngnathidae

The family Syngnathidae is part of the Order Syngnathiformes and comprises seahorses (*Hippocampus* sp.), pipefish and seadragons. The oldest Syngnathid fossils date back from the Middle Miocene (Lower Sarmatian, Coprolithic Horizon) and were discovered in Slovenia (Zalohar et al., 2009). The evolutionary processes led to the transformation of a conventionally shaped fish into one that does not look like a fish anymore (Zalohar et al., 2012). One of the morphologic characteristics used for the taxonomic classification of Syngnathids is the fusion of cranial and feeding structures. This highly atypical group of fish defined the Order Syngnathiformes, sharing as common characteristic the fact that all families comprise individuals with fused jaws.

The family Syngnathidae is one of the largest famlies whose members use one of the rarest reproductive strategies in the animal world: male pregnancy/gestation, defining gestation as the process of incubating embryos inside the body after the merger between the egg and the spermatozoon (Stolting & Wilson, 2007).

The seahorse is one of the most charismatic species in the Black Sea and, despite that apparently it has no economic significance (it is not a species usable as food source for people), it raises interest for use as bioresource for traditional medicine, either for the developing aquarium business, or it is simply sold illegally as souvenir or curio. In Romanian Black Sea waters, literature sources (Lourie et al., 2004; Foster & Vincent, 2004) indicate the occurrence of three species, namely *H. guttulatus* (Cuvier, 1829), *H. hippocampus* (Linnaeus, 1758) and *H. fuscus* (Rüppel 1838).

Additionally, in the Black Sea, several species of the subfamily Syngnathinae are accounted for, belonging to two genera, *Syngnathus* and *Nerophis* (*S. typhle, S. schmidti, S. variegatus, S. tenuirostris, S. abaster, N. ophidion*) (Radu & Radu, 2008). Individuals belonging to all these species were identified during thethree years of performing this research in by-catches of the fishing points located along the Romanian Black Sea coast, namely *S. typhle, S. schmidti, S. variegatus, S. tenuirostris, S. abaster, N. Ophidion*. However, with reference to seahorses, the only species identified was *H. guttulatus*, which was also confirmed by the results of the genetic analyses performed (*Subchapter 3.3. Genetic analyses for the species Hippocampus guttulatus of the Romanian Black Sea coast*).

Calculation of the length-weight relationshiop for Hippocampus guttulatus of the Romanian Black Sea coast

The main conclusion of this research was that *H. guttulatus* specimens measured and weighed during this study indicated an overall negative allometric growth (b = 2.72), which means that the length of individuals increases faster compared to their weight. When performing a comparison of the mean lengths of *H. guttulatus* individuals of different marine areas, it was found that the lengths of seahorses from the Romanian Black Sea coasts are comparable with the ones from the south-eastern corner of the Black Sea, while specimens from the Aegean Sea (Izmir Bay) are significantly larger, with sizes comparable to those measured on the Atlantic coast (Arade Estuary, Portugal). With reference to the weight of *H. guttulatus* specimens, it resulted that individuals from the Aegean Sea - Izmir Bay are much heavier, the difference between the latter and specimens from the Black Sea (both from the north-west and the south-east) being statistically significant. It was noted that individuals from the Black Sea, regardless of the collection area, recorded similar weights.

No available data were found in FishBase (Froese & Pauly, 2004) for the species *H. guttulatus*. This aspect, along with IUCN's classification of *H. guttulatus* as Data Deficient (DD), accounts for the need of future research, aiming at obtaining new information for the Black Sea.

Genetic analyses for the species Hippocampus guttulatus of the Romanian Black Sea coast

In Romanian Black Sea waters, literature sources (Lourie et al., 2004; Foster & Vincent, 2004) indicate the occurrence of three species, namely *H. guttulatus* (Cuvier, 1829), *H. hippocampus* (Linnaeus, 1758) and *H. fuscus* (Rüppel 1838), while more recent papers report a single species (Radu & Radu, 2008).

The results of this investigation led to the conclusion that *H. guttulatus* individuals sampled from the Romanian Black Sea coast have a katyotype with diploid values 2n = 44 chromosomes, and that there are no sexually differentiated chromosomes. The microscope analysis of the chromosome type concluded that the species *H. guttulatus* displays *42 acrocentric chromosomes* and *2 meta-submetacentric chromosomes*, thus resulting in a fundamental number of 46 chromosome arms, 2 sm-m+42 a; FN = 46.

It was concluded that all individuals analyzed by PCR-RFLP belong to the same species, namely *H. guttulatus*. Despite the fact that no polymorphism was identified at specimens analyzed in this study, the results might have been influenced by the small number of individuals available for testing, as well as by the fact that they were all collected from the same sampling area. Consequently, it is necessary to continue these studies further, using seahorse individuals caught from various locations along the Romanian Black Sea coast, for further mtDNA amplifications and nucleotide sequencing, aiming at settling the controversy on the existence of one or several species of the genus *Hippocampus*.

Determination of the effects of different live feed diets on the seahorse Hippocampus guttulatus

As a follow-up of the experiment performed during the doctoral research, concerning length measurements it was noted that length increase in *H. guttulatus* individuals was linear in all three feeding regimes, the final length (+10 days) being higher than the +5 days length and initial length, respectively. The maximum increase in length was reported for the combined diet (Tank C, combined *Brachionus plicatilis* + *Artemia salina* diet), followed closely by the *A. salina* diet (Tank B). The minimum increase was registered by seahorses fed with *B. plicatilis* (Tank A). With reference to weight variation, the maximum increase was reported for the combined diet (Tank C), followed by the brine shrimp diet (Tank B) and the rotifer diet (Tank A).

The preliminary conclusions of this research are that a combined diet of rotifers (*B. plicatilis*) and brine shrimp (*A. salina*) is the most recommended for rearing seahorses (*H. guttulatus*) in captivity, due to the advantages that the two invertebrates have separately. On the one hand, rotifers develop greater densities and have a higher protein content (reflected in the protein content of the batches analyzed), while brine shrimps have a higher lipid content and are easier to prey on, being larger and more visible in the tanks. Other diets should also be tested in captive conditions, as studies have indicated that Amphipoda, Anomura, Decapoda and Mysidacea seem to be the dominant prey categories in the wild (Kitsos et al., 2008; Gurkan et al., 2011). Future research will focus on analyzing the stomach content of *H. guttulatus* individuals obtained from by-catches of the fishing points along the Romanian Black Sea coast, aimed at accurately determining the type of prey preferred by this species in the Black Sea.

On the other hand, seawater temperature and salinity influence the metabolism of *H. guttulatus.* The increase of temperature above 23°C and salinity drop below 14 PSU‰ are likely to have caused the changes in the biochemical composition of *H. guttulatus*. Further research is required during a longer time frame (at least 30 days), in order to monitor how the length-weight relationship develops in time in correlation with the diet applied, as well as the variation of the biochemical composition of *H. guttulatus* under different feeding regimes, also considering the influence of environmental factors. In addition, sex differences should be considered, as well as different developmental stages (juveniles vs. adults). The ultimate aim is to find the optimal diet for rearing seahorses in captivity using live prey that can be easily obtained in artificial environments.

Investigation of pollutant accumulation in the seahorse Hippocampus guttulatus whole tissue

Starting from the knowledge gaps on the manner and the extent to which pollutants reaching the marine environment influence seahorses at the Romanian Black Sea coast, this study aimed at assessing the bioaccumulation of pollutants in the whole tissue of the species *H. guttulatus*. In addition, some biochemical parameters were determined (dry weight, moisture, ash, crude protein, crude lipid).

The overall conclusion of this first research attempt on the bioaccumulation level in the Black Sea *Hippocampus guttulatus* confirms the hypothesis that seahorses, like most of marine species, absorb the xenobiotics reaching the aquatic environment (Nenciu et al., 2014b). Synthetically, the organochloriene pesticide (OCP) values in seahorse tissue revealed that, despite its low lipid content compared to other marine organisms (mussels, for instance), seahorses still bioaccumulate organochlorine compounds, which are usually absorbed by fatty tissues. Higher overall OCP concentrations were recorded in the Cazino-Constanța Station. With reference to polycyclic aromatic hydrocarbons (PAHs) in seahorse tissue, the analyzed samples showed a higher contamination degree in the Cazino-Constanța Station, decreasing southwards (probably due to the proximity of the Constanța Port). For heavy metals, however, the peak values (copper) were recorded in one of the southern stations, Saturn-Venus, most likely due to vessel traffic in the area.

All these compounds detected as a follow-up of the investigations performed are highly toxic, even in low concentrations, especially if they accumulate in the metabolically active sites, and they could significantly influence the survival of a sensitive species such as the long-snouted seahorse. It is, thus, essential to perform future research aimed at analyzing the extent to which OCPs, PAHs and heavy metals influence the metabolic and enzymatic activity of *H. guttulatus*.

Studies on heavy metal bioaccumulation in Black Sea coast seahorse Hippocampus guttulatus and pipefish Syngnathus acus whole tissue expressed by the Bioconcentration Factor (BCF) and Biota-Sediment Accumulation Factor (BSAF)

The results obtained proved that both seahorses and pipefish collected from shallow coastal waters do bioaccumulate heavy metals at different rates, significant differences ($p < 0.05$, T test) being recorded also between sampling stations. For instance, in 2014, copper recorded the peak values in both species. High values were also recorded in seahorse tissue for nickel and chrome (Costinești), and in pipefish tissue for lead (Constanța).

In 2014, the Bioconcentration Factor (BCF) recorded high values for copper (>5000, *very bioaccumulative*) and nickel (>2000, *bioaccumulative*) in both species in the Constanța Station. In the Costinești Station, results were similar for copper (>5000, *very bioaccumulative*) in both species, while nickel and chrome recorded high values (>5000, *very bioaccumulative*) only in seahorse tissue. The values of the Biota-Sediment Accumulation Factor (BSAF) recorded by pipefish were low (<1) while for the seahorse tissue exceedings were recorded only for cadmium (Constanța) and (Costinești) (BSAF>1). In samples from 2013, exceedings of the BCF threshold values were recorded for Cd, Pb, Cu and Ni, while exceedings of the BSAF threshold values were reported only for Cd and Cu. For both bioaccumulation indices, the lowest values were recorded in the Cazino-Constanța Station, which raises some questions, whereas this station is located in the proximity of the Constanța Port, where anthropogenic influence is severe (Nenciu et al., 2014a). The highest BCF and BSAF values were calculated in Saturn-Venus (very high BCF for Cd), where high concentrations were also reported in *H. guttulatus* tissue (Cu).

The results obtained proved that *H. guttulatus* and *S. acus* intake heavy metals from the surrounding environment, being bioaccumulative and bioconcentrator species. With reference to the inter-species differences, due to their lower mobility and smaller habitat ranges they cover compared to pipefish, seahorses tend to accumulate more chemicals.

Some of these metals are normally found in the body (Cu, Cr), performing certain physiological functions, yet they may become toxic when concentrations increase due to anthropogenic impact. Other metals (Cd, Pb) are toxic even in low concentrations. The extremely high values recorded by copper is some marine organisms may be explained by the presence of haemocyanin, a respiratory protein in the form of metalloproteins containing two copper atoms, irreversibely bound by an oxygen molecule (Rainbow et al., 1989). The most well-known example of marine animal in which oxygen supply is copper-based is the North-American horseshoe crab *Limulus polyphemus*, but also other mollusks and most arthropods have copper-based blood (this could explain the high copper values recorded in *Rapana venosa* and the other analyzed mollusks from the Black Sea). Even though iron is usually more efficient than copper, under low temperature circumstaces, copper-based oxygenation is more efficient. However, it is not the case of Syngnathids and the other fish species in the Black Sea, which, as vertebrates, have their blood based on the haemoglobin respiratory pigment (Rainbow et al., 1989). Consequently, the copper source is probably external and, most certainly, of anthropogenic origin, caused by vessel traffic in the sampling area, whereas copper is used as antifouling agent on vessel hulls (Nenciu et al., 2014b).

Nevertheless, the influence of other factors on heavy metal bioaccumulation should not be overlooked: intake from food (biomagnification), age, maturity degree, physiological state, diseases etc. Heavy metal bioaccumulation by marine species can be an excellent indicator of the marine environment quality, providing additional knowledge to the data resulting after analyzing the water column and sediments alone.

Research on polycyclic aromatic hydrocarbons (PAH) contamination of Syngnathids in Romanian coastal waters

The main pathways for introducing PAHs in the marine environment are atmosphere deposits, wastes and industrial or oil spills reaching directly the marine environment. Once having reached the water, the compounds which do not evaporate tend to adhere to the surface of organic and inorganic particles, due to their highly hydrophobic character, being subsequently transported in the water column and finally accumulating in sediments. The contamination of marine waters and particularly sediments affects the marine benthos, due to the fact that these compounds are deposited and accumulate in the substrate, becoming a major pool of toxicants in the marine environment.

The results obtained as a follow-up of the analyses performed on the tissue of the two Syngnathid species (*H. guttulatus* and *S . acus*) confirmed the fact that they accumulate polycyclic aromatic compounds from the marine environment, all samples analyzed being rated as contaminated to a certain extent (a *highly contaminated* sample - seahorse tissue in Costinești 1341.8 ± 84.1 ng/g WW) and the other three *moderately contaminated*, with values over 100 ng/g WW.

On species, seahorses accumulated polycyclic compounds to a greater extent, on the one hand due to their higher concentration of lipids compared to pipefish and on the other hand due to their lower mobility.

Whereas polycyclic aromatic hydrocarbons (PAHs) are rated by the Water Framework Directive as "prioritary hazardous substances", they are regulated by the Framework Directive on Wastes, they are highly stable compounds persisting for a long time in the marine environment (persistent organic pollutants), the PAH contamination of marine organisms can serve as environmental quality indicator. Additionally, the potential negative effects on marine organisms cannot be ignored, as they may endanger the biodiversity of an ecosystem in such a fragile balance such as the Black Sea.

Consequently, with the aim of obtaining additional data on the biology and ecology of Syngnathids of the Romanian Black Sea coast and the way these species are affected by anthropogenic substances reaching the marine environment, we suggest the following ***research directions***:

- Increasing the sample collection efforts (particularly *H. guttulatus*) from by-catches of the fishing points along the Romanian Black Sea coast, with the view to performing measurements and calculating the growth parameters (separately on locations, seasons etc.), as well as stomach contents and pollutant bioaccumulation analysis in the tissue;
- Performing mtDNA amplification on a larger number of seahorse specimens, collected from different locations along the Romanian Black Sea coast (from by-catches of the fishing points, thus not exerting additional pressure on stocks), in order to confirm the preliminary hypothesis of this research (namely that *H. guttulatus* is the only species inhabiting the Romanian Black Sea coast, the other two documented by literature, *H. hippocampus* and *H. fuscus,* not being reported throughout the performance of research for this thesis);
- Resuming the experiment for the identification of the best diet for rearing seahorses in captivity, expanding its duration, as well as the food array provided to the animals;
- Performing toxicity tests on *Syngnathus* sp. specimens (with no conservation status and easy to collect from the natural environment), particularly using oil products, which can easily reach the shallow areas inhabited by these species;
- Applying the study and analysis methods on other marine living organisms in the Black Sea, aiming at adding-up information on their response to human activity impacts.

SELECTED REFERENCES

1. Başusta A., Özer E., Girgin H., Serdar O., Başusta N. (2014), Length-Weight Relationship and Condition Factor of *Hippocampus hippocampus* and *Hippocampus guttulatus* Inhabiting Eastern Black Sea, in Pakistan J. Zool., vol. 46(2), pp. 447-450;
2. Bănărescu P. (1964), Fauna Republicii Populare Române, Pisces - Osteichthyes (Peşti ganoizi şi osoşi), vol. XIII, Ed. Academiei R.P.R., 959 p.;

3. Butler P.A. (1971), Monitoring organisms, in Food and Agricultural Organisation Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing, Rome, 1970. Supplement I: Methods of detection, measurement and monitoring of pollutants in the marine environment. M. Ruivo (ed.), London, Fishing News (Books) Ltd., pp. 101-112;
4. Caldwell I.R., Vincent A.C.J. (2012), A sedentary fish on the move: effects of displacement on long-snouted seahorse (*Hippocampus guttulatus* Cuvier) movement and habitat use. Environmental Biology of Fishes. 96(1), pp. 67-75;
5. Carlander, K.D. (1977), Handbook of freshwater fishery biology, Volume 2. The Iowa State University Press, Ames, Iowa, 431 p.;
6. Coatu V., Ţigănuş D., Oros A., Lazăr L. (2013), Analysis of Hazardous Substance Contamination of the Marine Ecosystem in the Romanian Black Sea Coast, Part of the Marine Strategy Framework Directive (2008/56/EEC) Implementation, in Cercetări Marine / Recherches Marines, INCDM Constanţa, vol. 43, ISSN: 0250-3069, pp. 174-186;
7. Foster S.J., Vincent A.C.J. (2004), Life history and ecology of seahorses: implications for conservation and management. Journal of Fish Biology 65, pp. 1-61;
8. Froese R. (2006), Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology, 22, pp. 241-253;
9. Froese R., Pauly D. (2004), FishBase. World Wide Web electronic publication. http: //www.fishbase.org.status: July 2004;
10. Grasshoff K., Kremling K., Ehrhardt MG (1999), Methods of Seawater Analysis (3rd Edition). VCH Publishers. 632 pp;
11. Gurkan S., Taşkavak E. (2007), Length-Weight relationships for Syngnathid fishes of Aegean Sea, Turkey. Belg. J. Zool., 137, pp. 219-222;
12. Gurkan S., Taşkavak E., Sever T.M., Akalin S. (2011), Gut contents of two European seahorses *Hippocampus hippocampus* and *Hippocampus guttulatus* in the Aegean Sea, Coasts of Turkey. Pakistan J. Zool., vol. 43 (6), pp. 1197-1201;
13. Hafez R., Labat R., Quillier R. (1981), Recherches sur les chromosomes surnumeraires de lablette (*Alburnus alburnus* L.). Cybium, 5, pp. 81-87;
14. Haug A.S. (1974), Estimation of heavy metal pollution in two Norwegian fjord areas by analysis of the brown alga (*Ascophyllum nodosum*). Environ. Pollut., 7, pp. 179-192;
15. INCDM, (2012a), Evaluarea iniţială a apelor româneşti ale Mării Negre, cerinţă a Directivei Strategia pentru Mediul Marin: Boicenco L., Alexandrov L., Anton E., Coatu V., Cristea M.,Cristescu T., Diaconeasa D., Dumitrache C., Filimon A., Lazăr L., Malciu V., Marin O., Mateescu R., Micu D., Mihailov M., Nicolaev S., Niță V., Oros A., Radu G., Spînu A., Stoica E., Tabarcea C., Ţigănuş D., Timofte F., Zaharia T., 219 p. http://www.mmediu.ro/beta/wp-content/uploads/2012/07/2012-07-17_evaluare_impact_planuri_evaluareinitialamediumarin.pdf;
16. Kasapoglu N., Duzgunes E. (2014), Some Population Characteristics of Long-snouted Seahorse (*Hippocampus guttulatus* Cuvier, 1829) (Actinopterygii: Syngnathidae) in the Southeastern Black Sea, Acta Zool. Bulg., 66 (1), pp. 127-131;
17. Kitsos, M.S., Tzomos, T.H., Anagnostopoulou, L., Koukouras, A. (2008), Diet composition of the seahorses, *Hippocampus guttulatus* Cuvier 1829 and *Hippocampus hippocampus* (L., 1758) (Teleostei, Syngnathidae) in the Aegean Sea. J. Fish Biol., 72, pp. 1259-1267, DOI 10.1111/j.1095-8649.2007.01789.x;
18. Kleiber, D., Blight, L., Caldwell, I., Vincent, A. (2011), The importance of seahorses and pipefishes in the diet of marine animals. Reviews in Fish Biology and Fisheries. 21, pp. 205-223;
19. Léger P., Bengtson D.A., Sorgeloos P., Simpson K.L., Beck A.D. (1987), The nutritional value of Artemia: a review. Artemia Research and its Applications. 1987. Vol. 3. Ecology, Culturing, Use in aquaculture. Sorgeloos P., Bengtson D. A., Decleir W., and Jaspers E. (Eds). Universa Press, Wetteren, Belgium, 556 p.;
20. Lourie S.A., Foster S.J., Cooper E.W.T., Vincent A.C.J. (2004), A Guide to the Identification of Seahorses. Project Seahorse and TRAFFIC North America, University of British Columbia and World Wildlife Fund, Washington D.C;
21. Lowry O.H., Rosenbroufg N.J., Lewis-Farr A., Randall R.J. (1951), Protein measurement with the Folin Phenol reagent, in Journal of Biological Chemistry, 193, pp. 265-275;
22. Ludwig A., Debus L. et al. (2002), A Molecular Approach to Control the International Trade in Black Caviar. International Review of Hydrobiology 87(5-6), pp. 661-674;
23. Mamaev V., Aubrey D., Eremeev V., (eds.) (1995), Black Sea Bibliography 1974-1994, United Nations Publications, New York, 364 p.;
24. **Nenciu (Zaharia) M.-I.,** Roșioru D., Coatu V., Oros A., Roșoiu N. (2013), Characterization of the Environmental Conditions of the Long-Snouted Seahorse Habitat at the Romanian Coast, in Journal of Environmental Protection and Ecology, ISSN 1311-5065, Vol. 14, No. 4, pp. 1695-1702 - Thomson Reuters ISI Web of Knowledge, factor impact 0,338, http://www.jepe-journal.info/vol14-no4-2013;
25. **Nenciu M.I.,** Roșioru D., Oros A., Galațchi M., Roșoiu N. (2014a), Bioaccumulation of Heavy Metals in Seahorse Tissue at the Romanian Black Sea Coast, in 14th SGEM GeoConference on Water Resources. Forest, Marine And Ocean Ecosystems, www.sgem.org, SGEM2014 Conference Proceedings, ISBN 978-619-7105-14-8 / ISSN 1314-2704, June 19-25, 2014, Vol. 2, pp. 547-554, DOI: 10.5593/SGEM2014/B32/S15.072 - indexată BDI (Thomson Reuters ISI Web of Knowledge, SCOPUS, CrossRef Database (DOI for each paper), CrossRef Cited By Linking, ProQuest & GeoRef, EBSCO, Mendeley, CiteULike, UlrichsWeb, Google Scholar), http://www.sgem.org/sgemlib/spip.php?article4518;
26. **Nenciu M.-I.,** Coatu V., Oros A., Roșioru D., Ţigănuș D., Roșoiu N. (2014b), Pollutant Bioaccumulation in the Long-Snouted Seahorse at the Romanian Coast, in Journal of Environmental Protection and Ecology, ISSN 1311-5065, Vol. 15, No. 4, pp. 1650-1659 - Thomson Reuters ISI Web of Knowledge, factor impact 0,838, http://www.jepe-journal.info/journal-content/vol-15-no-4-2014;
27. **Nenciu M.-I**., Roșoiu N., Petcu C. L., Zaharia T., Niță V., Maximov V., Roșioru D. (2015), Effects of Different Live Feed Diets Applied to the Long-Snouted Seahorse (*Hippocampus guttulatus* Cuvier, 1829), in Turkish Journal of Fisheries and Aquatic Sciences (TrJFAS), Central Fisheries Research Institute, Trabzon, TURKEY, 15, pp. 406-415, ISSN 1303-2712, DOI: 10.4194/1303-2712-v15_2_26 - Thomson Reuters ISI Web of Knowledge, factor impact 0,384, http://www.trjfas.org/new.html;
28. Onără D., Taflan E., Cociaş Ş., Bercsényi M., Szűcs R., Suciu R. (2012), Comparative genetic diversity study of two European populations of pikeperch using PCR-RFLP, Scientific Annals of the Danube Delta Institute Tulcea, Romania, vol. 18/2012, ISSN 1842 - 614X; on-line ISSN 2247 - 9902 203, doi: 10.7427/DDI.18.10, pp. 203 - 210;
29. Onciu T. (1998), Cultura unor nevertebrate utilizate în hrana peştilor şi crustaceelor cu valoare economică - obiect al mariculturii româneşti, Teză de Doctorat, Institutul de Biologie din Bucureşti, 180 p.;
30. Oros A.N., Pecheanu I., Mihnea R. (2003), Some aspects concerning trace metals bioaccumulation in *Mytilus galloprovincialis* along the Romanian Black Sea coastal area, in Journal of Environmental Protection and Ecology, Volume 4, Issue 4, pp. 850-856;
31. Oros A.N. (2009), Contribuții la cunoașterea consecințelor poluării cu metale grele asupra ecosistemelor marine costiere de la litoralul românesc al Mării Negre, Teză de Doctorat, Universitatea „Ovidius“ Constanța, Facultatea de Științe ale Naturii și Științe Agricole, Specializarea Ecologie și Protecția Mediului, 212 p.;

32. Oros A.N., Gomoiu M.-T. (2012), A review of metal bioaccumulation levels in the Romanian Black Sea biota during the last decade - A requirement for implementing Marine Strategy Framework Directive (Descriptors 8 and 9), in Journal of Environmental Protection and Ecology, Volume 13, Issue 3A, pp. 1730-1743;
33. Petcu L.C. (2011), Analiza statistică cu SPSS - Note de curs, Ed. Ovidius University Press, ISBN: 978-973-614-641-1, 303 p.;
34. Phillips D.J.H., (1980), Trace metals. In: The International Mussel Watch. National Academy of Sciences, Washington DC, pp. 78-132;
35. Qiang L., Lin J., Lu J., Li B. (2008), Biochemical Composition of Six Seahorse Species, *Hippocampus* sp., from the Chinese Coast, in Journal of the World Aquaculture Society, Vol. 39, No. 2, pp. 225-234;
36. Rainbow P.S., Moore P.G., Watson D. (1989), Talitrid amphipods (Crustacea) as biomonitors for copper and zinc. Estuarine Coast. Shelf Sci., 28, pp. 567-582;
37. Radu G., Radu E. (2008), Determinator al principalelor specii de pești din Marea Neagră, Ed. Virom Constanța, 558 p.;
38. Ricker W.E. (1975), Computations and interpretation of biological statistics of fish populations. Fisheries Research Board Canada Bulletin, 191: p. 382;
39. Roșioru D. (2008), „Studiul biochimic al aglomerărilor de organisme marine în interacțiune cu mediul ambiant", Teză de Doctorat, Universitatea „Ovidius" Constanța, Facultatea de Științe ale Naturii și Științe Agricole, conducator științific prof. univ.dr. CS I Natalia Roșoiu, 210 p.;
40. Roșioru D., Coatu V., Oros A., Vasiliu D., Țigănuș D. (2012) , Marine environment quality of the growth and exploitation of the main mollusks from the Romanian Black Sea, in Journal of Environmental Protection and Ecology, Volume 13, Issue 3A, pp. 1799-1805;
41. Roșioru D., Abaza V., Dumitrache C., Filimon A., Timofte F. (2014) Ecological and biochemical aspects of *Mytilus galloprovincialis* from the Romanian Black Sea Coast, 14th SGEM GeoConference on Water Resources. Forest, Marine and Ocean Ecosystems, Vol. 2, No. SGEM2014 Conference Proceedings, ISBN 978-619-7105-14-8 / ISSN 1314-2704, June 19-25, 2014, Vol. 2. (2014), pp. 569-576, doi:10.5593/SGEM2014/B32/S15.075;
42. Sericano J.L., Atlas E.L., Wade T.L., Brooks J.M. (1990), NOOA's status and trends mussel watch program: chlorinated pesticides and PCBs in oysters (*Crassostrea virginica*) and sediments from the Gulf of Mexico, 1986-1987. Marine Environ. Res., 29, pp. 161-203;
43. Sneath P. H. A., Sokal R. R. (1973), Numerical taxonomy. Ed. Freeman, San Francisco CA. (abstract);
44. Stolting K.N., Wilson A.B. (2007), Male pregnancy in seahorses and pipefish: beyond the mammalian model. BioEssays. 29, pp. 884-896;
45. Taggart J.B., Hynes, R.A., Prodohl, P.A., Ferguson, A. (1992), A simplified protocol for routine total DNA isolation from salmonid fishes, Journal of Fish Biology 40: pp. 963 - 965;
46. Teske, P.R., Beheregaray, L.B. (200), Evolution of seahorses' upright posture was linked to Oligocene expansion of seagrass habitats. Biology Letters. 5, pp. 521-523;
47. Ţigănuş D., Coatu V., Lazăr L., Oros A., Spînu A. D. (2013). Identification of the sources of polycyclic aromatic hydrocarbons in sediments from the Romanian Black Sea sector, Cercetări Marine, INCDM Constanța, vol. 43, ISSN: 0250-3069, pp. 187-196;
48. Ţigănuş D. (2015), Hidrocarburi Aromatice Polinucleare, în Golumbeanu M. & Nicolaev S. (editori) (2015), Study on Integrated Coastal Zone Management, Editura Ex Ponto Constanța, versiunea originală în limba engleză, ISBN 978-606-598-397-7, 454 p, Anexa 3, pp. 439-445;
49. Varanasi U., Casillas E., Arkoosh M.R, Hom T., Misitano D., Brown D.W., Chan S., Collier T.K., McCain B.B., Stein J.E. (1993), Contaminant Exposure and Associated Biological Effects in Juvenile Chinook Salmon (*Oncorhynchus tshawytscha*) from Urban and Nonurban Estuaries of Puget Sound, WA, NOAA Tech. Memo NMFS-NWFSC-8. U.S. Department of Commerce, Rockville, MD;
50. Veiga, P., Machado D., Almeida,C., Bentes L.,Monteiro P., Oliveira F., Ruano M., Erzini, K, Gonçalves J.M.S. (2009), Weight-length relationships for 54 species of the Arade estuary, southern Portugal. J. appl. Ichthyol., DOI: 10.1111/j.1439-0426.2009.01230.x, 25: pp. 493-496;.
51. Woodall L. (2012). *Hippocampus guttulatus*. The IUCN Red List of Threatened Species. Version 2015.2. <www.iucnredlist.org>. Downloaded on 23 August 2015;
52. Zalohar, J., Hitij, T., Križnar, M. (2009), Two new species of seahorses (Syngnathidae, Hippocampus) from the Middle Miocene (Sarmatian) Coprolitic Horizon in Tunjice Hills, Slovenia: The oldest fossil record of seahorses, Annales de paléontologie. Elsevier, pp. 71-96;
53. Zalohar, J., Hitij, T. (2012), The first known fossil record of pygmy pipehorses (Teleostei: Syngnathidae: Hippocampinae) from the Miocene coprolitic horizon, Tunjice Hills, Slovenia, Annales de Paléontologie. Elsevier, pp. 131-151;
54. *** IAEA-MEL (1995). Marine Environmental Studies Laboratory, Training Manual on the Measurement of Organochlorine and Petroleum Hydrocarbons in Environmental samples;
55. *** IAEA-MEL (1999). Standard operating procedures for trace metals analyses

SCIENTIFIC PUBLICATIONS ACHIEVED DURING THE DOCTORAL PROGRAMME

A. PAPERS PUBLISHED IN ISI-RATED JOURNALS WITH IMPACT FACTOR

1. ZAHARIA TANIA, MAXIMOV VALODIA, MICU DRAGOȘ, NIȚĂ VICTOR, NEDELCU M.ARIUS, GANEA GABRIEL, URSACHE CORNEL, GOLUMBEANU MARIANA, **NENCIU MAGDA**, 2012, Romanian Marine Fisheries and Natura 2000 Network, in **Journal of Environmental Protection and Ecology**, Volume 13, Issue 3A, pp. 1792-1798 - **Thomson Reuters ISI Web of Knowledge, factor impact 0,259,** http://www.jepe-journal.info/journal-content/vol13-no-3a;

2. **NENCIU (ZAHARIA) MAGDA-IOANA**, ROȘIORU DANIELA, COATU VALENTINA, OROS ANDRA, ROȘOIU NATALIA, **Characterization of the Environmental Conditions of the Long-Snouted Seahorse Habitat at the Romanian Coast**, 2013, in **Journal of Environmental Protection and Ecology**, ISSN 1311-5065, Vol. 14, No. 4, pp. 1695-1702 - **Thomson Reuters ISI Web of Knowledge, factor impact 0,338,** http://www.jepe-journal.info/vol14-no4-2013;

3. GOLUMBEANU MARIANA, OROS ANDRA, **NENCIU MAGDA**, ZAVATARELLI MARCO, DRAGO ALDO, 2014, Contribution of Environmental Indices in Meeting the Objectives and Principles of the Marine Strategy Framework Directive (MSFD), in **Journal of Environmental Protection and Ecology**, ISSN 1311-5065, Vol. 15, No. 3, pp. 1130-1138 - **Thomson Reuters ISI Web of Knowledge, factor impact 0,838,** http://www.jepe-journal.info/journal-content/https-sites-google-com-a-jepe-journal-info-jepe-journal-vol-15-no-3-2014;

4. MAXIMOV VALODIA, ŢIGANOV GEORGE, PARASCHIV MARIAN, **NENCIU MAGDA-IOANA**, ZAHARIA TANIA, (2014), Preliminary Data on the Monitoring of Sturgeon Species in Romanian Marine Waters, in **Journal of Environmental Protection and Ecology**, ISSN 1311-5065, Vol. 15, No. 3, pp. 933-943 - **Thomson Reuters ISI Web of Knowledge, factor impact 0,838,** http://www.jepe-journal.info/journal-content/https-sites-google-com-a-jepe-journal-info-jepe-journal-vol-15-no-3-2014;

5. GOLUMBEANU MARIANA, ZAHARIA TANIA, RADU GHEORGHE, MAXIMOV VALODIA, **NENCIU MAGDA**, 2014, Contributions of the Mareframe Project to Ecosystem-Based Fisheries Management Solutions for European Fish Stocks, in **Journal of Environmental Protection and Ecology**, ISSN 1311-5065, Vol. 15, No. 2, pp. 655-659 - **Thomson Reuters ISI Web of Knowledge, factor impact 0,838,** http://www.jepe-journal.info/vol-15-no-2-2014;

6. ZAHARIA TANIA, MAXIMOV VALODIA, RADU GHEORGHE, ANTON EUGEN, SPÎNU ALINA, **NENCIU MAGDA,** 2014, Romanian marine fisheries vs. Marine protected areas. Reconciling fisheries and habitat protection in coastal Romanian marine protected areas, in **Scientia Marina**, ISSN 0214-8358 Instituto de Ciencias del Mar de Barcelona (CSIC), Spain, pp. 95-101 - **Thomson Reuters ISI Web of Knowledge, factor impact 1,493,** http://www.icm.csic.es/scimar/index.php/secId/6/IdArt/4251/;

7. **NENCIU MAGDA-IOANA**, COATU VALENTINA, OROS ANDRA, ROŞIORU DANIELA, ŢIGĂNUŞ DANIELA, ROŞOIU NATALIA, 2014, **Pollutant Bioaccumulation in the Long-Snouted Seahorse at the Romanian Coast**, in **Journal of Environmental Protection and Ecology**, ISSN 1311-5065, Vol. 15, No. 4, pp. 1650-1659 - **Thomson Reuters ISI Web of Knowledge, factor impact 0,838,** http://www.jepe-journal.info/journal-content/vol-15-no-4-2014;

8. **NENCIU MAGDA-IOANA**, ROŞOIU NATALIA, PETCU CRISTIAN LUCIAN, ZAHARIA TANIA, NIŢĂ VICTOR, MAXIMOV VALODIA, ROŞIORU DANIELA, **Effects of Different Live Feed Diets Applied to the Long-Snouted Seahorse (*Hippocampus guttulatus* Cuvier, 1829)**, 2015, in **Turkish Journal of Fisheries and Aquatic Sciences (TrJFAS)**, Central Fisheries Research Institute, Trabzon, TURKEY, 15, pp. 406-415, ISSN 1303-2712, DOI: 10.4194/1303-2712-v15_2_26 - **Thomson Reuters ISI Web of Knowledge, factor impact 0,566,** http://www.trjfas.org/new.html.

B. PAPERS PUBLISHED IN ISI-RATED JOURNALS WITH NO IMPACT FACTOR

1. **NENCIU MAGDA-IOANA**, ROŞIORU DANIELA, OROS ANDRA, GALAŢCHI MĂDĂLINA, ROŞOIU NATALIA, 2014, **Bioaccumulation of Heavy Metals in Seahorse Tissue at the Romanian Black Sea Coast**, in **14th SGEM GeoConference on Water Resources. Forest, Marine And Ocean Ecosystems, www.sgem.org, SGEM2014 Conference Proceedings,** ISBN 978-619-7105-14-8 / ISSN 1314-2704, June 19-25, 2014, Vol. 2, pp. 547-554, DOI: 10.5593/SGEM2014/B32/S15.072 - **IDB indexed** (Thomson Reuters ISI Web of Knowledge, SCOPUS, CrossRef Database (DOI for each paper), CrossRef Cited By Linking, ProQuest & GeoRef, EBSCO, Mendeley, CiteULike, UlrichsWeb, Google Scholar), http://www.sgem.org/sgemlib/spip.php?article4518;

2. GALAŢCHI MĂDĂLINA, ROŞIORU DANIELA, **NENCIU MAGDA**, ZAHARIA TANIA, COPREAN DRAGOMIR, 2015, **Aspects of Romanian Black Sea Coast Anchovy (*Engraulis encrasicolus*, Linnaeus, 1758) Biology during 2013-2014**, in 15th International Multidisciplinary Scientific GeoConference SGEM 2015, www.sgem.org, SGEM2015 Conference Proceedings, ISBN 978-619-7105-37-7 / ISSN 1314-2704, June 18-24, 2015, Book 3 Vol. 2, 603-610 pp, DOI: 10.5593/SGEM2015/B32/S15.080 - **IDB indexed** (Thomson Reuters ISI Web of Knowledge, SCOPUS, CrossRef Database (DOI for each paper), CrossRef Cited By Linking, ProQuest & GeoRef, EBSCO, Mendeley, CiteULike, UlrichsWeb, Google Scholar), http://www.sgem.org/sgemlib/spip.php?article6088;

C. PAPERS PUBLISHED IN IDB INDEXED JOURNALS

1. NIŢĂ VICTOR, ZAHARIA TANIA, **NENCIU MAGDA**, CRISTEA MĂDĂLINA, ŢIGANOV GEORGE - Current State Overview of the Vama Veche - 2 Mai Marine Reserve, Black Sea, Romania, 2012, in **Aquaculture, Aquarium, Conservation & Legislation International Journal of the Bioflux Society**, Volume 5, Issue 1, February 2012, pp. 44-54, AACL Bioflux - http://www.bioflux.com.ro/aacl - **C.N.C.S.I.S. B+** (indexed in Thomson Reuters Scientific - ISI Web of Knowledge, Scopus - Elsevier; Sciverse, Scimago - Journal Rank, EBSCO - EBSCOhost Online Research Databases), http://www.bioflux.com.ro /docs/AACL_5.1.10.pdf;

2. **NENCIU MAGDA**, OROS ANDRA, ROŞIORU DANIELA, GALAŢCHI MĂDĂLINA, FILIMON ADRIAN, ŢIGANOV GEORGE, DANILOV CRISTIAN, ROŞOIU NATALIA, 2016, **Heavy Metal Bioaccumulation in Marine Organisms from the Romanian Black Sea Coast**, in **Annals of Academy of Romanian Scientists. Series on Biological Sciences**, on-line ISSN 2285 - 4177 Open Acces, printed ISSN 2285 - 4169, accepted for publication, *in press.*

D. PAPERS DEFENDED DURING INTERNATIONAL SCIENTIFIC EVENTS AND PUBLISHED AS ABSTRACT

1. **NENCIU (ZAHARIA) MAGDA-IOANA**, ROŞIORU DANIELA, COATU VALENTINA, OROS ANDRA, ROŞOIU NATALIA, 2013, **Characterization of the Environmental Conditions of the Long-Snouted Seahorse Habitat at the Romanian Coast**, in Book of Abstracts of the UAB-B.EN.A. International Conference “Environmental Engineering and Sustainable Development“, Alba Iulia, Romania, 23-25 May 2013, ISBN 978-606-613-067-7, pp. 95-96;

2. **NENCIU MAGDA**, ROŞIORU DANIELA, LUPAŞCU NALIANA, ROŞOIU NATALIA, 2013, **Identifying the Major Threats Seahorses are Facing at the Romanian Coast**, in Book of Abstracts of the 1st International U.O.C.-B.EN.A Conference “The Sustainability of Pharmaceutical, Medical and Ecological Education and Research“ - SPHAMEER 2013, 20-23 June 2013, Constanța, Romania“, ISBN 978-973-614-784-5, p. 166;

3. **NENCIU MAGDA**, ROŞIORU DANIELA, OROS ANDRA, COATU VALENTINA, CRISTEA MĂDĂLINA, GOLUMBEANU MARIANA, ROŞOIU NATALIA, 2013, **Major Threats on Seahorses at the Romanian Black Sea**

Coast, in Book of Abstracts of the "Marine Research Horizon 2020 - MARES 2020" International Conference, Hotel "Admiral", 17-21 September 2013, Golden Sands, Bulgaria, ISBN 978-954-92-787-8-1, p. 154;

4. **NENCIU MAGDA-IOANA**, COATU VALENTINA, OROS ANDRA, ROȘIORU DANIELA ȚIGĂNUȘ, ROȘOIU NATALIA, 2014, **Pollutant Bioaccumulation in the Long-Snouted Seahorse at the Romanian Coast**, in Proceedings of the International Conference "New Tools for the Sustainable Management of Aquatic Resources", Book of Abstracts, Bucharest, 17-18 January 2014, editors Dr. Deak Gyorgy, Prof. Dr. Fokion Vosniakos, ISBN 978-973-741-357-4, p. 103;

5. **NENCIU MAGDA-IOANA**, COATU VALENTINA, OROS ANDRA, ROȘIORU DANIELA, ȚIGĂNUȘ DANIELA, ROȘOIU NATALIA, 2014, **Xenobiotic Intake of *H. guttulatus* at the Romanian Black Sea Coast**, in Abstracts Book of the International Congress on "Green Infrastructures and Sustainable Societies/Cities" GreInSus 2014, Izmir, Turkey, 8-10 May 2014, ISBN 978-605-85204-0-0, p. 260;

6. **NENCIU MAGDA-IOANA**, OROS ANDRA, ROȘIORU DANIELA, GALAȚCHI MĂDĂLINA, ROȘOIU NATALIA, 2014, **Heavy Metal Bioaccumulation Indicators in Seahorse Tissue**, in Book of Abstracts of the 1st International Medical Conference "Environment and Public Health" MEDENV 2014, Constanta, Romania, 12-14 September 2014, ISBN 978-606-8066-486, p. 98;

7. **NENCIU MAGDA**, **Bioacumularea de metale grele în țesutul de căluț de mare (*Hippocampus guttulatus* Cuvier, 1829)**, în Volumul de Rezumate al Sesiunii Ştiinţifice de Toamnă a Academiei Oamenilor de Știință din România, 19-20 septembrie 2014, Constanța, România, p. 70;

8. **NENCIU MAGDA-IOANA**, ROȘIORU DANIELA, NIȚĂ VICTOR, MAXIMOV VALODIA, ROȘOIU NATALIA, 2014, **Effects of Different Live Feed Diets Applied to the Long-Snouted Seahorse (*Hippocampus guttulatus* Cuvier, 1829)**, in Abstract Book of the FABA 2014 International Symposium on Fisheries and Aquatic Sciences, Trabzon, Turkey, 25-27 September 2014, p. 296;

9. **NENCIU MAGDA-IOANA**, ROȘIORU DANIELA, ZAHARIA TANIA, ONĂRĂ DALIA, TAFLAN ELENA, HOLOȘTENCO DANIELA, ROȘOIU NATALIA, 2015, **First Attempt of Genetic Analyses for the Species *Hippocampus guttulatus* Cuvier 1829 in the Romanian Black Sea**, (2015), in Deltas and Wetlands (Book of Abstracts) (ed. L. Torok), vol. 3, ISSN 2344-3766, Danube Delta Technological Center Publishing House, Tulcea, Romania, 19-21 April 2015, p. 18, DOI 14592/DDI.D.W.03.2015;

10. **NENCIU MAGDA-IOANA**, OROS ANDRA, COATU VALENTINA, ȚIGĂNUȘ DANIELA, ROȘIORU DANIELA, ROȘOIU NATALIA, 2015, **Overview of Pollutants Identified in Long-Snouted Seahorse (*Hippocampus guttulatus*, Cuvier 1829) Samples from the Romanian Black Sea Coast**, in Book of Abstracts of the International U.A.B. - B.EN.A. Conference Environmental Engineering and Sustainable Development, Alba Iulia, Romania, May 28-30th, 2015, ISSN 245702829, p. 126;

11. **NENCIU MAGDA**, OROS ANDRA, ROȘIORU DANIELA, URSACHE CORNEL, ROȘOIU NATALIA, 2015, **Heavy Metal Bioconcentration in Syngnathids from the Romanian Black Sea**, în Abstracts Book of the 1st International Conference on Sea and Coastal Development in the Frame of Sustanability, MACODESU 2015, Trabzon, Turkey, 18-20 September 2015, p. 83;

12. **NENCIU MAGDA-IOANA,** ȚIGĂNUȘ DANIELA, OROS ANDRA, ROȘIORU DANIELA, ROȘOIU NATALIA, 2015, **Hydrocarbon Contamination of Syngnathids in Romanian Black Sea Waters**, în Proceedings Book of the 1st International Conference on "Ecology and Protection of Marine and Freshwater Environments - ECOPROWATER 2015", Viterbo, Italy, 1-3 October 2015, ISBN: 9788890755361, p. 40;

13. **NENCIU MAGDA**, OROS ANDRA, ROȘIORU DANIELA, GALAȚCHI MĂDĂLINA, ȚIGANOV GEORGE, DANILOV CRISTIAN-SORIN, FILIMON ADRIAN, ROȘOIU NATALIA, 2015, **Bioacumularea metalelor grele la specii din familia Syngnathidae de la litoralul românesc al Mării Negre**, în Volumul de Rezumate al Sesiunii Ştiinţifice de Toamnă 2015 a Academiei Oamenilor de Știință din România, 24-26 septembrie 2015, Iași, România, p. 53-54;

14. ROȘIORU DANIELA MARIANA; OROS ANDRA, **NENCIU MAGDA**, LUMINIȚA LAZĂR, 2015, **Heavy Metals Contamination in *Mytilus galloprovincialis*, Water And Sediments from the Romanian Black Sea Coast**, în Book of Abstracts of the International Symposium PROTECTION OF THE BLACK SEA ECOSYSTEM AND SUSTAINABLE MANAGEMENT OF MARITIME ACTIVITIES - PROMARE 2015, 7th Edition, Constanta, Romania, 29-31 October 2015, editors S. Nicolaev, F.K. Vosniakos, T. Zaharia, M. Golumbeanu, M. Nenciu, ISBN: 978-606-8066-52-3, p. 57.

15. **NENCIU MAGDA-IOANA**, ROȘIORU DANIELA, ZAHARIA TANIA, ONARĂ DALIA, TAFLAN ELENA, HOLOȘTENCO DANIELA, ROȘOIU NATALIA, 2015, **Preliminary Genetic Analyses for the Species *Hippocampus guttulatus* (Cuvier, 1829) in the Romanian Black Sea**, în Book of Abstracts of the International Symposium PROTECTION OF THE BLACK SEA ECOSYSTEM AND SUSTAINABLE MANAGEMENT OF MARITIME ACTIVITIES - PROMARE 2015, 7th Edition, Constanta, Romania, 29-31 October 2015, editors S. Nicolaev, F.K. Vosniakos, T. Zaharia, M. Golumbeanu, M. Nenciu, ISBN: 978-606-8066-52-3, p. 71.

PARTICIPATION IN INTERNATIONAL SCIENTIFIC CONFERENCES

1. UAB-B.EN.A. International Conference "Environmental Engineering and Sustainable Development", Alba Iulia, Romania, 23-25 May 2013 - oral presentation;
2. U.O.C.-B.EN.A Conference "The Sustainability of Pharmaceutical, Medical and Ecological Education and Research" - SPHAMEER 2013, Constanța, Romania, 20-23 June 2013 - oral presentation;
3. "Marine Research Horizon 2020 - MARES 2020" International Conference, Hotel "Admiral", Golden Sands, Bulgaria, 17-21 September 2013 - poster;
4. 4th Black Sea Scientific Conference - Black Sea, Challenges towards Good Environmental Status, Constanța, Romania, 28-31 October 2013 - poster;
5. International Conference "New Tools for the Sustainable Management of Aquatic Resources", București, România, 17-18 January 2014 - oral presentation + poster;
6. International Congress on "Green Infrastructures and Sustainable Societies/Cities" GreInSus 2014, Izmir, Turcia, 8-10 May 2014 - poster;
7. 1st International Medical Conference "Environment and Public Health" MEDENV 2014, Constanța, Romania, 12-14 September 2014 - poster;
8. BSB Net-Eco-"Deltas and Wetlands", 15-17 September 2014, Tulcea, Romania - oral presentation;
9. Autumn Scientific Session of the Romanian Scientists Academy, Constanța, Romania, 19-20 September 2014 - oral presentation;
10. FABA 2014 International Symposium on Fisheries and Aquatic Sciences, Trabzon, Turkey, 25-27 September 2014 - poster;
11. The 24th Symposium "DELTAS and WETLANDS", 19-21 May 2015, Tulcea, Romania - oral presentation;
12. International U.A.B.- B.EN.A. Conference Environmental Engineering and Sustainable Development, Alba Iulia, Romania, 28-30 May 2015 - oral presentation;
13. 1st International Conference on Sea and Coastal Development in the Frame of Sustanability, MACODESU 2015, Trabzon, Turkey, 18-20 September 2015 - oral presentation;
14. Autumn Scientific Session of the Romanian Scientists Academy 2015, Iași, Romania, 24-26 September 2015 - oral presentation;
15. 1st International Conference on "Ecology and Protection of Marine and Freshwater Environments - ECOPROWATER 2015", Viterbo, Italia, 1-3 October 2015 - oral presentation;
16. International Symposium PROTECTION OF THE BLACK SEA ECOSYSTEM AND SUSTAINABLE MANAGEMENT OF MARITIME ACTIVITIES - PROMARE 2015, 7th Edition, Constanța, Romania, 29-31 October 2015 - oral presentation;
17. Autumn Scientific Session of the Romanian Scientists Academy - Constanța Branch, Constanța, Romania, 21 November 2015, with the paper "Heavy Metal Bioaccujulation in Marine Living Organisms of the Romanian Black Sea Coast" - Magda Nenciu, Andra Oros, Daniela Roșioru, Natalia Roșoiu - oral presentation;
18. Closing Meeting of the Project CoCoNet - A Coast to Coast NETwork of protected areas: from the shore to the deep sea, Salento University, Lecce, Italy, 1-2 December 2015 - Young Scientists Section, with the paper: "Preliminary Genetic Analyses of *Hippocampus guttulatus* (Cuvier, 1829), charismatic species of Romanian MPAs" - oral presentation.

AWARDS

1st Prize for the *Best Poster* within the International Conference "Environment and Public Health" - MED ENV 2014, Constanța, Romania, 12-14 September 2014, with the paper "Heavy Metal Bioaccumulation Indicators in Seahorse Tissue", **NENCIU MAGDA**, OROS ANDRA, ROȘIORU DANIELA, GALAȚCHI MĂDĂLINA, ROȘOIU NATALIA (2014);

Recognition for *Best Oral Presentation* within the International Conference U.A.B.- B.EN.A. Conference Environmental Engineering and Sustainable Development, Alba Iulia, Romania, May 28-30th, 2015, with the paper "Overview of Pollutants Identified in Long-Snouted Seahorse (*Hippocampus guttulatus*, Cuvier 1829) Samples from the Romanian Black Sea Coast", **NENCIU MAGDA-IOANA**, OROS ANDRA, COATU VALENTINA, ȚIGĂNUȘ DANIELA, ROȘIORU DANIELA, ROȘOIU NATALIA (2015);

3rd Prize for *Best Oral Presentation* în cadrul Simpozionului Internațional PROTECTION OF THE BLACK SEA ECOSYSTEM AND SUSTAINABLE MANAGEMENT OF MARITIME ACTIVITIES - PROMARE 2015, 7th Edition, Constanța, România, 29-31 October 2015, with the paper "Preliminary Genetic Analyses for the Species *Hippocampus guttulatus* (Cuvier, 1829) in the Romanian Black Sea", **MAGDA-IOANA NENCIU**, DANIELA ROȘIORU, TANIA ZAHARIA, DALIA ONARĂ, ELENA TAFLAN, DANIELA HOLOȘTENCO, NATALIA ROȘOIU (2015).

PARTICIPATION IN RESEARCH CONTRACTS

1. **PN 09320207 Obținerea informațiilor actualizate necesare extinderii rețelei ecologice europene NATURA 2000 (arii speciale de conservare) în zona marină românească** (coordonator INCDM „Grigore Antipa“);

2. **PN II - Capacități, Modul III: Intelligent Sensing and Information Processing Technology for Fish Product during Cold Chain Management, Contract nr. 623/01.01.2013** (bilaterala România - China), beneficiar UEFISCDI București, coordonator Universitatea „Dunărea de Jos“ Galați;

3. **PNII - Parteneriate PCCA 2013**, direcția de cercetare 5: **Sistem informatic pentru trasabilitatea produselor pescărești bazat pe tehnologia cloud computing** (TraSiPesc), coordonator Universitatea „Dunărea de Jos“ din Galați, contract nr. 167/19.08.2014;

4. **Crearea unei metodologii de utilizare a dispersanților în Marea Neagră, care să poată constitui baza elaborării unui cadru legislativ privind utilizarea dispersanților în Marea Neagră**, contract nr. ROVOCON13-011/28.02.2013, beneficiar ExxonMobil Exploration and Production Romania Limited Nassau (Bahamas), sucursala București (coordonator INCDM „Grigore Antipa“);

5. **Asistență pentru obținerea acordurilor de mediu pentru perimetrele EX 27 - Muridava și EX 28 - Est Cobălcescu, Craiova S, Craiova N**, Contract de consultanță nr. 18/07.01.2013, beneficiar MELROSE RESOURCES ROMANIA B.V. AMSTERDAM (PETROCELTIC SRL), sucursala București (coordonator INCDM „Grigore Antipa“);

6. **Contract de servicii pentru parcurgerea procedurii de obținere a acordului de mediu și a avizului de gospodărire a apelor**, Contract de servicii ROVOCON12-016 din data de 19.06.2012, beneficiar ExxonMobil Exploration and Production Romania Limited Nassau (Bahamas), sucursala București (coordonator INCDM „Grigore Antipa“);

7. **Servicii pentru Monitorizarea stării de conservare a speciilor marine și a habitatelor costiere și marine de interes comunitar din România**, Contract nr. 1208/30.08.2012 (INTEGRA III), Anexa nr. 1 (coordonator INCDM „Grigore Antipa“);

8. **„Studiul privind managementul integrat al zonei costiere”** în cadrul proiectului „Îmbunătăţirea managementului integrat al zonei costiere în regiunea Mării Negre, ICZM, contract nr. 84/28.08.2014, beneficiar ABADL Constanța (coordonator INCDM „Grigore Antipa“);

9. **Servicii de obţinere a acordului de mediu pentru sonde offshore**, Contract nr. 99001716/01.07.2014, beneficiar OMV Petrom SA (coordonator INCDM „Grigore Antipa“);

10. **CE/PC7-OCEAN-2011: Policy-oriented Marine Environmental Research for the Southern European Seas (PERSEUS),** Grant Agreement no. 287600/21.12.2011 (INCDM „Grigore Antipa“ partener);

11. CE/PC7 - OCEAN-2011: **A Coast to Coast NETwork of protected areas:from the shore to the deep sea (CoCoNET)**, Grant Agreement no. 287844 (INCDM „Grigore Antipa“ partener);

12. **DG Environment: Integrated regional monitoring implementation strategy in the South European Seas Grant Agreement 07.0335/2013/659540/SUB/C2 IRIS-SES** (INCDM „Grigore Antipa“ partener);

13. **CE/PC: Co-creating ecosystem-based fisheries management solutions MARE FRAME**, Grant Agreement PC7 KBBE:2013-7- single-stage No. 613571 (INCDM „Grigore Antipa“ partener).