THE STUDY OF SOME PROTEOLYTIC ENZYMES FROM *PALAEMON ELEGANS*

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ABSTRACT

The paper presents the activity of pepsin, trypsin and chymotrypsin of *Palaemon elegans Ratke 1837* from Black Sea in experimental conditions.

The experiment had in view the activity evolution of these proteolytic enzymes in correlation with individuals density and food regime.

It were used juvenile animals obtained by natural – controlled reproduction at 4 weeks age and all parameters have been determined after every 2 weeks, during 6 weeks.

Also, were analysed the proteins, glucides and lipids content, dry substance, organic substance, mineral residue, growth and survive rate of this marine organism.

The compared results with the same animal from natural marine environment grown in natural conditions showed that *Palaemon elegans* is influenced by density and type of food.

The data are usefully for mariculture system and also in ecological marine biochemistry.

KEY WORDS: trypsin, chymotrypsin, pepsin, biochemical parameters, experimental conditions, *Palaemon elegans*,

INTRODUCTION

The ecological studies showed that the protease activities (pepsin, trypsin, chymotrypsin) are firstly correlated with food quality and quantity. They are in inverse relation. When the quantity of food is small, the protease activities are high and when the quantity of food is large, the protease activities are low. This kind of relation is more evident for organisms during the growth process (LE GAL, 1988).

The experiment aims were to get some preliminary data regarding proteases (trypsin, chymotrypsin and pepsin) reaction to the different kind of food and individual density, in case of a marine invertebrate organism being in a growth process.

The elegant shrimp *Palaemon elegans* Ratke, 1837(GOMOIU, SKOLKA, 1998), one of the 12 species of Black Sea shrimp, is a mass bottom crustacean living in shallow waters. It is one of the main food resources for gobies and other benthic fish (ZAITSEV, MAMAIEV, 1997).

P. elegans is a crustacean, a rocky shrimp, everywhere found at the Romanian littoral, on the sandy bottoms, which are covered by algae. Is eurihaline, euribionte specie and forms dense populations even at dam rocks of Sulina. It has a nocturnal activity. During the storms is spread to the off shore or is hidden under stones, so is rarely thrown out on the shore together with other crustaceans. Supports the winter better than *P. adspersus* and disappears at 0-1^{o}C.

Are not influenced by high water turbidity. It lives in agglomerations, 200-300 individuals/square meter on the stony walls, in vertical position. *P. elegans* has a carnivorous regime, eating residue animals.

The breeding is in May- September period, having yellow - green eggs.

P. elegans is also found in Atlantic Ocean (Spain area) and Adriatic Sea (BACESCU,1967).

MATERIALS AND METHODS

The experiment was organized as following:

EXPERIMENT: 6 weeks

- Shrimps - juvenile animals obtained by natural – controlled reproduction
- Control: shrimps from natural marine environment, feed with natural food
- Initial age of shrimps: 4 weeks
- Final age of shrimps: 10 weeks
- Data were achieved after every 2 weeks: 0 week, 2 weeks, 4 weeks, 6 weeks.
- Daily feed: 10 % from total weight of individuals
- Daily temperature of marine water in basins: 18.8 ^{o}C -22.7 ^{o}C

Variants	Control	Basin 1	Basin 2	Basin 3	Basin 4	Basin 5	Basin 6
Density	500 ex/m^3	4000 ex/m^3	2000 ex/m^3	1000 ex/m^3	4000 ex/m^3	2000 ex/m^3	1000 ex/m^3
Food regime	natural	marine small fish	marine small fish	marine small fish	90% marine small fish 10% Tetra prima	90% marine small fish 10% Tetra prima	90% marine small fish 10% Tetra prima

FOOD COMPOSITION

- Marine small fish: 16.53 % proteins, 10.62 % lipids, 0.7 % glucides
- Tetra prima (food for tropical fish, made in Germany):
  - 47.6 % proteins, 6.5 % lipids, 2 % cellulose, 10,5 % ash, 6% humidity,
  - ingredients: fish, extracts from vegetal proteins, cereals, mollusks, crustaceans, yeast, minerals, oils and fats, colorants

The analyses of proteolytic enzymes and proteins content were made in total proteic extracts.

The extraction ratio was 1g tissue for 10 ml bidistilled water. The resulted homogenates were preserved for 1 hour at +4C^{o} for completion of protein extraction and thereafter were centrifuged at 4000 x g for 15 minutes. The resulted supernatants in this way were considered as total protein extracts (MIRCEA *et al*.,2005; ROSOIU, SERBAN,2002).

Determination of protein concentration in the total protein extracts was carried out by method of LOWRY *et al.* (IORDACHESCU, DUMITRU,1988), glucides content by method of DUBOIS *et al*. (RAZET *et al.* 1996) and lipids content by method of MARSH and WEINSTEIN (RAZET *et al.* 1996).

Dry substance, organic substance and mineral residue were analyzed in entirely organisms by a method cited by MANESCU (1978).

Proteolytic enzymes have been analyzed with methods cited by ROSOIU, SERBAN (2002); ROSOIU *et al.*(1981).

Pepsin activity was determined by the Anson method, using hemoglobin at pH=2.0 as a substrate; trypsin and chymotrypsin activities were also determined by the Anson method, using adulterated hemoglobin at pH =7.2 for trypsin and pH= 8 for chymotrypsin. The

enzymatic activity was expressed in nmoles tyrosine/mg protein /minute at 37°C (ROSOIU, SERBAN, 1992).

RESULTS AND DISCUSSIONS

The experiment has proposed to analyze the specific activity of proteolytic enzymes: trypsin, chymotrypsin and pepsin, of shrimp *P. elegans* in different individuals density and food regime. Also have been determined biochemical parameters: proteins, glucides and lipids content, dry substance, organic substance, mineral residue.

Important was to be taken into account the survival and growth rate of shrimps, which are influenced by density and food regime. These last parameters are significant also for mariculture system.

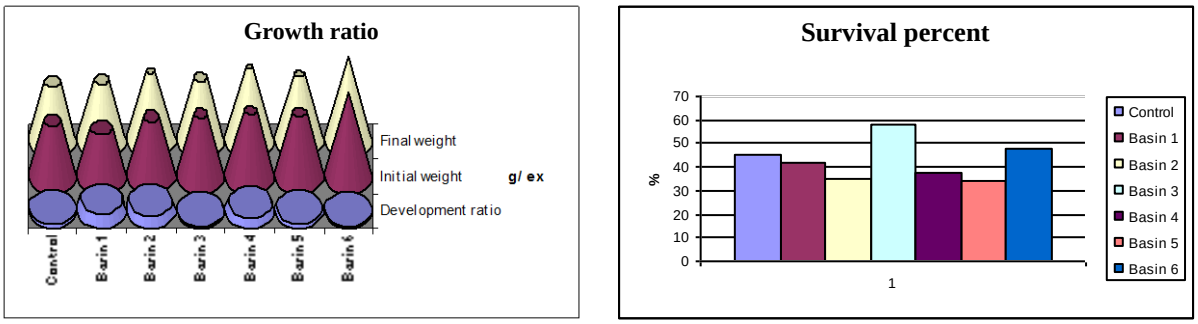

Fig1- Growth ratio and survival percent of shrimps

For both food regime of experiment were achieved the following results (Fig.1):

Growth spore was maximum in basins with 4000 ex/m^3 and minimum in basins with 1000 ex/m^3

Survival rate was maximum in basins with 1000 ex/m^3 and minimum for 2000 ex/m^3

Closer by Control as survival rate was Basin 1 (-3%) and Basin 6 (+3%).

The maximum survival of shrimps was for 1000 ex/m^3 and the growth spore was minimum.

Regarding proteases specific activities, at 0 week in all situations, the registered values have been smaller than Control. Generally, they were situated at the middle of values for all period of observation. (Fig.2).

For the others parameters: proteins, glucides, lipids (Fig.3), dry substance, organic substance, mineral residue (Fig.4), Control recorded the highest values, and at 0 week all values were at the middle of the interval.

After 2 weeks (Fig.2), trypsin specific activity was low in Basin1 (4.26 nmoles Tyr/mg protein /minute at 37°C) and Basin 4 (4.43 nmoles Tyr/mg protein /minute at 37°C), basins with 1000 ex/m^3 density of individuals, and high in Basin 3 (19.7 nmoles Tyr/mg protein /minute at 37°C and Basin 5 (8.32 nmoles Tyr/mg protein /minute at 37°C), basins with 2000 ex/m^3 (Fig. 2).

Chymotrypsin activity (Fig.2) was low in Basin 1(2.23 nmoles Tyr/mg protein /minute at 37°C) and Basin 5 (0.11 nmoles Tyr/mg protein /minute at 37°C), and high in Basin 2 (11.48 nmoles Tyr/mg protein /minute at 37°C) and Basin 6 (2.4 nmoles Tyr/mg protein /minute at 37°C).

Pepsin activity (Fig.2) was low in Basin 3 (3.63 nmoles Tyr/mg protein /minute at 37°C) and Basin 5 (12.6 nmoles Tyr/mg protein /minute at 37°C), high in Basin 2 (18.12 nmoles Tyr/mg protein /minute at 37°C) and Basin 6 (18.99 nmoles Tyr/mg protein /minute at 37°C).

Proteins, glucides, and lipids (Fig.3) had the same evolution for the basins with fish food regime, the achieved data were minimum for Basin 3 (proteins 95.77 μg/mg w.w., glucides 7.17 μg/mg w.w., lipids 62.11 μg/mg w.w.) and maximum for Basin 1 (proteins 183.79 μg/mg w.w., glucides 14.84 μg/mg w.w., lipids 98.21 μg/mg w.w.). For basins with

mixed food, only glucides and lipids had the same tendency, minimum for Basin 4 (glucides 3.79 μg/mg w.w., lipids 49.72 μg/mg w.w.) and maximum for Basin 6 (glucides 10.02 μg/mg w.w., lipids 109.39 μg/mg w.w.).

In case of dry substance, organic substance and mineral residue (Fig. 4), for basins with fish regime the results were the same, minimum in Basin 2 (dry subst. 26.48 %, organic subst.23.1 %, mineral residue 3.38 %) and maximum in Basin1 (dry subst.31.36%, organic subst. 27.33 %) and Basin 3 (mineral residue 4.6 %). For the rest of basins minimum was in basin 6 (dry subst. 28.18 %, organic subst. 25.15%, mineral residue 3.03 %) and maximum in Basin 4 (dry subst. 29.64 %, organic subst.26.01%, except mineral residue with min in Basin 5, 4.3 %).

After 4 weeks (Fig.2), all proteolytic enzymes registered minimum in Basin1 (trypsin-2.8 nmoles Tyr/mg protein /minute at 37^{o}C, chymotrypsin- 0.35 nmoles Tyr/mg protein /minute at 37^{o}C, pepsin- 4.13 nmoles Tyr/mg protein /minute at 37^{o}C) and maximum in Basin 3 (trypsin-24.87 nmoles Tyr/mg protein /minute at 37^{o}C, chymotrypsin-12.2 nmoles Tyr/mg protein /minute at 37^{o}C, pepsin -17.67 nmoles Tyr/mg protein /minute at 37^{o}C) in situation of food based on fish. In case of mixed food, trypsin and chymotrypsin had maximum in Basin 6 (trypsin-24.18 nmoles Tyr/mg protein /minute at 37^{o}C, chymotrypsin 15.82 nmoles Tyr/mg protein /minute at 37^{o}C), and pepsin minimum (25.61 nmoles Tyr/mg protein /minute at 37^{o}C).

Proteins, glucides and lipids (Fig.3) for basins feed with marine fish showed almost the same behavior, minimum in Basin 2 (proteins 142.67 μg/mg w.w, glucides 4.6 μg/mg w.w, lipides 91.13 μg/mg w.w) and maximum in Basin1 (proteins 162.86 μg/mg w.w , glucides 10.01 μg/mg w.w, except lipids 127.18 μg/mg w.w -min in Basin 3). For the basins with mixed food was not a homogeneity.

For the last biochemical parameters (Fig. 4), achieved data presented an uniformity in variation as following: minimum in Basin 3 (dry subst. 28.87 %, organic subst. 25.02 %, mineral residue 3.85 %), Basin 6 (dry subst 29.52 %, organic subst 25.61 %, mineral residue 3.91 %) and maximum in Basin 1 (dry subst. 34.25 %, organic subst. 30.00 %, mineral residue 3.91 %) and Basin 5 (dry subst 35.42 %, organic subst 29.73 %, except mineral residue 4.46 % – max in Basin 2).

At the end of experiment, after 6 weeks, all studied enzymes (Fig. 2) showed the same evolution for all variants, minimum in Basin 3 (trypsin-20.86 nmoles Tyr/mg protein /minute at 37^{o}C, chymotrypsin 12.21 nmoles Tyr/mg protein /minute at 37^{o}C, pepsin 4.52 nmoles Tyr/mg protein /minute at 37^{o}C) Basin 4 (trypsin-16.95 nmoles Tyr/mg protein /minute at 37^{o}C , chymotrypsin-2.3 nmoles Tyr/mg protein /minute at 37^{o}C, pepsin-1.01 nmoles Tyr/mg protein /minute at 37^{o}C) and maximum in Basin 2 (trypsin- 56.55 nmoles Tyr/mg protein /minute at 37^{o}C, chymotrypsin-21.22 nmoles Tyr/mg protein /minute at 37^{o}C, pepsin 22.63, pepsin) Basin 6 (trypsin 33.73 nmoles Tyr/mg protein /minute at 37^{o}C, chymotrypsin- 28.22 nmoles Tyr/mg protein /minute at 37^{o}C, except pepsin-14.82 nmoles Tyr/mg protein /minute at 37^{o}C - max in Basin1).

Proteins, glucides, lipids (Fig. 3) content had a variability regarding distribution in basines for both type of food regime.

Also, dry substance, organic substance, mineral residue (Fig. 4) recorded minimum and maximum values for the same basins: min-Basin1 (dry subst. 25.38 %, organic subst. 21.26 %, exception, mineral residue with maximum in Basin1- 4.12 %.), Basin 5 (dry subst. 25.71 %, organic subst. 23.03 %, mineral residue 2.68 %), max- Basin 3 (dry subst. 29.33 %, organic subst. 25.84%, except mineral residue- max in Basin1- 4.12 %) Basin 6 (dry subst. 31.29 %, organic subst. 26.78 %, mineral residue 4.51 %).

By comparison among basins, in the case of the same type of food, the trypsin specific activity has increased progressively in all situations during the entirely period of experiment (except Basin 3, in which decrease after 6 weeks). For basins with mixed food, the trypsin activity values were smaller than in basins with marine small fish as food (except Basin 6 after 6 weeks).

In basins feed with fish, chymotrypsin specific activity increased progressively in basin 1 and basin 2 (decreased in Basin 3) and for mixed food, enzyme showed a progressively increasing for all experiment.

In case of basins 1,2,3, trypsin and chymotrypsin had the same sense of evolution during the experiment. The values of specific activity in basins with mixed food were lower than in basins feed with fish, except Basin 6 in which was higher after 4 and 6 weeks.

Pepsin specific activity in case of basins with fish food, after 2 and 4 weeks decreased and after 6 weeks increased progressively. In situation of basins with mixed food, enzyme manifested an opposite behavior to the previous situation.

Regarding individuals density, trypsin and chymotrypsin specific activity recorded the low values in basins with 1000 ex/m^3 (Basin 3, Basin 6) and high values in basins with 2000 ex/m^3 (Basin2, Basin5).

Pepsin specific activity in case of basins with fish food, for 1000 ex/m^3, Basin 3 (except week 4), the values were low, and for mixed food, the values were high for 1000 ex/m^3, Basin 6.

For all studied enzymes, in basins feed with marine small fish with 1000 ex/m^3 were achieved low values for specific activities.

At the end of experiment, for Basin 3 with 1000 ex/m^3 feed with marine small fish proteins and lipids content decreased and glucides content increased. For the Basin 6 with mixed food the biochemical parameters manifested an opposite distribution.

Dry and organic substance for basin with 1000 ex/m^3, after 4 weeks the values increased and after 6 weeks decrease in both type of food. The same evolution showed mineral residue for Basin 6 and opposite on for Basin 3.

Concerning the survival of shrimps, the maximum was achieved for 1000 ex/m^3 and the growth spore was minimum.

CONCLUSIONS

- For all situations, in case of the basins feed with mixed food, the achieved specific activity of proteolytic enzymes was lower than in basins feed with marine small fish, even the mixed food content a high quantity of proteins.
- By comparison with other marine organism like *Mytillus galloprovincialis* from natural marine environment, the values of proteolytic activity have been lower (twice less for trypsin, three time less for chymotrypsin, and ten times less for pepsin) [6].
- In basins with low density of individuals, the survival rate is maximum and growth rate is minimum. It seems that density of individuals is very important first for shrimp survive, even the growth rate is low.
- Is possible to appears a competition for food, because in basins with high density of shrimps, specific activity of trypsin, chymotrypsin and pepsin showed high values.
- Marine small fish contains smaller quantity of protein than Tetra prima from mixed food, but at the low density of shrimps (1000 ex/m^3), the total proteins content in shrimps is

higher and proteolytic enzyme activities are lower. Shrimps accumulated more proteins from fish, but the proteolytic enzymes are not very active.

- The final conclusion is that the food used in experiment was not the proper one in this system.

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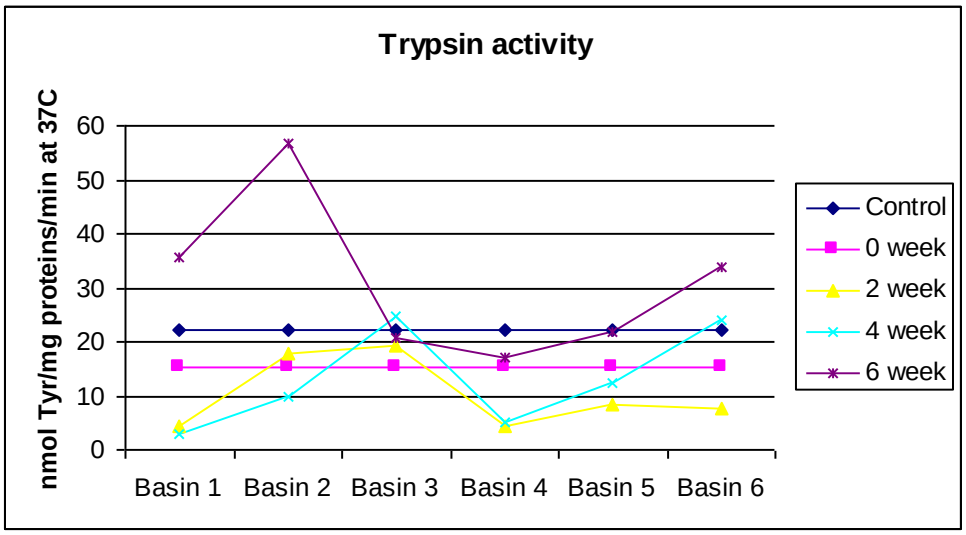
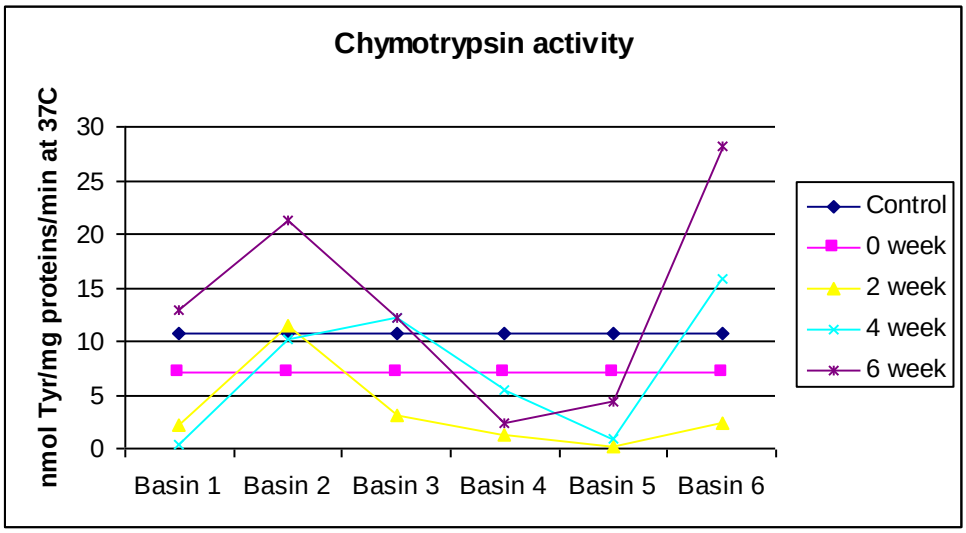
Fig. 2.- *Palaemon elegans* - specific activity of trypsin, chymotrypsin and pepsin





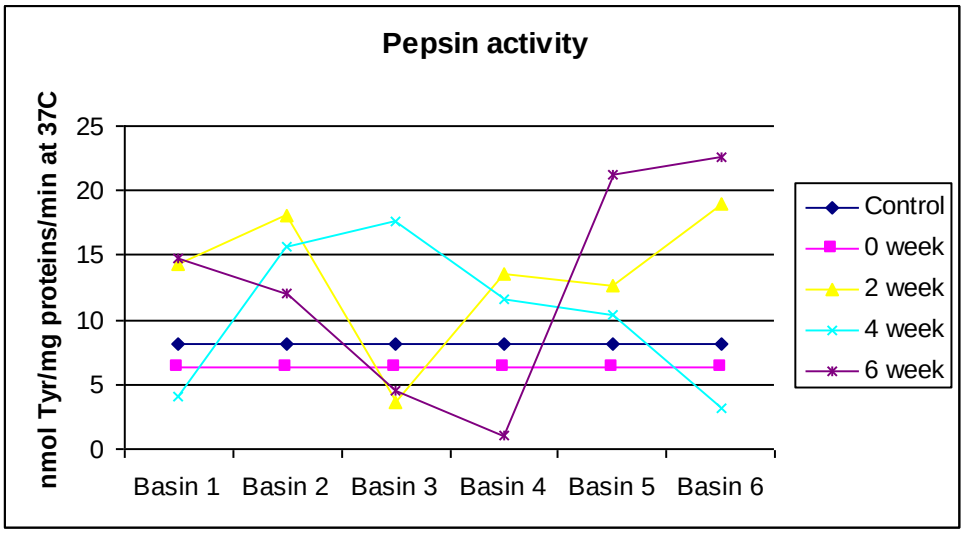
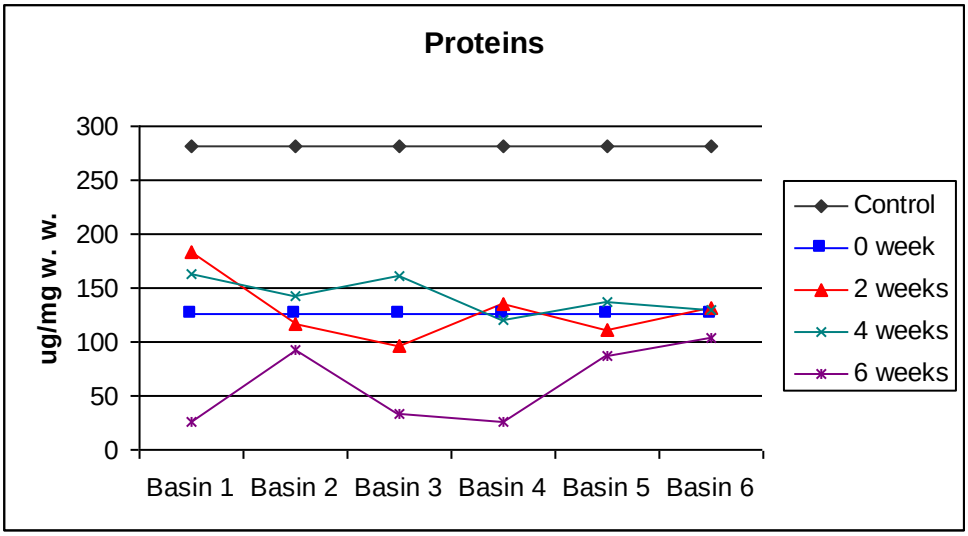
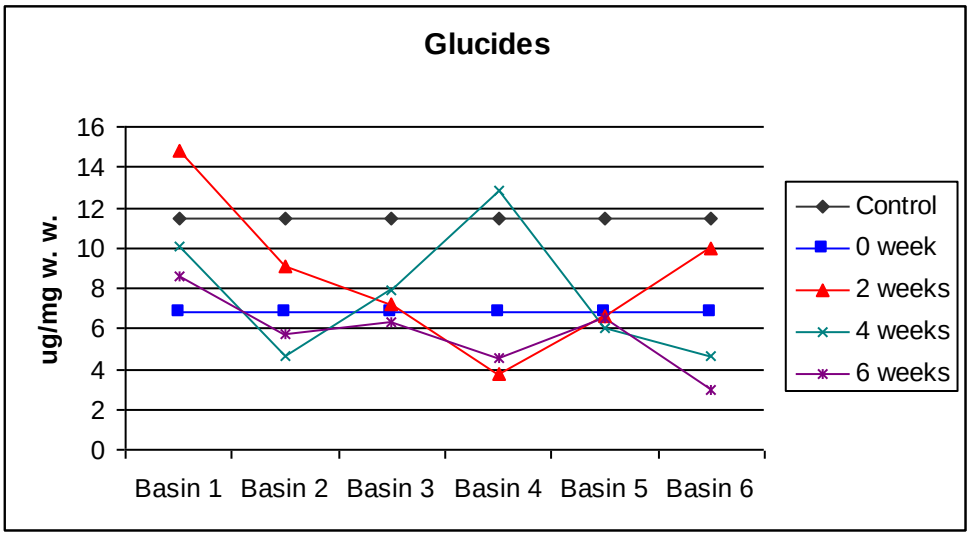


Fig. 3.- *Palaemon elegans* – proteins, glucides, lipids content





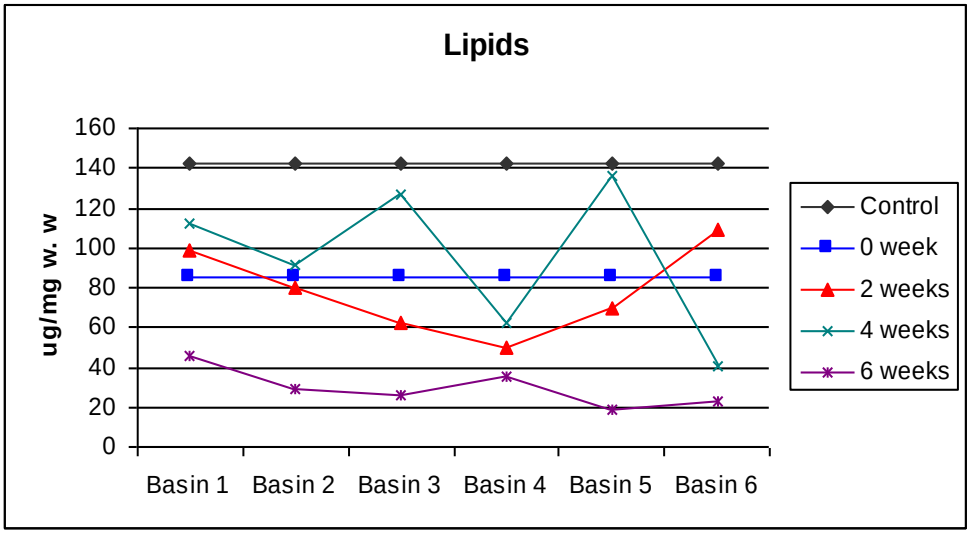


Fig. 4 - *Palaemon elegans* – dry substance, organic substance, mineral residue content