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ORIGINAL ARTICLE

Diel feeding of the transparent goby *Aphia minuta* (Pisces, Gobiidae) in the Northwestern Adriatic Sea in spring time

Valentina Tirelli[1], Sara Legovini[1], Diego Borme[1], Elena Di Poi[1] & Mario La Mesa[2]

1 Sezione di Oceanografia, OGS (Istituto di Oceanografia e di Geofisica Sperimentale), Trieste, Italy
2 CNR-Consiglio Nazionale delle Ricerche, ISMAR Istituto di Scienze Marine in Ancona, Ancona, Italy

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Correspondence
Valentina Tirelli, Istituto di Oceanografia e di Geofisica Sperimentale, Sezione di Oceanografia, Via A. Piccard 54, 34151, Trieste, Italy.
E-mail: vtirelli@ogs.trieste.it

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Abstract

The present study provides the first estimate of the daily ration for a goby species in the Mediterranean Sea, using a new approach to determine the mass of fish stomach contents through the sum of individual prey dry mass derived indirectly from prey size. Diel feeding activity and daily ration of the pelagic goby *Aphia minuta* were studied under natural conditions in the coastal waters off Comacchio (Northwestern Adriatic Sea) during the spring season. A total of 318 individuals of *A. minuta*, collected by 14 trawls carried out on 4 consecutive days, was examined for gut contents. The diel pattern of the vacuity index and the stomach fullness index indicated that *A. minuta* has nocturnal feeding behaviour. The daily ration, computed over a period of 16 h, was equivalent to 2.23% wet body mass.

Introduction

The transparent goby *Aphia minuta* (Risso, 1810) is a small pelagic species, widely distributed in inshore and estuarine waters in the Northeast Atlantic from Gibraltar to Norway and the Baltic Sea and throughout the Mediterranean, the Black Sea and the Azov Sea (Tortonese 1975). *Aphia minuta* forms a monotypic genus within the family Gobiidae, one of the largest groups of fish inhabiting inshore marine, estuarine and freshwater environments (Miller 1986). Despite its small size (<6 cm in total length), the transparent goby is the seasonal target species of small-scale artisanal fisheries in the western and central Mediterranean (off Spain and Italy), locally yielding more than 100 metric tons per fishing season (La Mesa *et al.* 2005).

Aphia minuta is known to be a food item of several demersal fish in the Black Sea, such as *Merlangius merlangus* (Linnaeus, 1758), *Mullus barbatus* Linnaeus, 1758, *Platichthys flesus* (Linnaeus, 1758), *Scorpaena porcus* Linnaeus, 1758, *Solea* spp. Quensel, 1806, and *Trachinus* spp. Linnaeus, 1758 (Fortunatova 1949), as well as of *Merluccius merluccius* (Linnaeus, 1758), *Thunnus thynnus* (Linnaeus, 1758) and *Trachurus trachurus* (Linnaeus, 1758) in Spanish waters and *Serranus cabrilla* (Linnaeus, 1758) and *Serranus hepatus* (Linnaeus, 1758) in Greek waters (Iglesias 1995; Labropoulou & Eleftheriou 1997; Cartes *et al.* 2004). In the Northeastern Atlantic (Galician waters), it is preyed on by cephalopods as well (Guerra & Rocha 1994).

Contrary to most of the Mediterranean gobies, which are generally benthic, the transparent goby shows pelagic habits for most of its life cycle (Iglesias *et al.* 1997), a feature shared in the Mediterranean Sea with other two gobies, namely *Crystallogobius linearis* (Dűben, 1845) and *Pseudaphya ferreri* (de Buen & Fage, 1908) (La Mesa 2011). *Aphia minuta* is a neritic species, living from the surface to 80−100 m depth (Tortonese 1975). Planktonic larvae of *A. minuta* hatch from demersal eggs and are followed by a ‘pelagic phase’ made up of post-larval specimens up to 15−20 mm total length (TL) that live in shallow waters, very close to shore. From 20 to about 35 mm TL, juvenile specimens settle to the bottom, forming very large schools (‘aggregate phase’). In spring, from 35 mm TL onwards and in coincidence with gonad maturation, there is a progressive offshore migration and dispersal of adults that lie close to the bottom, forming the ‘demersal phase’ (references in La Mesa *et al.* 2005). The spawning period for *A. minuta* largely varies across its zoogeographical distribution; in the Central Adriatic Sea the breeding season lasts at least 6 months, from April to September (La Mesa *et al.* 2005). Adults of this species exhibit a series of larval characters such as the scarcity of melanophores, the persistence of the swimbladder and the presence of a short, straight alimentary tract. These characteristics have been pointed out as an adaptation to pelagic or semi-pelagic life or, more likely, to the planktivorous behaviour of these species (Miller 1973, 1989).

Despite great interest in the biology of *A. minuta* (La Mesa *et al.* 2005), few studies have been carried out on its feeding ecology. The diet of *A. minuta* is poorly described and often vaguely reported, with copepods and cirripede and mysid larvae thought to be the main food items (Hesthagen 1971; Miller 1986). Chesalin *et al.* (2004) provided qualitative data on the feeding habits of *A. minuta* from the Black Sea, where they found that it fed on small copepods, sharing this food resource with sprat and anchovy. Recent studies in the Northwestern Adriatic have confirmed previous data (La Mesa *et al.* 2008).

Diet description is a first step in the study of the feeding ecology of a fish. Nevertheless, qualitative information on fish diet needs to be combined with evaluations of diel feeding patterns and food consumption rates, in order to determine the ecological role of the fish species in food webs, as well as the influence of the trophic environment on its growth.

Estimates of daily rations for pelagic fishes are rather infrequent because of methodological problems. Stomach content must be assessed *in situ* at regular intervals over 24-h periods in order to couple the temporal pattern of stomach fullness with estimates of gut evacuation rate. The problems increase in the case of small (cm) planktivorous fish like *A. minuta* because of the very low prey mass contents in their stomach. It is almost impossible to directly measure wet or dry mass for prey that are as small as a few hundred μm in length and often squashed and deformed by predation. Bearing in mind such limitations, it is not surprising that the daily food consumption of *A. minuta* and other small-sized planktivorous fish is presently unknown.

In order to fill this gap, we decided to utilize the data of La Mesa *et al.* (2008) and estimate fish stomach contents as the sum of individual prey mass derived from prey size. This approach allowed us to determine the daily feeding activity pattern of *A. minuta* and to obtain the first estimation of its daily ration.

Material and Methods

Sample collection

The sampling was carried out in the Northwestern Adriatic Sea off Comacchio (locality Po di Goro, south of Po River delta) aboard RV *G. Dalla Porta* from 17 to 20 May 2003 (Fig. 1). Given the constraints in sampling over the 24 h, trawling was carried out at different times of day in an attempt to cover the entire 24 h. Fish were collected with a small-meshed semi-pelagic trawl with 4-mm cod end mesh size, hauled on average at a rate of 3.0 knots for 30 min; the net was also equipped with a temperature/depth recorder VEMCO MINILOG TD. Overall, 14 trawls were carried out at depths ranging from 7 to 30 m, covering the entire daily cycle of 24 h (Table 1). During the sampling the sun rose approximately at 05:30 h (UTC+2) and set at 20:30 h (UTC+2). The mean seawater temperature was 12.4 ± 1.7 °C. Catches were immediately preserved on board in formaldehyde-sea water solution (4% final concentration) for laboratory analysis.

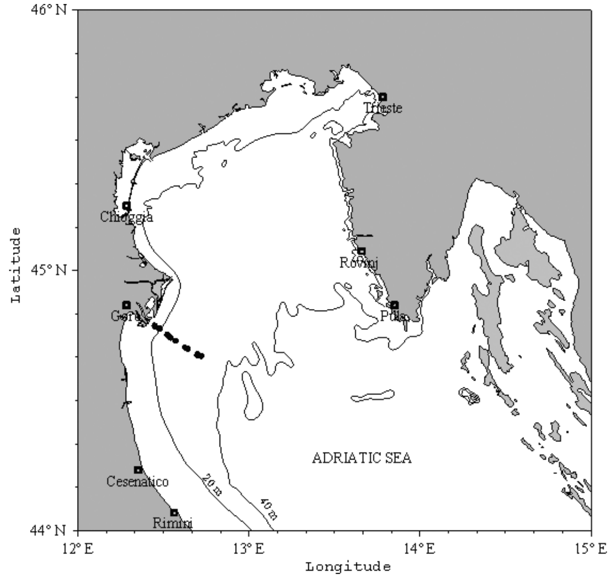

Fig. 1. Study area in the north-western Adriatic Sea. The sampling transect is represented by the dotted line.

Stomach content analysis: feeding periodicity and daily ration

In the laboratory, each fish specimen was measured to the nearest mm (total length, TL) and sexed. As fish underwent shrinkage as a result of fixation in formaldehyde, their length was converted into initial length at sea by applying the correction factor of 2.5% according to Fey (1999). The fish wet body mass (BM, mg) was obtained applying the total length/body mass relationship estimated on fresh individuals (La Mesa 1999).

Analysis of the stomach contents was carried out using a stereomicroscope at 70× magnification. Prey items were identified, where possible, to the species level. The prosome length of all copepods and the maximum dimensions of other zooplankters were measured using

Table 1. Sampling data of *Aphia minuta* from the Northwestern Adriatic Sea. Total lengths (mean value ± standard deviation) were obtained by applying a correction factor of 2.5% (for shrinkage because of fixation in formaldehyde) to the initial length

Date	Trawl number	Time of catch	Sample size (n)	Total length (mm)	Vacuity index (%)
17 May	2[a]	10:19	20	37 ± 4	35
17 May	3	12:10	30	33 ± 4	56
17 May	4	15:06	20	42 ± 6	5
17 May	6	22:22	20	47 ± 9	25
17 May	7	23:54	20	39 ± 4	5
18 May	8[a]	01:09	34	35 ± 4	26
18 May	9[a]	10:12	41	36 ± 4	51
18 May	11[b]	15:10	4	36 ± 8	
18 May	12	16:34	20	51 ± 5	20
18 May	13	22:00	20	41 ± 8	45
18 May	14	23:24	23	38 ± 6	8
19 May	15[a]	01:05	15	35 ± 4	13
20 May	16	02:50	20	37 ± 4	25
20 May	18	12:51	35	44 ± 9	31

[a]Trawls pooled (9 and 2, 15 and 8) for daily ration estimation.
[b]Samples excluded from the analysis because of low sample size.

an ocular micrometer, with an accuracy of ±14 μm. Further details on the methodology applied for prey identification and to evaluate prey dry masses are given in La Mesa *et al.* (2008). The stomach content (dry mass, DM) was estimated as the sum of individual prey dry mass. As often only the cephalosome of some copepod species was recovered, we calculated the DM of these incomplete prey as the DM of undamaged individuals of the same size (for each prey species the ratio of cephalosome to prosome length was obtained by measurements on undamaged specimens from plankton samples collected in the study area). A loss of about 30% of the dry mass as a result of fixation was assumed (Boettger & Schnack 1986; Alcaraz *et al.* 2003), then stomach content was expressed as wet mass by applying a water content value of 87%, *i.e.* that of copepods of the species *Acartia clausi* from the Northern Adriatic Sea (Borme 2006).

To determine the feeding periodicity of *Aphia minuta*, the vacuity index (V%) and the fullness index (F) (Hureau 1970) were calculated for each trawl, as follows:

$$V = \frac{E}{A} \times 100 \quad (1)$$

where E is the number of empty stomachs and A is the total number of stomachs analysed per trawl.

$$F = \frac{S}{BM} \times 100 \quad (2)$$

where S is the wet mass of the stomach content (mg) and BM is the wet body mass of fish (mg).

The daily ration was calculated following the model of Eggers (1979):

$$C_t = (W_t - W_0) + \overline{W} R_{max} t \quad (3)$$

where C_t is the consumption over the considered feeding interval, W_t is the amount of food at time t, W_0 is the amount of food at the beginning of the time interval, $\overline{W}$ is the mean mass of food content over the entire interval, R is the instantaneous gastric evacuation rate (h^{-1}) and t is the interval duration. The model of Eggers (1979) was preferred to Elliott & Persson's (1978) model because of the irregularity of sampling (Boisclair & Leggett 1988). The standard error of consumption estimate and the evacuation rate were expressed as a function of the standard errors of the parameters used in the models, according to the method proposed by Worobec (1984). Empty stomachs were not considered, as recommended by Bromley (1994).

The instantaneous gastric evacuation rate (R) was estimated from field data by an exponential model. One instantaneous evacuation rate (R_{ti}) was computed for each considered time interval (t_i):

$$R_{ti} = \frac{(\ln W_t - \ln W_0)}{t_i} \quad (4)$$

where W_0 is the amount of food at the beginning of the time interval, W_t is the amount of food at time t and t_i is the duration of the time interval expressed in hours. According to Héroux & Magnan (1996), the estimated instantaneous evacuation rate (R) corresponds to the highest rate observed over all the time intervals considered as:

$$R = \text{Maximum value of } R_{ti} \quad (5)$$

Daily ration as a percentage of wet body mass was calculated from:

$$C\% = (C_t/\overline{BM}) * 100 \quad (6)$$

where $\overline{BM}$ is the mean body mass of *A. minuta*.

Results

A total of 318 individuals of *Aphia minuta* (259 females and 59 males) was examined for gut contents. Females were slightly smaller than males, ranging from 26 to 57 mm (TL) (mean TL±SE = 38 ± 0.4 mm) and from 31 to 57 mm (TL) (mean TL ± SE = 45 ± 1 mm), respectively. The average body mass was 349 ± 14 mg (mean ± SE).

The vacuity index varied from 5% to 56.7% (Table 1). At both afternoon and night the VI was generally low,

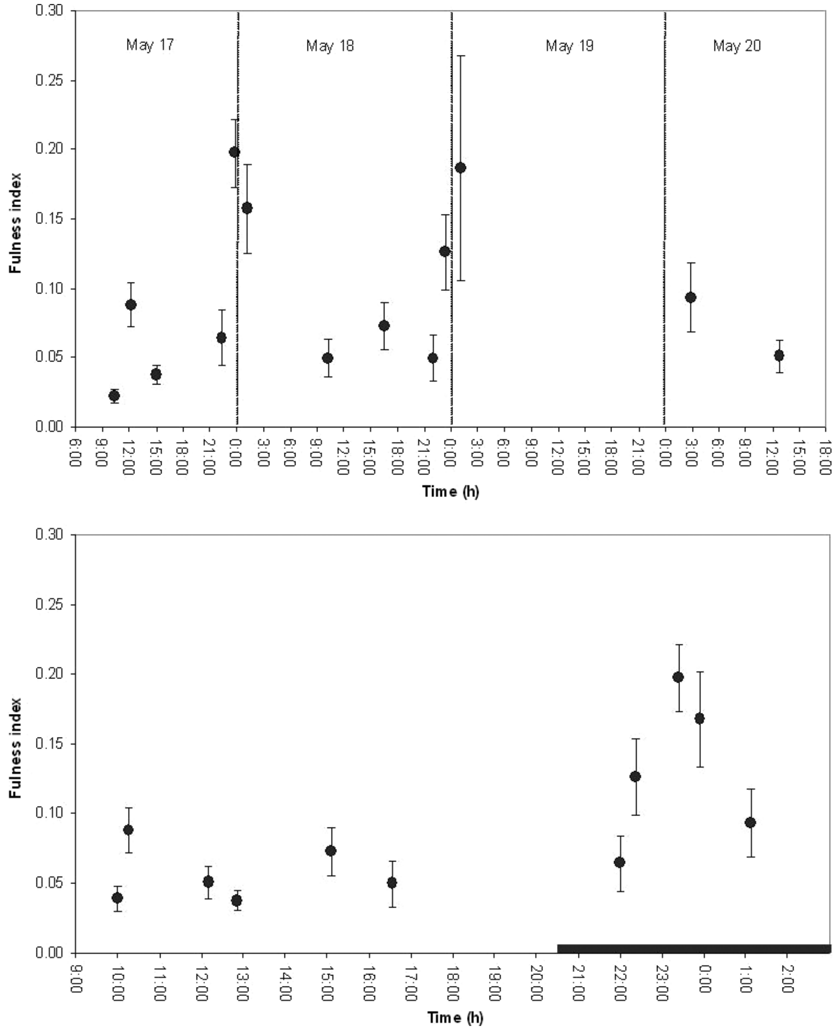

Fig. 2. Mean values (±SE) of A. minuta stomach fullness index against time of the day. In the lower graph samples collected in the 4 days of sampling were pooled to 11 consecutive hauls. In two occasions tows were made at the same hour of different days and fish obtained in the two catches were pooled together (trawls 9 and 2; trawls 15 and 8; see also table 1). Black bar on the x-axis indicates the darkness period.

ranging from 5% to 26%, except for the value recorded at 22:00 h (about 45%). In the morning the number of empty stomachs increased, suggesting a lower feeding activity of *A. minuta* during this period.

Feeding periodicity was also observed by plotting mean fullness index against time of day. Occasionally, the catch was not positive for transparent goby or too few specimens were recovered (*i.e.* trawl 11); hence, samples collected in the 4 days of sampling were pooled to 11 consecutive trawls (lower graph in Fig. 2; Table 1). The diel pattern of stomach fullness (F) confirmed the nocturnal feeding behaviour of *A. minuta*. F was generally low during the day, increasing progressively at dusk to peak during the night, then decreasing again before sunrise.

In order to calculate the daily ration, samples were pooled over a 24h sampling period as shown in Fig. 2 for the fullness index. When tows were carried out at the same hour of different days, fish obtained in the two catches were pooled together (trawls 9 and 2; trawls 15 and 8) (Table 1).

The highest value of R computed was $1.67 \pm 0.27\ h^{-1}$.

The food consumption and the relative daily ration were: $\sum_{02:50}^{10:16} C_t = 7.80 \pm 0.35$ mg wet mass = 2.23% wet body mass $t^{-1.}$

Discussion

The present study provides the first estimate of the daily ration for a goby species in the Mediterranean Sea. It is a widespread practice to derive the zooplankton biomass (particularly for copepods) from body size, as the body dry mass relative to body length has been determined in many species (Mauchline 1998) and is commonly used in fish diet analysis. Nevertheless, this is the first time that the daily ration of a planktivorous fish has been calculated using prey dry mass derived from prey size. This approach enabled us to obtain an estimate of daily consumption of *Aphia minuta* in the Northwestern Adriatic Sea during spring time.

Unfortunately, no data are currently available on food consumption of *Crystallogobius linearis* (Düben, 1845) and *Pseudaphya ferreri* (de Buen & Fage, 1908), the other two species of pelagic gobies living in the Mediterranean Sea; therefore, it was possible to refer only to literature related to benthic gobies. Comparing the daily rations of small gobies from shallow inshore waters, such as *Pomatoschistus*, which exhibits life history traits similar to those of *A. minuta* (Miller 1986), they were consistently higher than that of *A. minuta*, although with strong variability in relation to local environmental conditions. In *Pomatoschistus microps* (Krøyer, 1838), for example, the daily ration varied from 4.8−6.5% at 14−20.5 °C in the Baltic Sea (Meyer-Antholz 1987), to 7.2−14.5% of body ash-free dry mass (Hampel & Cattrijsse 2004) and 27.3% body mass in the Northern Wadden Sea (del Norte-Campos & Temming 1994). Similarly, the daily ration estimated for *Pomatoschistus minutus* (Pallas, 1770) was approximately 5−12% body dry mass in the Gullam Fjord (Evans 1984) and 13% body mass in the Northern Wadden Sea (del Norte-Campos & Temming 1994). The only study reporting similar values to our results is that of Hamerlynck *et al.* (1993), who calculated a daily ration of 1% of body ash-free dry mass for *Pomatoschistus lozanoi* (de Buen, 1923); however, in the same study the authors also reported a daily ration of about 5% obtained the successive year.

The lower value of daily ration obtained for *A. minuta* is probably a result of differences in the feeding behaviour and diet of gobies. Diel feeding activity is a complex process influenced by environmental, behavioural and physiological features (Cortés 1997). As vision plays a fundamental role for prey detection, fish food intake may be dependent on the light−dark cycle. Nevertheless, feeding activity may also be influenced by several other factors, such as hunger, the risk of predation reproductive strategies and territorial defence (Magnhagen 1988). Despite numerous studies carried out on the feeding behaviour of benthic gobies, the diel feeding pattern of pelagic gobies remained unknown prior to the present study. Based on the present data, *A. minuta* fed more intensively at dusk and during the night, when it exhibits a selective feeding behaviour (La Mesa *et al.* 2008). These results seem to be in agreement with some observations on other gobies, such as *Pomatoschistus lozanoi* and *Pomatoschistus minutus*, also indicating higher feeding activity at night (Berge *et al.* 1983; Hamerlynck *et al.* 1993). Despite the closer link with the bottom, characterizing its demersal phase, *A. minuta* showed a fully zooplanktonic diet (La Mesa *et al.* 2008), suggesting active searching for food exclusively along the water column. *Aphia minuta* is considered to be a generalist feeder, with a relatively broad niche width composed of several rare prey caught occasionally but feeding largely on prey that are abundant in the environment, such as the copepods *Temora longicornis* (Müller O.F., 1785) and *Acartia clausi* Giesbrecht, 1889 (La Mesa *et al.* 2008). Conversely, benthic gobies generally rely on macrofaunal and benthic organisms. As an example, copepods numerically dominated the diet of *Pomatoschistus lozanoi*, but mysid shrimps and infaunal amphipod were the most dominant prey items in terms of biomass (Hampel & Cattrijsse 2004). In the Baltic Sea, the most frequent and numerous prey of *Pomatoschistus minutus* included amphipods, copepods and mysids (Zloch *et al.* 2005), which represent valuable prey in terms of high abundance (Wiktor 1993) and energetic value (Szaniawska 1993). In the Wadden Sea, *P. minutus* fed on meiofauna (mainly on harpacticoids), as well as on mysids, polychaetes and molluscs (del Norte-Campos & Temming 1994).

van der Lingen (1998) observed that the daily ration of sardines [*Sardinops sagax* (Jenyns, 1842)] was influenced by fish diet, being lower when sardines fed on zooplankton (daily ration: 0.99% to 2.52%) than on phytoplankton (daily ration: 2.97% to 7.58%). The authors ascribed this difference to the important difference in rates of gastric evacuation for fish fed on these prey types, *i.e.* phytoplankton was evacuated at a much faster rate than zooplankton. The daily ration of *A. minuta* is in the range of daily ration observed for *Sardinops sagax* (van der Lingen 1998) and *Sardina pilchardus* (Walbaum, 1792) (2.02−3.67% total weight for adult sardines in the North Aegean Sea, Nikolioudakis *et al.* 2011) but it is much lower than the values reported for other planktivorous fish, such as adult anchovies, which exhibit a daily ration of 3−4% of their body wet mass (Tudela & Palomera 1995; Plounevez & Champalbert 2000) or even higher (16−20%, Bulgakova 1993). In our case, the gut evacuation rate cannot be considered to be slow and the low daily ration of *A. minuta* is more likely to be the result of other factors. One of the hypotheses that can be drawn is that *A. minuta* was possibly food limited during our sampling. This explanation is also supported by the particularly low fullness index measured (maximal F value = 0.20%). A further hypothesis is that our study was carried out when the feeding activity of *A. minuta* was minimal. In fact, it has been shown that the feeding activity of some gobies can vary seasonally (Zloch *et al.* 2005).

Summary

In the present study field data were used to analyse the diel feeding of *Aphia minuta*, a planktivorous species with poorly known feeding behaviour and metabolic rates. The diel pattern of the vacuity index and the stomach fullness index indicated nocturnal feeding behaviour for *A. minuta*. The daily ration was estimated by calculating the prey dry mass from prey size, an approach that is possible only with accurate prey identification. Hence, we wish to stress once again the importance of high taxonomic resolution of prey in feeding studies of planktivorous fish such as the transparent goby. Compared with other gobiids, the daily ration of *A. minuta* was comparatively low, suggesting a rather low metabolic demand despite their pelagic lifestyle. In order to validate and interpret this result, similar studies are required in different seasons.

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