Nutritional conditions of jellyfish revealed by nucleic acid determinations- a case study on *Mnemiopsis leidyi*

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Running Head: Gelatinous zooplankton nutrition

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1 **Abstract**

2 Recent increases in reported jellyfish blooms along coastal areas have raised

3 awareness on their ecological impacts on pelagic communities. Understanding their

4 nutritional state that determines dietary statuses, energy adequacy and food

5 inadequacy per capita in a population is mandatory to estimate their actual role and

6 potential threat in marine ecosystems. In this study RNA and DNA measurements

7 have been used to describe nutritional condition of the ctenophore *Mnemiopsis leidyi*

8 under feeding and starvation conditions. A non-linear increase in DNA and RNA

9 concentrations was observed in fed animals, whereas starved organisms represented a

10 linear decrease in both nucleic acid concentrations. The observed DNA increase was

11 not in accordance with the somatic growth and is likely attributed to sexual maturation

12 stimulated under good nutritional condition. Although the RNA: DNA ratio showed a

13 treatment effect, the same pattern of changes in fed and starved animals was observed.

14 In this case study, correspondence plots and related analyses support the conclusion

15 that nucleic acid determination on jellyfish has merit and is likely to provide

16 complementary information on nutritional state of this group.

17 Key words:

18 gelatinous zooplankton, Mnemiopsis leidyi, RNA: DNA, nutritional condition

19

Introduction

Due to a global increase in abundance of gelatinous zooplankton, their future ecological role became a matter of concern for marine ecologists (Condon et al. 2012; Jackson 2008). One of the current challenges of marine ecologists is to determine the "in situ" eco-physiological and nutritional state of gelatinous zooplankton to gain a better understanding of physiological state and trophic interactions of this group. RNA:DNA ratios are one of the most used proxy in marine ecology that have been applied successfully as indicators of nutritional condition, growth and trophic interactions in fish and marine invertebrate (Chicharo and Chicharo 2008 and references there in, Koop et al. 2011). Principally RNA content provides information about the metabolic activity of the organism and varies with age, size, life stage or abiotic conditions. The amount of DNA is constant under changing environmental situations in all somatic tissue cells and reflects the cell number (Chicharo and Chicharo 2008; Dortch et al. 1983). Thus, a higher RNA: A DNA ratio reflects a better nutritional condition of an organism (Clemmesen 1994).

Impact of gelatinous plankton and magnitude of their predatory potential became highly conspicuous when the comb jelly *Mnemiopsis leidyi*, was introduced into the Black Sea, presumably via ballast water tanks, leading to a massive development of this species (Shiganova et al. 2001). *M. leidyi* is known as a generalist predator due to its high clearance rate on different types of food, high reproductive potential (self-fertilizing hermaphrodite, several 100 eggs/ ind per day under optimal conditions), wide tolerance limits for salinity (2-38) and temperature (0-32°C) and also high tolerance for low oxygen concentration (Costello et al. 2012; Purcell et al. 2001).

To our knowledge there is still no proof if nucleic acid measurements would be applicable to gelatinous carnivores especially to *M. leidyi*. The purpose of this first

45 study was to determine whether RNA and DNA comprise a measurable portion of the

46 cellular mass in *M. leidyi* and how their concentrations vary as a result of starvation

47 and feeding traits.

48 **Method**

49 Individuals of *M. leidyi* were collected with "RV Polarfuchs" in the inner Kiel fjord

50 (54°19,6' N; 10° 9,2' E) by means of a plankton net (HydroBios WP3 , 1000 μm mesh

51 size and 80 cm diameter). The net was towed in a vertical profile over the whole water

52 column from 18 m depth resulting in 120 healthy *M. leidyi* of the same size (1 ± 0.2

53 cm) to be selected for the experiment. Our experimental design was composed of one

54 factor "nutritional condition" with two levels of (i) starvation and (ii) feeding. Due to

55 the handling problems and space limitation, 60 individuals per each treatment were

56 kept together in a 50 liter bucket that was filled with filtered see water (5μm). *M.*

57 *leidyi* in the "feeding" treatment received daily copepods (copepodites and adults of

58 *Acartia tonsa*), taken from our continuous standard culture, at a final concentration of

59 2 mg Carbon per *M. leidyi* per day over the experimental period. *M. leidyi* in the

60 "starvation" treatment did not receive any food. Salinity and temperature were kept

61 similar to ambient conditions in the Kiel Fjord (salinity 17; temperature 12°C). Five

62 individuals of *M. leidyi* from each bucket were sampled for DNA and RNA

63 measurements every 2 days over 14 days. To avoid possible bias by gut content,

64 sampled individuals were kept in filtered seawater for 1-2 hours to allow for gut

65 evacuation. After measurements of total length, sampled individuals were stored at -80

66 °C and freeze dried prior to the nucleic acid analysis. Dry weight ± 0.001 μg was

67 quantified. DNA and RNA content of all individuals was determined by fluorescence

68 technique described by Clemmesen 1993; Clemmesen 1988 and modified by Malzahn

69 et al. 2003 and Belchier et al. 2004 eliminating some purification steps.

70   Statistical analysis- Our initial data exploration was carried out following the protocol

71   described in (Zuur 2007; Zuur 2010) on response variables RNA, DNA and the ratio

72   (RNA: DNA) and explanatory variables namely length, dry weight, time (date) and

73   condition (treatment). Due to the experimental design one would expect a dependency

74   structure as the same bucket is repeatedly sampled over time, potentially causing a

75   'site' effect. The next step was thus to check for the "bucket" effect. The follow up

76   analysis (Pearson correlations), showed that there is no inherent correlation between

77   observations from different days ruling out a "site" effect. Pearson correlation

78   coefficients analysis however showed a co-linearity between length and dry weight.

79   This is expected as both variables indicate changes in growth. Additionally a negative

80   relationship between dry weight and date was detected. Therefore we removed length

81   and dry weight from the model and only considered date and treatment for the final

82   analysis using the following three models:

83   *RNA ~ (Date + Treatment)*

84   *DNA ~ (Date + Treatment)*

85   *RATIO ~ (Date + Treatment)*

86   We have applied a generalized additive model (GAM) on DNA due to non-linear

87   relationship between response and explanatory variables.   Since we detected

88   heterogeneity in the residuals obtained from the GAM model on RNA and the Ratio;

89   we applied a Generalized Additive Mixed Model (GAMMs, Woods 2006) on these

90   data sets. All statistical analyses were performed in the software R (Development Core

91   Team, 2011). Our model validation did not show any heterogeneity patterns, serious

92   deviation from normality, violation of independence (assessed by ACF plot) and

93   finally no indication of autocorrelation in any of the models performed.

94   **Results**

95   Starved *M. leidyi* reduced their dry weight significantly by approximately 80% of the

96   mean starting value over 14 days (from 3 to 0. 5 mg ind^{-1} in average, $F_{(1, 8)} = 54.1$,

97   P<0.01), whereas the body length decreased by approx. 50% from 13.6 to 6.8 mm (F

98    $_{(1, 8)} = 24.3$ P< 0.01). In fed organisms however, neither the dry weight nor the body

99   length showed a significant change over time ($F_{(1, 8)} = 2.7$, P >0.5 and $F_{(1, 8)} = 0.01$, P

100   >0.5 respectively).

101   *DNA*- Our model output indicates a significant treatment effect on DNA

102   concentrations (R=0.6, T= -7.296, P< 0.001). As can be seen from the Fig. 1 starved

103   animals have a significantly lower amount of DNA compared to fed ones ($F_{3.768}$=

104   15.996, P<0.001). It is apparent from the Fig. 1 that a non-linear relationship between

105   DNA and "date" in fed treatment exists. Under feeding conditions, DNA concentration

106   increased up to day 10 when it reached its highest values of 4.9 ± 0.8 µg ind^{-1}

107   (mean±SD). Thereafter it decreased over the last two sampling dates. In contrast, the

108   smoother representing the relationship for the starved condition showed a negative and

109   significant decrease of DNA over the experimental period ($F_{1.0}= 4.188$, P= 0.04).

110   *RNA*- Similar to the DNA pattern we observed a significant treatment effect on RNA

111   concentrations (R = 0.81, T= -9.153, P<0.001). Fig. 2 presents the results obtained

112   from GAMM model. RNA concentration in fed animals showed a significant non-

113   linear changes over the experimental period ($F_{2.136}=2.947$, P = 0.05) where RNA

114   increased up to day 12 reaching highest value of 22.6 ± 12.4 µg ind^{-}1(mean±SD) and

115   then decreased. In starved animals however, RNA changed linearly over time with a

116   significant decrease ($F_{1.222}=50.964$, P < 0.001). Notice that the variation in the RNA

117   concentrations in the feeding group is larger compared to the RNA concentrations

118   found in starved animals ($\sigma^2_1= 45.61$ for fed animals; $\sigma^2_2 = 11.86$ for starved animals).

119 *RNA:DNA-* The GAMM model revealed no significant interaction between
120 "treatment" and "date"; therefore one smoother was capable in capturing the Ratio-
121 date relationship. There was a significant effect of "treatment" on the Ratio ($R = 0.75$,
122 $T = -5.397$, $P < 0.001$) where starving animals represented lower concentration than the
123 fed animals. The effect of "date" alone was significantly different in both treatments
124 ($F_{2.832} = 15.72$, $P < 0.001$) due to a decrease in the Ratio starting at day 8.

Discussion

126 The significant differences between DNA and RNA concentrations in starved and fed
127 animals provides compelling evidence that this method can be applied to gelatinous
128 zooplankton and specifically on *M. leidyi* as a measure of the nutritional condition.
129 The most striking result emerging from our data is an increase of both DNA and RNA
130 concentrations over the time of the experiment in fed animals, without any significant
131 increase in dry weight or length. Given that every somatic cell contains the same
132 amount of DNA, DNA concentrations can reflect cell numbers and the amount of
133 RNA (relative to DNA) is a proxy for metabolic activity and consequently the
134 nutritional condition of the individual (Dortch et al. 1983, Clemmesen 1994).
135 Therefore an increase in DNA concentration often is reflected in an increase in
136 somatic growth (Buckley et al. 1999, Chicharo & Chicharo 2008). Here, we observed
137 a different and non-linear response where fed *M. leidyi* showed cell replication without
138 increase in somatic growth. This resulted likely from the production of genital cells
139 between days 6 to 10 under good nutritional conditions. Fed animals were performing
140 reproductive growth in combination with an increased RNA content i.e. increased
141 metabolic activity of their somatic cells, without an increase in somatic growth. This
142 hypothesis is supported by previous experiments indicating that sufficient food supply
143 (200 copepods l^{-1}) can initiate egg production in ctenophores even in larval phases

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144 (Jaspers et al. 2012; Martindale 1987; Reeve et al. 1989). Similar results have been

145 observed for Washington clam where increase in DNA content was correlated with

146 sexual maturation in male and female clams (Kim et al. 2005).

147 Under starved conditions, we observed a significant reduction in both dry weight and

148 body length, which was fairly well reflected in DNA and RNA concentrations. *M.*

149 *leidyi* appears to compensate a nutritional depletion by somatic degeneration. Here we

150 observed a decrease of metabolic activities that starts at 4 days after starvation

151 continues to decrease similar to results observed in larval fish indicative by a sharp

152 reduction of RNA (Clemmesen 1994). This is in line with previous findings that *M.*

153 *leidyi* is able to survive under starvation situations by reducing its body size (Anninsky

154 et al. 2005) and might explain the ability of overwintering population.

155 Of all studied nucleic acid derived indices to determine the nutritional condition or the

156 physiological state of many groups of animals, the RNA:DNA ratio has been the one

157 mainly used. Although the RNA: DNA ratio in this study was significantly higher in

158 the feeding group, the same pattern of changes in the ratio over time in both starved

159 and fed organisms was observed. Some studies have shown better results when only

160 RNA derived indices were used. This was specially the case when juvenile fish and

161 invertebrates at a further developmental stage were analyzed and RNA concentrations

162 and RNA: protein ratios were shown to be a better predictor of growth rate, than the

163 RNA: DNA ratio (Foster et al. 1992, Rooker and Holt 1996, Houlihan et al. 1990).

164 One explanation for the lack of response in the RNA:DNA ratio is the change in cell

165 size or number due to the different forms of tissue being formed with development and

166 the different reaction patterns of the RNA:DNA ratio in dependence of developmental

167 stage (Buckley et al. 1999) and the tissue type (Olivar et al. 2009). For *M. leidyi* this

168 could be caused by the formation of gametes occurring already in an early stage of

169 development. Future studies should relate RNA to dry weight or protein content to

170 evaluate if this is a better indicator, especially at times when gamete production

171 occurs.

172 **Comments and recommendations**

173 Our finding is conclusive proof that nucleic acid derived indicators are able to describe

174 the nutritional condition of gelatinous zooplankton and specifically *M. leidyi* and

175 therefore can help understanding the physiological state which is needed for a better

176 understanding of trophic interactions. To be able to apply this method directly to field

177 collected organisms further laboratory experiments are needed to (i) calibrate these

178 indicators in response to egg production and somatic growth rates (ii) characterize the

179 reaction of *M. leidyi* to starvation on a detailed histological level and (iii) define

180 thresholds and critical values for survival, like recently have been shown for larval

181 fish (Meyer et al. 2012).

182

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Figures and figure legends

Figure 1- Estimated smoothing curves generated for DNA concentration of *M. leidyi* under and (a) feeding and (b) starvation conditions. Fitted values obtained by GAM model. Dotted lines are 95% point-wise confidence bands.
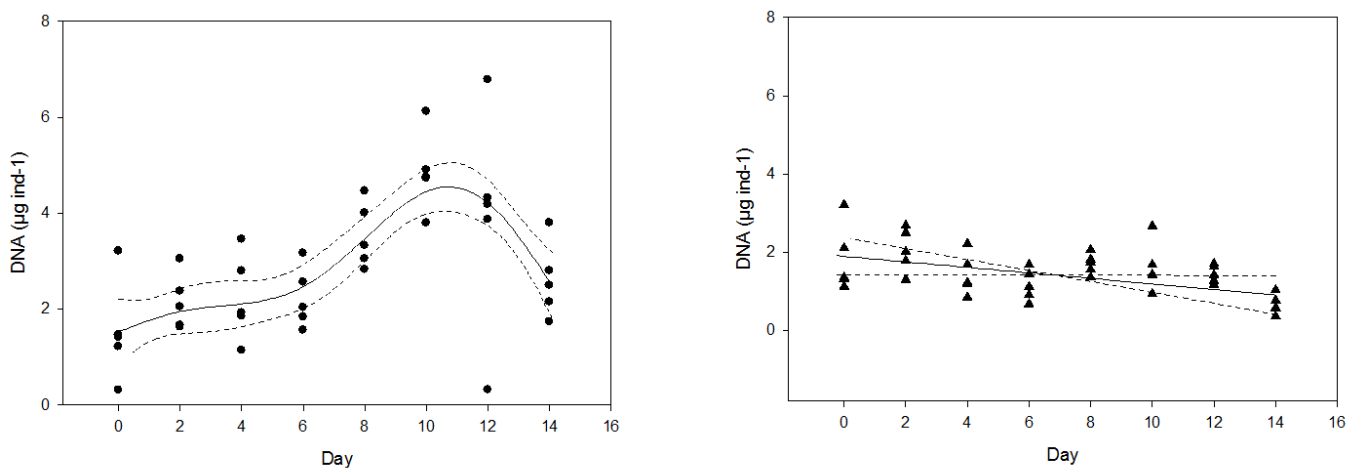

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Figure 2- Estimated smoothing curves generated for RNA concentration of *M. leidyi* under and (a) feeding and (b) starvation conditions. Fitted values obtained by GAMM model. Dotted lines are 95% point-wise confidence bands.
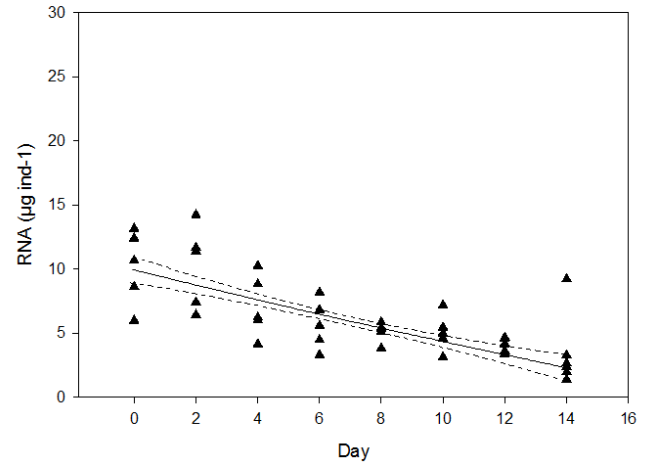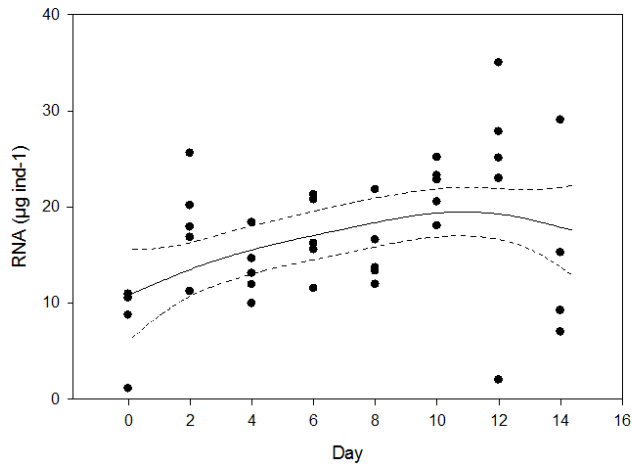
Figure 3- Estimated smoothing curves generated for RNA: DNA ratio of *M. leidyi* under and (a) feeding and (b) starvation conditions. Fitted values obtained by GAMM model. Dotted lines are 95% point-wise confidence bands.