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Research article

Ingestion-rate method for measurement of clearance rates of the ctenophore *Mnemiopsis leidyi*

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Abstract

Clearance rates of small (about 12 mm oral-aboral length) *Mnemiopsis leidyi* were measured by means of the ingestion-rate method in order to compare with rates estimated from an equation (derived from Decker et al. 2004) used in previous studies. About 3 times higher clearance rates measured in the present study indicate that the estimated predation impact of small ctenophores may previously have been considerably underestimated. The prey-capture efficiency measured in *M. leidyi* depends on type and size of prey organism, and the study indicates that the clearance of slow swimming prey (e.g. mussel larvae) captured by auricular flow fields is higher than the clearance of fast swimming/jumping prey (copepods) captured by the lobes. The ingestion-rate method presented may open up not only more precise measurements of clearance rates, but also provide for digestion rates of *M. leidyi* offered different types of prey organisms.

Key words: *Mnemiopsis*, clearance rate, prey-capture efficiency, digestion time, zooplankton

Introduction

The invasive ctenophore *Mnemiopsis leidyi* A. Agassiz, 1865 is native to estuaries along the eastern coast of North and South America (Purcell et al. 2001). High feeding-, growth- and reproduction rates enable the ctenophore to rapidly reach high abundances (Purcell et al. 2001). The accidental introduction of this species to the Black Sea, via ballast water, in the early 1980s had serious consequences for zooplanktivorous fish due to the ctenophore's predation impact on the zooplankton (Waggett and Costello 1999; Kideys and Romanova 2001; Purcell et al. 2001; Shiganova et al. 2001). Occurrence for the first time of *M. leidyi* in Danish waters was described by Tendal et al. (2007) who concluded from numerous reports that the ctenophore was widely distributed in all inner Danish waters in the summer of 2007, and in certain areas, including Limfjorden, it exhibited mass occurrence. The abundance of *M. leidyi* in Limfjorden in August and September 2007 was recorded by Riisgård et al. (2007, 2010) who also evaluated the ctenophore's possible predation effects on zooplankton. Likewise, Huwer et al. (2008) evaluated the predation impact of *M. leidyi* on zooplankton and eggs in the central Baltic Sea. In both cases, the calculated predation impact used a clearance-rate equation derived from empirical estimates by Decker et al. (2004) for estimating the individual filtration rate (= clearance rate) of small *M. leidyi* feeding on copepods.

In the present study we measured the clearance rate (Cl) by means of the "ingestion-rate method" (Riisgård and Madsen 2010) as: Cl = I/C, where I = ingestion rate of a certain type of prey, C = concentration of prey organisms in the ambient water. The aim was to measure the clearance rate of small *Mnemiopsis leidyi* by means of the ingestion-rate method in order to evaluate this method, and secondly to compare the measured clearance rate using adult copepods as prey with that estimated from the clearance rate equation derived from Decker et al. (2004). Further, the relative retention efficiency of different species of prey (mussel veligers, rotifers, brine shrimps, adult and nauplii copepods) was measured by means of the ingestion-rate method, and finally, the estimation of the digestion time of prey in the predator's gut was estimated.

Materials and methods

Measured clearance rate**.** Lobate ctenophores used in the clearance experiments were offered different types of cultivated prey organisms: brine shrimps (*Artemia salina*), nauplii and adult copepods (*Acartia tonsa*), rotifers (*Brachionus plicatilis*), and mussel veligers (*Mytilus edulis*). All prey organisms were fed a monoculture of *Rhodomonas salina*. Lobate ctenophores were collected with a 2 mm-mesh plankton net (1.77 m^2 mouth area) in the Great Belt (Denmark) in July and August 2009.

A known number of ctenophores (usually 12) were carefully transferred in a beaker with water to the experimental tank containing a certain volume of bio- filtered seawater (60 or 70 l) without zooplankton organisms and allowed to adapt to the ambient water salinity and temperature (20 psu, 12.4ºC, diffuse dim light) for some hours, until the undisturbed jellyfish were freely swimming around in the water. Subsequently at time zero, 3 types of prey organisms were added in the same concentration (C) of 10 prey l^{-1}. After 10 min, 2 ctenophores were taken out and then again every 10 min another 2 ctenophores were taken out, and the number of each type of prey observed in the stomach were counted using a stereo-microscope so that the ingestion rate (I, ind. min^{-1}) could be determined from the slope of a regression line based on the number of prey in the stomach as a function of time. The clearance rate (Cl, 1 h^{-1}) was then determined as:

$$Cl = I \times 60/C \qquad \text{Eq. (1)}$$

A precondition for using the equation is that the prey concentration remains constant throughout the experimental time. This was approximated by using a large tank volume so that the prey concentration remained approximately constant during the experimental period, and in no case more than 15% of the prey organism had been removed by the end of the experiment.

Because it was important to count all the prey ingested, the optimum concentration for the experimental procedure was initially determined. Ctenophores may regurgitate their stomach contents if the prey concentration is too high. Preliminary tests with different concentrations of *Artemia salina* nauplii showed that regurgitation did not occur at or below 10 prey l^{-1}. To ensure that the number of each prey type in the stomach could be identified, the experimental period was 60 min. Prey organisms were classified as digested when no solid material was seen in the stomach.

Estimated clearance rate. Oral-aboral length (L, mm) and body volume (B, ml) were measured on 20 *M. leidyi* ranging from 7 to 31 mm and the following relationship was obtained (Riisgård et al. 2007):

$$B = 0.0226L^{1.72} \qquad \text{Eq. (2).}$$

For ctenophores offered copepods as prey the following equation was used to estimate the individual clearance rate (Cl_{est} , l d^{-1}) of ctenophores from the mean body volume (B, ml):

$$Cl_{est} = 2.64B \qquad \text{Eq. (3).}$$

This equation was derived from empirical estimates as follows: Decker et al. (2004) measured filtration rates (= clearance) of small *Mnemiopsis leidyi* (mean volumes 2.3 - 3.9 ml) feeding on the copepod *Acartia tonsa* at about 22°C in 90 l containers as 0.11 l h^{-1} per ml of ctenophore (= 2.64 l d^{-1} ml^{-1}, see Table 1 in Decker et al. 2004).

Steady-state experiment and digestive time. In steady state (i.e. ingestion rate of prey = digestion rate of prey), the ingestion rate (I, prey h^{-1}), clearance rate (Cl, l $ind.^{-1}$ h^{-1}), number of prey in the gut (G), prey concentration in the ambient water (C, prey l^{-1}), and prey-digestion time (E, h) are interconnected and can be expressed by means of the following equations:

$$I = G/E \qquad \text{Eq. (4)}$$

$$Cl = I/C \qquad \text{Eq. (5)}$$

The number of prey organisms (*Artemia salina* nauplii) in the gut of *Mnemiopsis leidyi* (21±2 mm) was monitored during a 3-h experiment where 6 ctenophores were removed every 60 min from a 70-l aquarium (12.4ºC) with 10 prey l^{-1}.

Results

Figure 1 shows two examples of ingestion rate measurements where the slope of the regression line for number of prey in the stomach as a function of time was used for estimation of clearance rate. The measured and estimated clearances rates are shown in Table 1. The mean clearance rate using adult copepods as prey was Cl=0.6±0.4 l h^{-1} which may be compared to the mean estimated clearance rate Cl_{est}= 0.19 ± 0.01.

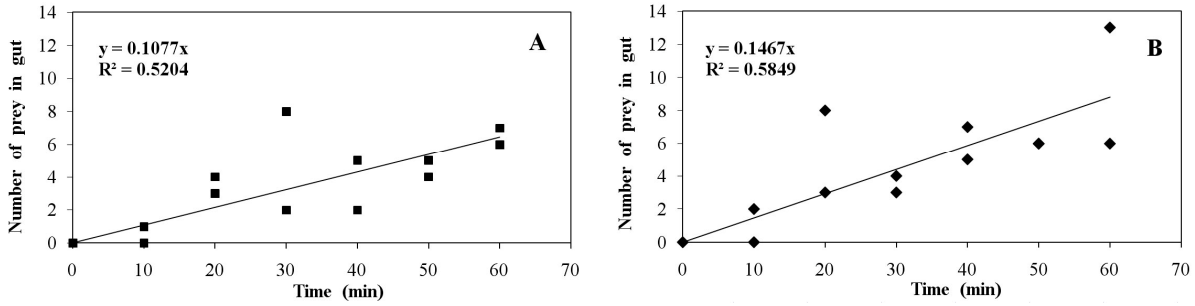

Figure 1. *Mnemiopsis leidyi* (L =33 ± 2 mm). Number of prey organisms (*Artemia* nauplii) in the gut of ctenophores as a function of feeding time at constant prey concentration (10 ind. l^{-1}, 12.4ºC) in two experiments (A and B). Linear regression lines and their equations are shown.

Table 1. *Mnemiopsis leidyi*. Clearance rate (Cl =I/C) of lobate ctenophores measured in ingestion-rate experiments using 3 types of prey organisms (# 1 to #4), 2 types (#5 and #6), and one type (#7 and #8). L = mean (±SD) oral-aboral length; B = mean (±SD) bio-volume of individual ctenophores; V = volume of water in experimental aquarium (12.4 ºC); C =concentration of prey; I = ingestion rate with [r2] for the linear regression line where slope expresses the ingestion rate; Clest = estimated clearance rate using Eq.(3).

Exp. #	*L* (mm)	*B*(ml)	*V*(l)	Prey	Prey size (mm)	*C* (ind. l^{-1})	*I* (ind. min^{-1})	*Cl* (l h^{-1})	Cl_{est} (l h^{-1})
				Copepods	adult	3.3	0.013 [0.548]	0.2	0.18
				Copepods	nauplii		0.008 [0.375]	0.1	
1	11.8±1.3	1.6±0.3	60	*Artemia*	0.7		0.017 [0.729]	0.3	
				Copepods	adult	3.3	0.015 [0.563]	0.3	0.17
				Copepods	nauplii		0.008 [0.656]	0.1	
2	11.6±1.1	1.5±0.2	60	*Artemia*	0.7		0.023 [0.803]	0.4	
				Copepods	adult	3.3	0.062 [0.916]	1.1	0.20
				Copepods	nauplii		0.014 [0.711]	0.3	
3	12.7±1.8	1.8±0.4	60	Rotifers			0.051 [0.834]	0.9	
				Copepods	adult	3.3	0.032 [0.765]	0.6	0.19
				Copepods	nauplii		0.007 [0.333]	0.1	
4	13.1±1.8	1.7±0.7	60	Rotifers			0.041 [0.527]	0.7	
				Artemia	0.7	5	0.046 [0.612]	0.5	
5	12.5±1.2	1.7±0.3	70	Mussel veligers			0.103 [0.763]	1.2	
				Artemia	0.7	5	0.054 [0.961]	0.7	
6	11.9±1.3	1.6±0.3	70	Mussel veligers			0.093 [0.931]	1.1	
7	13.5±2.4	2.0±0.6	70	*Artemia*	0.7	10	0.108 [0.799]	0.6	
8	13.1±2.3	1.9±0.5	70	*Artemia*	0.7	10	0.147 [0.846]	0.9	

Table 2. *Mnemiopsis leidyi*. Relative retention efficiency of different prey organisms in experiments using the ingestion-rate method for measurement of clearance. The mean clearance rates Cl (± SD) are obtained from experiments shown in Table 1.

Prey	*Cl* (l h^{-1})	Relative retention (%)
Mussel veligers	1.2 ± 0.1	100
Rotifers	0.8 ± 0.1	70
Artemia (0.7 mm)	0.6 ± 0.2	47
Copepods (adults)	0.6 ± 0.4	47
Copepods (nauplii)	0.2 ± 0.1	14

Thus, the actually measured clearance rate using the ingestion-rate method is about 3 times higher than the estimated, cf. Eq.(3).

The relative retention efficiency by which *Mnemiopsis leidyi* clear the ambient water for different prey organisms is shown in Table 2. Mussel veligers were captured with the highest efficiency, rotifers and adult copepods were captured less efficient, 70% and 47%, respectively, whereas copepod nauplii were captured with only 14 % efficiency.

In the steady-state experiment, the mean (± SD) number of prey (*Artemia salina* nauplii) in the gut was 15.0 ± 5.3 (n = 6), 14.5 ± 4.8 (6), and 16.5 ± 5.4 (6) after 1, 2 and 3 h, respectively, and the overall mean in the steady-state period was G = 15.3 ± 1.0 (n = 3) ind. l^{-1}. The mean clearance was (cf. previous experiment, Figure 1, Table 1) measured to be *Cl* = 0.6 l h^{-1} so that $I = Cl \times C = 0.6 \times 10 = 6$ prey h^{-1}, and thus, the prey-digestive time was estimated at $E = G/I = 15.3/6 = 2.6$ h.

Discussion

In previous studies by Huwer et al. (2008) and Riisgård et al. (2007, 2010) Eq.(3) was used for assessing the predation impact of *Mnemiopsis leidyi*, but the approximately 3 times higher clearance rates measured by means of the ingestion-rate method (Table 1) indicate that the estimated predation impact of small ctenophores in the above studies may have been underestimated. Although the temperature difference between Decker et al. (2004; cf. Eq. (3)) and the present study is considerable (about 10ºC), this cannot explain the approximately 3 times higher clearance rate obtained at the lower temperature used in this study.

The prey-capture efficiency measured in *Mnemiopsis leidyi* (Table 2) varies considerably, depending on type and size of prey organism. This is highly relevant for understanding the predation impact on various zooplankton taxa, and for evaluating the mortality of , for example bivalve veliger larvae in coastal shellfish areas where this ctenophore may occasionally occur in large numbers (Purcell and Decker 2005; Purcell et al. 2001, Purcell 2009; McNamara et al. 2010). *M. leidyi* has two methods of catching prey that functions synergistically, and this enables the ctenophore to predate on a wide range of zooplankton taxa. Capture via auricular flow-field entrainment is thought to be the mechanism of the capture of non-motile (e.g. fish eggs) and slowly swimming prey (e.g. bivalve veligers). Capture of highly motile prey (e.g. copepods, cf. Viitasalo and Rautio 1998) is utilized by the collision of the prey with the interior of the oral lobes (Waggett and Costello 1999). *In situ* studies by Larson (1987) showed that slowly swimming prey such as veligers were captured more frequently than adult copepods. This agrees with the high retention efficiency found for mussel veligers relative to copepods in the present study where mussel veligers were captured with the highest efficiency (Table 2).

The ingestion-rate method used in the present work was also used in a recent work by Riisgård and Madsen (2010) for measuring clearance rates in small medusae of the common jellyfish *Aurelia aurita* offered different types of prey. It was found that fish larvae and *Artemia salina* nauplii were cleared with nearly same rate whereas "mixed zooplankton", rotifers, copepods, and not least ciliates were retained with increasingly lower efficiency (Riisgård and Madsen 2010, Table 7 therein) which probably reflects the importance of both prey-escape behavior and prey size for predation efficiency. The relative retention efficiency of different prey organisms measured in the present work (Table 2) differ from this pattern possibly because the prey-capture mechanisms are very different in the two species, but the results show that the design of the ingestion-rate method used in this work may be a useful tool in futures studies of prey-capture efficiency in *Mnemiopsis leidyi,* which should also include fish eggs and larvae, cf. Sullivan et al. (2001), Purcell et al. (1994), Cowan and Houde (1992, 1993), Monteleone and Duguay (1988).

The prey-digestive time of 2.6 h for *Artemia salina* nauplii estimated in this work is in good agreement with the actual digestion time observed (i.e. no solid material could be seen in the stomach), and thus, the present study has demonstrated that the ingestion-rate method may open up not only more precise measurements of clearance rates, but also provide digestion rates of *Mnemiopsis leidyi* offered different types of prey organisms. The estimated prey-digestion time of $E = 2.6$ h in the present work is longer than the observed digestion time of 1.6 to 1.9 h for complete digestion of 8 *A. salina* nauplii in the gut of *Aurelia aurita* (umbrella diameter 3.5 to 14.5 mm, 9.5ºC) reported by Martinussen and Båmstedt (1999), who did however show that a

smaller meal of one or two *A. salina* nauplii was digested more rapidly, within 1.1 to 1.2 h. The digestion time in the present work, where the steady-state meal was 15 *A. salina* nauplii, may likewise be shorter if smaller meals of nauplii were offered.

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