Fish foraging periodicity correlates with daily changes of diet quality

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Abstract. We tested the adaptive feeding hypothesis of Taborsky and Limberger (1980; Pubbl. Staz. zool. Napoli (I: Mar. Ecol.) 1: 143–153) by comparing two populations of *Parablennius sanguinolentus* (observed ca. 20 km north of Trieste, Italy, and near Calvi, France, during August and September 1989), each of which fed on different algae: turf and sea lettuce (*Ulva lactuca*). Turf energy peaked in the afternoon, *U. lactuca* energy around noon. In both fish populations, feeding paralleled the energy content of their food source, which is strong evidence for the adaptive feeding hypothesis. Turf feeders took an average of 2 to 7 times more bites in the afternoon than at other times of the day, whereas *U. lactuca* feeders bit 1.4 to 15 times more often around noon. We checked for chemical components of algae, but our data did not suffice to reduce the diel pattern of energy content to the short-term distribution of either starch, protein or ash.

Introduction

Diel foraging periodicity with peak feeding in the afternoon has been observed in several marine-littoral fish: tropical and temperate blenniid fish, Caribbean and Pacific damselfish, and some West Indian Ocean surgeon fish (Polunin and Klumpp 1989). All 19 species recorded with this particular rhythm have one thing in common: they feed on algae.

The red-speckled blenny *Parablennius sanguinolentus* was among the first examples in which this marked diel periodicity was demonstrated in the field (Montgomery 1980, Taborsky and Limberger 1980, Nursall 1981). Its feeding activity increased markedly from morning to late afternoon, when it reached the highest intensity, then decreased quickly before dusk. No other fish activity in the areas investigated showed a similar pattern, and no correlations were found when feeding was compared to environmental factors such as diel changes in light intensity, wind speed, water and air temperature. Taborsky

and Limberger (1980) suggested that this feeding rhythm was an adaptation to the changing food quality of green algae during the course of the day. To date, this is the only explanation for the evolutionary cause of this type of feeding pattern (Polunin and Klumpp 1989).

Studies of the photosynthetic metabolism of unicellular algae (Euglenidae – Leedale 1959, Edmunds 1964, 1966) have indicated that there is an enrichment of storage products during the photophase, while cell division takes place during darkness. Storage products are used in cell division and respiration at night. The concentration per cell reaches its original low level at the beginning of the next photophase (Hitchcock 1980).

Parablennius sanguinolentus is a typical grazer on turf algae (mainly Chlorophyta and the genus *Cladophora*, mixed with Rhodophyta of the genera *Ceramium* and *Gelidium*) (Gibson 1968, Goldschmid et al. 1980, 1984, Horn 1989), but Patzner (1985) described a population in the Gulf of Trieste browsing on sea lettuce, *Ulva lactuca*. The latter alga seems to have a different pattern of storage and cell division: it requires four periods of darkness until the average cell has divided (Lovlie and Braten 1968, Britz and Seliger 1973).

These apparent differences between turf and *Ulva lactuca*, combined with the local preferences of *Parablennius sanguinolentus* populations, provided a unique opportunity to test the adaptive feeding hypothesis of Taborsky and Limberger (1980) under natural conditions in the field. Possible differences in the diel pattern of algae metabolism should be reflected by the circadian feeding pattern of *P. sanguinolentus*, if they result in diverging patterns of energy content and the enrichment of storage products like starch and proteins.

Methods

Study sites

There were two study sites, which differed with regard to the staple diet of *Parablennius sanguinolentus*: (1) World Wildlife Found marine park of Miramare and the harbour of the marine biological station

of Auresina, both about 20 km north of Trieste, Italy. There *Ulva lactuca* is the sole diet of *P. sanguinolentus* (Patzner 1985, authors' own observations and gut-content analyses of three specimens). (2) Harbour and rocky shore near the marine biological laboratory STARESO (Station de Recherches Sous-Marines et Océanographiques), in the northwest of Corse near Calvi, France, where *P. sanguinolentus* feeds almost exclusively on turf algae.

Fish behaviour

Field studies were conducted while snorkeling during the fishes' non-reproductive season in August and September 1989. Bite frequency and feeding time were determined by recording with event counters and stop watches the behaviour of each individual for 10 min, after allowing at least 5 min for the fish to become accustomed to the observer (Lobel and Ogden 1981). We chose the individuals independently from size, status and behaviour by arbitrarily selecting the first individual encountered at a previously chosen site, at a depth between 50 and 100 cm below the surface. Up to four different individuals were observed at each session. Sessions were repeated four times daily for three consecutive days. At Miramare the time schedule was limited by the opening hours of the park: observation hours were 09:00 to 10:00, 12:00 to 13:00, 15:00 to 16:00 and 18:00 to 19:00 hrs. At STARESO observations were made at 07:00 to 08:00, 11:00 to 12:00, 15:00 to 16:00 and 18:30 to 19:30 hrs, which embraced the entire activity period of this population at that time of year. Both feeding time and bite rate were recorded when the fish grazed on turf. If no bites were taken for 3 s in succession, the recording of feeding time was interrupted. Feeding time was not recorded when the fish browsed on *Ulva lactuca*, because fragments of variable size are torn from leaves and their handling times are often difficult to determine. There was no evidence from our observations that bite size varied with respect to time of day.

The sizes of the observed fish were estimated and assigned to one of three categories: small (<10 cm), medium (from 10 to 15 cm) and large (>15 cm). Tide level was taken from tables (Instituto Talassografico di Trieste). Weather and water motion were estimated on scale with four categories each. Water-temperature reading was taken 50 cm below the surface from the same locations for the duration of the observations.

Chemical and energy analyses

Sampling and field preparations

Algae were collected from sunrise (05:00 hrs at Auresina and 06:00 hrs at Calvi) to sunset (20:30 hrs at Calvi and 21:00 hrs at Auresina) five times per day on four consecutive days. At Auresina, one additional sample was taken in the middle of each night. Algae were collected where blennies had been seen feeding. Sampling locations remained constant during the day, but different between days. Turf algae were scraped off rocks, while *Ulva lactuca* leaves were picked at their base. The samples were put into 2-ml Eppendorf pipettes without washing, and only macroscopic epiphytic plants and animals were removed from *U. lactuca* leaves. The samples were immediately frozen in liquid nitrogen for transport to Vienna, where they were stored in a deep freezer at −70 °C until analysis.

Energy, ash and metabolic products

Caloric content was measured by combusting pellets of algae with a Metzler MK 2000 micro-bomb calorimeter. Calorimetric readings were made 10 min after ignition. Ash contents were determined by weighing the combustion residues. The energy content in J g^{-1} was calculated as the reading/(dry wt − ash − 0.072), where 0.072 g = weight of ignition wire. Starch was measured following Jones (1979). Protein samples were analysed according to Wollgiehn and Parthier (1964), a modified version of the Lowry method (1951).

Statistics

We calculated means of fish bite numbers ($N=2$ to 4) and the means of the algae analysis results ($N=3$) for each sampling point during the 4 d. Data on fish feeding and samples of algae were collected on different days. As fish foraging was not normally distributed, medians were calculated, whereas alga results were expressed in arithmetic means. For each time point we compared the median numbers of fish bites with means of the algae components. The times of day when algae were collected differed slightly from those when feeding was recorded; therefore, we weighted the data of energy content to compare them with fish feeding on a daytime basis. We tested whether the data were evenly distributed over the course of the day by Chi-square tests. For correlation analyses of fish bite rate and algae energy content we used a test of significance for the Spearman-Rank correlation coefficient. We predicted a positive correlation between these variables, due to the results of Taborsky and Limberger (1980) and previous data on unicellular green algae (Edmunds 1964, 1966). Therefore, we made a one-tailed analysis. A backward stepwise multiple regression analysis was performed for the complete data set of fish behaviour, environmental variables and algae components, with fish feeding as dependent variable.

Results

Fish foraging patterns

Turf and *Ulva lactuca* foragers fed in bursts, during which the food was taken into the mouth (separated by breaks in which the algae were chewed) and swallowed or partly rejected. Feeding time was closely correlated with bite rate when the fish grazed on turf (Spearman-Rank correlation, $r_s=0.944$, $p<0.01$). Therefore, only bite rate was used for further analyses.

Feeding on turf increased from sunrise until mid-afternoon, when it reached its maximum, decreasing again towards dusk. Feeding on *Ulva lactuca* also showed a marked temporal pattern; it increased until noon, when it reached its maximum, then decreased in the early afternoon. The two rhythms (Fig. 1 A, B) differed highly significantly from each other, and they were unevenly distributed over the day (in both cases $p<0.01$, Chi-square tests).

Algal metabolism

The results of all analyses (energy, starch, ash) in both turf and *Ulva lactuca* averaged for 1 d (Table 1) differed significantly from being evenly distributed over the day, and also from each other ($p<0.05$, Chi-square tests). No significant relation was found between energy content and starch, protein or ash in any combination using a correlation (Spearman-Rank test) and a multivariate technique (backwards stepwise multiple regression analysis).

We averaged the number of bites per time point for all observation days and compared these values with the weighted means from the metabolic data of algae. We found a significant correlation between the number of fish bites and the energy content of both turf and *Ulva lactuca* [$r_s=1.0$, $p<0.05$, $N=4$ time points; Spearman-Rank correlation analyses, one-tailed (see Fig. 1 A, B)].
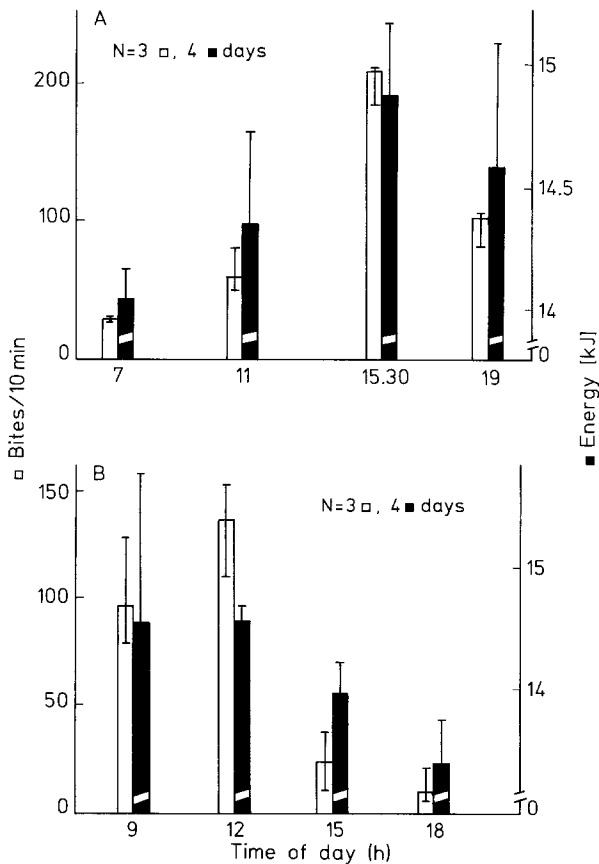
Fig. 1. *Parablennius sanguinolentus*. Relation between number of bites per 10 min ($N = 3$ d, up to four different individuals each) and energy content of the fish's staple diet (kJ g^{-1} ash-free dry wt, $N = 4$ d), for turf algae (A) and *Ulva lactuca* (B). Bars for number of fish bites represent medians with quartile ranges; bars for energy content are means with standard deviations

Table 1. Energy (kJ g^{-1}), ash and starch (% dry wt) in turf algae and *Ulva lactuca* at different times of day, and their standard deviations

Algae	Time	Energy	Ash	Starch
Turf	6:00	14.044 ± 0.099	29.6 ± 4.0	45.7 ± 5.5
	9:30	14.390 ± 0.350	28.9 ± 5.1	44.0 ± 5.1
	12:30	14.513 ± 0.393	31.3 ± 6.5	52.2 ± 7.2
	16:00	15.014 ± 0.359	37.9 ± 10.0	56.1 ± 2.7
	20:30	14.498 ± 0.505	26.5 ± 5.5	58.5 ± 9.0
Ulva lactuca	5:00	13.462 ± 0.687	16.0 ± 0.8	57.6 ± 5.7
	9:00	14.539 ± 1.212	16.9 ± 0.9	57.1 ± 5.9
	13:00	14.148 ± 0.803	17.3 ± 2.2	57.0 ± 6.8
	17:00	13.578 ± 0.582	15.0 ± 2.2	76.4 ± 15.6
	21:00	13.653 ± 0.453	16.2 ± 2.6	62.8 ± 8.5
	1:30	12.850 ± 0.637	19.2 ± 1.2	61.1 ± 12.6

When the fish fed on turf the number of bites correlated positively with ash and starch ($r_s = 0.8$ each). When the fish fed on *Ulva lactuca*, feeding activity correlated weakly with tidal level ($r_s = 0.6$). These correlations could not be tested for significance with a two-tailed test, because of the small sample sizes ($N = 4$ time points per day in each case).

Discussion

The foraging periodicity of *Parablennius sanguinolentus*, as demonstrated by Taborsky and Limberger (1980), has been confirmed in the present study for fish grazing on turf. It turf is not available, e.g. because of pollution (Stirn et al. 1974, Zoufal unpublished data), *P. sanguinolentus* feeds on sea lettuce, *Ulva lactuca*, with a different feeding rhythm. A significant positive correlation exists between the energy content of algae and feeding activity of blennies. The pattern of algal energy content over the course of the day differed significantly between turf and *U. lactuca*, and the distribution of fish bites paralleled these changes exactly. This is strong evidence in support of the adaptive feeding hypothesis of Taborsky and Limberger (1980).

In the Gulf of Trieste, where the differences between high and low tide are marked, *Parablennius sanguinolentus* might be additionally influenced by the tides, as has been suggested for other places with considerable tide variation (Gibson 1967, 1970, 1971, 1982). In our measurement period at Miramare, high tide was always around noon and therefore coincided with the period of maximum energy content of *Ulva lactuca*. But even though the fish at Miramare were less active at relatively low tide levels in the afternoon, the correlation between feeding and algal energy was unequivocal as compared to that between feeding and tides. Nevertheless, very low tide levels seem to impair all activities. Gibson (1982) attributed this to a higher risk of predation. It might also be due to higher costs of locomotion and the movements necessary to compensate for the surface water current. Ebb tide may also limit access to very shallow algal mats.

Compared to the data of other studies (Paine and Vadas 1969, Montgomery and Gerking 1980), the ash and energy content of *Ulva lactuca* was on average ca. 10% lower in our samples.

Starch does not seem to be the sole basis for the diel increase in energy content of both turf and *Ulva lactuca*, since starch content is still increasing when energy content is already declining. The energy variation is probably also influenced by soluble monocarbohydrates, such as glucose, which may be already reorganised into cell-wall polysaccharides when cell division starts later in the day. The earlier decline in energy content of *U. lactuca* compared to that of turf may be caused by earlier light saturation and light inhibition in the former. Starch content in *U. lactuca* corresponded well to the reference data (Montgomery and Gerking 1980). We could not directly compare our turf analysis results to any previous data, as turf is a mixture of several algae. Protein levels seem to be very low (0.41% of dry weight in turf, 0.60% of that in *U. lactuca*) when compared to the values of Montgomery and Gerking (1980); therefore, we did not take these data into consideration. We suggest either that the cell membranes were damaged by freezing, resulting in a loss of protoplasm, or that a chemical component used for the analysis was unsuitable for this purpose.

Comparison of the diel distribution of feeding with ecological variables other than tidal level in the Gulf of Trieste (i.e., water temperature and weather) did not

suggest any relationships. This confirms the results of previous investigations (Taborsky and Limberger 1980, Polunin and Klumpp 1989).

Taborsky and Limberger have suggested that this feeding periodicity is not endogenous. The present study provides evidence for that hypothesis. The prevalence of *Ulva lactuca* in the Gulf of Trieste has probably been established only during the last 20 yr (see Stirn et al. 1974). It is unlikely that a genetically based alteration of this feeding periodicity could have occurred via natural selection with 10 to 20 generations.

This study suggests that the dependence of food quality on time may be another very important factor for foraging efficiency, along with food quantity. It should therefore be considered a variable of great potential importance for predictions derived from foraging models, perhaps not only for algae feeders (Jarman 1977).

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