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Biochemical composition of *Mnemiopsis leidyi* in the southern Caspian Sea

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Abstract

The biochemical composition of the invasive ctenophore, *Mnemiopsis leidyi* from the southern Caspian Sea was analysed in terms of total fractions of protein, lipid, ninhydrin–positive substances (NPS), polysaccharides (PS) and low molecular- weight carbohydrates (LMC). The sum of organic substances per gram dry weight was 84.2 mg g^{-1}. Protein was the major organic fraction followed by lipid and NPS. The NPS/ protein and LNC/PS ratio were 0.45 and 0.50 respectively. Starvation of *M. leidyi* for a 5-day period resulted in a 32.7% reduction of total organic content. These data will be important in evaluating the feeding condition of *M. leidyi* specimens from the field.

Introduction

The ecosystem of the Caspian Sea has been faced with many several problems of anthropogenic origin during last decades, such as pollution, eutrophication and illegal fishing. However, the impact caused by the recent invader *Mnemiopsis leidyi* is uncomparable at magnitude to these previous problems.

This voracious plankton consumer ctenophore *Mnemiopsis leidyi* was first transported to the Black Sea via ballast water in the 1980s causing sharp decreases on the valuable planktivorous fish stocks. This ctenophore, with its high growth and fecundity rates and capacity for self-fertilization, is able to sudden population outbursts and hence can severely deplete zooplankton resources, which include mainly the copepods (Reeve and Walter 1978).

Since its transport to the Caspian Sea at the end of 1990s, this invaluable ecosystem already seems to be even worse affected than in the Black Sea due to greater sensitivity of this closed basin (Kideys 2002; Kideys et al. 2004). Very significant decreases in the quantity of mesozooplankton in the northern (Shiganova et al. 2001) and southern Caspian Sea (A. Roohi, unpublished data) were reported. Most notable decreases were observed in the pelagic (mainly kilka *Clupeonella* spp.) fishery of all Caspian countries: almost a 50 % decrease in the kilka catches of both Iranian and Azerbaijan fisheries has occurred during 1999 and 2001 that resulted in great economic losses (Kideys et al. 2001a, b). By 2003, fishing on kilka almost stopped due to very low catch in the southern Caspian (personal observation). Russian catches were also reported to decrease remarkably (Shiganova et al. 2001). There have been also a few mass mortality events for the endemic Caspian seal (*Phoca caspica*) which feeds mainly on these small fish.

Monitoring the condition of ctenophores in the sea coupled with studying their physiological features is needed to quantify the ecological impact of these species on the ecosystem. Therefore investigation of major biochemical components of *M. leidyi* may provide insights to the feeding conditions in this sensitive ecosystem. A few studies have been published on chemical composition and metabolism of ctenophores from other regions (Hoeger 1983, Morris et al. 1983) including the Black Sea (Anninsky et al. 1998 and 2004) but not from the Caspian Sea. In the present paper, the major biochemical fractions of *M. leidyi* were investigated and compared with the other ctenophore species. Additionally an experiment was undertaken in order to investigate changes in biochemical composition of *M. leidyi* with respect to starvation to evaluate the feeding condition of ctenophores in the field for the future studies.

Table 1. Biochemical composition of field (N=95) and 5 days starved (N=88) samples of *Mnemiopsis leidyi*. NPS : Ninhydrine-Positive Substances, PS: Polysaccharides, LMC = Low Molecular – Weight Carbohydrates.

Composition (as mg g^{-1} dry weight)	Field samples	5 days starved samples	% decrease
Protein	31.3 ±4.7	27.1 ± 3.2	13.4
Lipid	21.41 ± 3.20	11.5 ± 2.1	46.3
NPS	14.2 ± 3.2	7.1 ± 3.2	50.0
PS	10.0 ± 1.0	7.4 ± 0.8	26.0
LMC	4.9 ± 0.7	3.7 ± 0.5	24.5
Total biochemical fraction	84.2	56.7	32.7
Lipid/protein	0.77	0.42	
NPS/Protein	0.45	0.26	
LMC/PS	0.50	0.50	

Material and methods

Mnemiopsis leidyi were sampled from the southern Caspian Sea on 12 May 2002 using 500 (m mesh plankton net. The ctenophores were transferred to laboratory within 30 minutes of collection.

95 undamaged individuals of average size (10–20 mm in total length) were placed in plastic bags after draining the excess water in a 500 m plastic tray, and then frozen at −20 °C for the estimation of biochemical content of non-starved field samples. Meantime, an experiment was run for estimating biochemical content of 5-day starved ctenophores. A total of 88 specimens composed of average size *M. leidyi* were kept in a 20-liter aquarium for this experiment. The aquarium was equipped with aeration under room temperature. The water of aquarium was changed every day with freshly filtered seawater. At the end of 5th day, they were also placed in the plastic bags and kept at −20 °C until analysis. All samples were later were finely ground and lyophilized prior to chemical analyses.

Samples were analysed for their total fraction of protein, lipid, and polysaccharides (PS) as the major chemical compounds and their major degradation products (i.e. free amino-acids known as ninhydrin – positive substances, NPS and low molecular weight carbohydrates, LMC). Protein was measured by method of Lowry et al. (1951). For this, portions of samples were digested for 12 h in 0.1 m NaOH at 50 °C and then triplicate sub samples were taken for assay. For the analyses of NPS which are assumed to be mainly amino acids and low molecular weight peptides, the ninhydrin method were used according to Troll & Cannan (1953). Carbohydrate fractions (PS and LMC) were analysed using phenol-sulfuric acid reagent according to Dubois et al. (1956). Lipid was extracted from frozen samples with methanol/chloroform (Barnes and Blackstock 1973). All analyses were carried out in triplicate and the standard deviation (SD) was always less them 8% of the mean for the entire fraction.

Results and discussion

The biochemical composition of *Mnemiopsis leidyi* of the Caspian Sea in terms of dry weight is shown in Table 1. It is worthy to note that water content is extremely high in this species similar to other ctenophores or cnidarians. The dry matter content of *M. leidyi* from the northern Caspian with a salinity of 5.7 was reported to be on average only 0.78% (range 0.61–0.97%) of total wet weight (Shiganova et al. 2001). Unfortunately no dry matter content measurement was made in our study but it is well known that salinity of the ambient water in one of the most important factors affecting this parameter. Dry matter content was measured as 2.2% of total wet weight in the Black Sea having a salinity of 18 (Finenko et al. 1995) and about 1.3% in the Azov Sea at a salinity of 11-12 (Shiganova et al. 2001). The salinity in the southern Caspian (12-12.5) is very close to that in the latter and therefore, for comparison purposes based on wet weight, we could use the value obtained for the Azov Sea.

The total organic matter content in *M. leidyi* was overall low with an average value of 84.2 mg g^{-1}

Table 2. Composition of ctenophores from the North Sea (Hoeger 1983) and Black Sea (the last column; Anninsky et al. 2004).

Composition (as mg g^{-1} dry weight)	*Beroe gracilis*		*Pleurobrachia pileus*		*Bolinopsis infundibulum*		*Mnemiopsis leidyi*
Dry weight as % of wet weight	3.9	0.3	4.0	0.1	3.8	0.2	2.2
Protein	48.6	6.8	30.6	6.8	13.1		41.8
Lipid	42.4	7.8	17	3.4	4.97		4.5
NPS	28.8	5.2	9.1	3.0	4.16		1.8
PS	6.3	0.4	3.7	0.72	0.90		3.2*
LMC	2.5	0.4	0.69	0.21	0.14		*
Total biochemical fraction	128.4	15.8	61.3	12.9	23.5		51.8
Lipid/protein	0.87		0.54		0.37		0.1
NPS/Protein	0.59		0.29		0.33		0.04
LMC/PS	0.41		0.19		0.14		

*PS and LMC together.

Table 3. Nitrogen and carbon contents of some ctenophores

Species	Nitrogen % of dry weight	Carbon % of dry weight	Reference
Beroe gracilis	1.2	7.2	Hoeger 1983
Beroe cucumis & ovata	0.9–3.3		Curl 1962
Beroe cucumis		8–12	Reeve et al. 1978
Pleurobrachia pileus	0.71	3.4	Hoeger 1983
Pleurobrachia bachei	0.87–1.3		Reeve et al 1978
Pleurobrachia bachei		3.3–4.8	Reeve 1980
Bolinopsis infundibulum	0.33	1.5	Hoeger 1983
Bolinopsis infundibulum	0.2–0.6	1.2–2	Kremer, pers. comm.
Mnemiopsis leidyi	0.72	4.6	This study

DW for the field specimens (Table 1), however, this value was higher than *M. leidyi* of the Black Sea (41.8 mg g^{-1} DW; Anninsky et al. 2004) and some other ctenophore species from the North Sea (Hoeger 1983; see Table 2). This must be due to higher salt content in dry matter in these studies compared to the Caspian specimens. Excluding our study, within the ctenophore species, the highest organic matter was present in *Beroe gracilis* which is a predatory ctenophore feeding on other ctenophores most notably on *Bolinopsis infundibulum*. The latter presented the lowest organic matter content (23.5 mg g^{-1} DW; Table 2).

In the present study, protein was the major fraction (31.3 mg g^{-1} DW), followed by lipid and NPS similar to other studies with ctenophores. Carbohydrate consisted of the smallest part of the dry material. The most distinct differences in the balance of biochemical components among ctenophores existed in the lipid/protein ratio: our value of 0.77 was much higher than that found from the Black Sea (0.11) for the same species. The reason for such discrepancy could be related with the nutritional state of ctenophores between the two regions. After analysing the lipid fraction of *Beroe cucumis* and *Pleurobrachia bachei*, Lee (1974) found that the main fraction comprising about 70% of the total lipids was in the form of phospholipids which are structural lipids since they are mainly located in the cell membranes (Fox, 1972). Only small amounts (2–12%) of total lipids were storage compounds in the form of triglycerides and wax esters (Lee 1974). That is why Lee (1974) mentioned that ctenophores require constant feeding. However, the scarcity of any specific storage material in most ctenophores including Caspian *Mnemiopsis* may have the advantage that nutritional energy is immediately available for tissue growth and egg production and thus contributing to the rapid increase of ctenophore populations during bloom times both in the Caspian (S. Bagheri and A.E.

Kideys, unpublished data) and Black Seas (Finenko et al. 2003).

Another important parameter is the rate between NPS to protein and LMC to PS. The higher levels of these intermediary products may indicate a higher turnover rate of proteins and polysaccharides and point towards a higher basal metabolic rate in these ctenophores (Hoeger 1983). These parameters in both *Beroe gracilis* and *M. leidyi* was higher compared with the others. Anninsky et al. (1998) noted that the content of low molecular compounds such as free aminoacids and monoscaccarides is reduced more notably in the Black Sea *Mnemiopsis*.

The ash-free dry-weight was not used as a measure the organic weight in the gelatinous, water rich animals because erroneously high values were obtained with this methods as compared to calculations based on carbon and nitrogen analysis (Reeve and Baker 1975). Nitrogen and carbon contents are also important characteristics in several ecological studies including modeling. In this study, the nitrogen content of the biochemical fraction was tentatively calculated by dividing the combined value of protein and NPS by 6.25 (Mayzaud and Martin 1975), thus a mean value of 0.72% of dry weight was obtained for *M. leidyi* (Table 3). The percentages of nitrogen from several North Sea ctenophores are shown in Table 3. Carbon content calculation was carried out assuming carbon contents of protein and carbohydrates to be 53% and, 40%, respectively (Dyson 1978) and of lipid as 66% (Altman and Dittmer 1964). Carbon content of 4.6% of dry material was obtained for *M. leidyi* which is at moderate levels compared to other ctenophores (Table 3).

As expected, starvation caused decrease in body size of *M. leidyi*. The influence of 5 days starvation on the biochemical composition of dry material is shown in Table 1. The sum of organic material decrease was calculated as 32.7% during this period. As it was mentioned before, some planktonic organisms including *M. leidyi* require constant feeding therefore during starvation there is often a sharp decrease in body weight. During 5 days of starvation all high molecular fractions were catabolized but not at the similar rates. The highest decrease was in NPS and lipids. This is in agreement with the study of Hoeger (1983), who observed rapid consumption of lipid until 5 day in *Pleurobrachia pileus*. At the end of 8 days starvation all fractions were decreased to 40–25% of their initial composition being lowest for protein (which is also consumed significantly after 11 days) The degradation of the whole body tissue provided the energy to maintain the metabolism of ctenophores during starvation.

The scarcity of storage compounds and decreased organic matter content of structural biochemical compounds give rise to the low biomass levels observed in the southern Caspian during winter and spring (S. Bagheri and A.E. Kideys, unpublished data). This shows that under low feeding conditions, *M. leidyi* is weak to compete with native zooplankton; a fact that could be used in decimating the impact of this species in the Caspian ecosystem. The results of this study are thus important in understanding the ecological impact of *M. leidyi* on the Caspian ecosystem, both for the mitigation and for the modeling purposes.

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