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STUDY OF THE NATURAL DIET OF ADULT DRAGONFLIES USING AN IMMUNOLOGICAL METHOD

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The diet spectra of 8 spp., belonging to several systematic and ecological groups, were analyzed by a serological method. It is demonstrated that the zygopterans possess rather uniform diets, consisting predominantly of chironomids. In contrast, the anisopterans have a more heterogeneous diet, which changes during the season; they forage on chironomids, mosquitoes, butterflies and horseflies, as well as on representatives of their own order. The food preferences of these odonates are connected with the peculiarities of the behaviour of the prey rather than its size. Thus, under conditions in West Siberia, they have a maximal effect on population sizes of chironomids and representatives of their own order.

INTRODUCTION

It is widely accepted that dragonflies, being abundant and highly active predators, perform an important role in trophic food webs of aquatic and near-aquatic habitats. However, up to now this point of view was based on fragmentary data, concerning mainly larval diet. Little is known of the qualitative aspects of adults' feeding habits (EDMAN & HAEGER, 1974; ALFORD, 1975; DEAN, 1978; DUNKLE, 1981). Estimates of food consumption have been made for several species using visual observations. (HIGASHI et al., 1979; BAIRD & MAY, 1988, 1989). However, this method has a serious drawback – small prey items, although observed to be captured, cannot be identified since they are heavily damaged by the damselfly's mandibles. To overcome this problem we employed the serological method widely used for the study of feeding of many invertebrates (SERGEEVA, 1982; TITOVA, 1973; SOBOLEVA-DOKUCHAEVA, 1975; REYNOLDS & SCUDDER, 1987; YOUNG, 1980, 1987; BOROVSKIKH & UZENBAEV, 1987; BUTCHER et al. 1988; REILLY & McCARTHY, 1990).

MATERIAL AND METHODS

All investigations were carried out in the surroundings of the Chani Lake (Novosibirsk region, West Siberia) from 1987 to 1989. We studied the diet spectra of eight species of dragonflies (*Libellula quadrimaculata, Sympetrum flaveolum, S. danae, S. vulgatum, Coenagrion lunulatum, C. pulchellum, Lestes sponsa, Sympecma paedisca*); the total number of specimens analyzed was 752. The feeding behaviour of other species was also recorded. A serological test of the gut contents of dragonflies was performed according to the method of SERGEEVA (1982) with some modifications.

Antigen material. – Water-soluble proteins of seven species of potential prey were used as antigens: *Aedes excurciens* (Culicidae), *Chironomus* sp. (Chironomidae), *Hybomitra ciureai* (Tabanidae), *Margarita sticticalis* (Pyralidae) and three species of dragonflies, *Coenagrion vernale, Lestes sponsa* and *Sympetrum flaveolum*. Storage of material and extraction of proteins were performed by conventional methods (SERGEEVA, 1982).

Preparation of antisera. – Rabbits were used for immunization since other animals are known to produce antisera of lower titres against insect proteins (BOREHAM & OHIAGU, 1978). Antigen was injected subcutaneously with Freund's complete adjuvant into six points in the interscapular area. 0.1 mg of protein was used in 0.5 ml of PBS and 0.5 ml of adjuvant per injection. Six immunizations were carried out at two-week intervals with stimulating blood-letting at the 35th day. Such a scheme of immunization allowed us to obtain antisera with titres 1:32 to 1:64 upon injection of a total of 0.6 mg protein. Our investigation did not imply determination of dragonfly influence on the insect species used; therefore it was not necessary to obtain species-specific antisera. Of the seven antisera used, one was specific at the order level (anti-Lepidoptera) three were specific at the family level and were called anti-Culicidae, anti-Chironomidae and anti-Tabanidae, and three were specific at the generic level (anti-Coenagrion, anti-Lestes, anti-Sympetrum).

Immunodiffusion assay. – Analysis of the gut content of the dragonflies was carried out using the Ouchterlony assay as it is the most suitable under field conditions. Glass slides were covered with a 1.5% layer of agar (Difco, USA) in PBS, containing 3% polyethylenglycol (m.w. 6000). Sodium oxide was used as a preservative. Taking into account the relatively low titres of the antisera used, the diameter of the wells for antisera were adjusted to 5 mm in diameter. This increased diameter of the wells allowed us to obtain clear precipitin lines, even with the use of antisera with titres as low as 1:8. The reaction was carried out at 4°C, and the results were scored at 24, 48 and 72 hours after the start of the reaction. Food smears were prepared from entire guts according to the standard method (LOUGHTON et al., 1963).

RESULTS AND DISCUSSION

During the period of investigation in the surroundings of the Chani Lake we found 35 species of dragonflies but only eight of them were abundant. The species were attributed to three groups according to the season of adult flight: spring-summer: *Coenagrion lunulatum, C. pulchellum, Libellula quadrimaculata*; – summer: *Lestes sponsa, Sympetrum danae, S. flaveolum*; – summer-autumn: *Sympecma paedisca, Sympetrum vulgatum.*

An analysis of the natural diet of two species of damselflies from the first group (*C. lunulatum, C. pulchellum*) demonstrated that 75% of individuals tested gave a positive reaction with anti-Chironomidae but only 2% with anti-Culicidae. In natural biotopes in this period, numbers of mosquitoes and chironomids were comparable, so there is a strong indication that dragonflies preferred chironomids. Obser-

vations of dragonfly feeding showed that this preference was associated with the different behaviour of mosquitoes and chironomids during daylight.

Mosquitoes spend the daylight period in the lower level of the herbage near the ground. At the same time chironomids reside in the middle and upper levels of the herbage as well as on the under side of leaves of trees and bushes. Unlike culicids, they make flights from time to time and at these moments they fall prey to dragonflies and damselflies. Serological tests demonstrated that, on average, 64% of individuals from populations of *L. quadrimaculata* and species of *Sympetrum* gave a positive reaction to anti-Chironomidae. Marked differences in the time of swarming of mosquitoes and chironomids also affect their foraging by dragonflies. Swarming of chironomids began at about 7 p.m., although upward flights of separate individuals could be observed much earlier.

Libellula quadrimaculata and *Aeshna* spp. (*A. serrata* and *A. cyanea*) were sepa-

Table I

Proportions of prey items in the diet of odonates

Species	Date of collection tested	No. of individuals	Positive reactions% * anti--Culicidae	anti--Chironomidae	anti--Tabanidae	anti--Lepidoptera	anti--Sympetrum	anti--Coenagrion	anti--Lestes
Zygoptera									
C. lunulatum	5-VI-1988	45	2	75	–	–	–	–	–
L. pulchellum	15-VI-1988	57	2	74	–	–	–	–	–
L. sponsa	10-VII/ 10-VIII-1988	30	11	70	–	–	–	–	–
S. paedisca	10-VII/ 20-VII-1988	30	3	38	–	–	–	–	–
Anisoptera									
Aeshna sp.	13-VIII/ 17-VIII-1988	32	28	19	15	–	66	–	25
L. quadrimaculata	10-VI-1988	102	2	36	–	–	–	4	–
	16-VI-1988	36	–	97	–	–	–	–	–
	27-VI-1988	75	3	64	–	–	–	18	1
	12-VII-1988	35	–	97	–	–	–	–	9
	17-VII-1988	30	3	22	18	–	81	–	77
S. danae	10-VIII-1988	66	–	46	–	2	–	–	2
S. flaveolum	20-VII-1988	48	2	71	–	2	–	–	–
	30-VII-1988	36	5	78	–	–	–	–	44
S. vulgatum	3-VIII-1988	30	–	64	6	–	6	–	18
	17-VIII-1988	50	14	17	20	–	–	–	17
	27-VIII-1988	50	4	38	17	–	–	–	–

* Sum of positive reactions exceeding 100% means that some individuals gave positive reactions with several antisera

rated in time and space. *L. quadrimaculata* actively foraged chironomids swarming from 1 to 3 m above ground from 4 to 8 p.m. In contrast, aeshnas were hunting in swarms of chironomids over the crowns of trees and bushes at a height of 5 to 15 m, and somewhat later (from 8 to 11 p.m.). After 11 p.m., when illumination substantially decreased, aeshnas foraged near the surface of the ground. At this time mosquitoes began to swarm and became their prey. For this reason all specimens of *Aeshna* captured between 11 p.m. and midnight always gave a positive reaction with anti-Culicidae.

On the whole, analysis of feeding of odonates by the serological method and observations in the field showed that the diets of damselflies of spring-summer, summer and summer-autumn groups were rather uniform and did not change during the season (Tab. I).

The data obtained from serological tests were confirmed by the method of withdrawal of a prey item at the moment of its capture by a dragonfly. In the case of damselflies these were exclusively chironomids. However, this method has two drawbacks. The first was a high expense of time and labour. For example, for two hours of observation we were able to withdraw prey items from only 8-10 dragonflies. The second was a scarcity of information. Thus, the presence of mosquitoes in the diet of 11% of individuals from the population of *L. sponsa* was revealed by serological testing but not by prey withdrawal (Tab. I).

The diet of anisopterans turned out to be more heterogeneous. The food of *S. flaveolum* and *S. danae*, which possess a tranquil flight and do not travel large distances, is quite uniform (Tab. I); a large proportion of it consists of chironomids (74.5% of positive reactions for *S. flaveolum* and 47% for *S. danae*). It should be noted that during the third decade of July up to 44% of the *S. flaveolum* population feed on *Lestes* spp., which by this time have almost finished their life cycle and are weak. A much lower proportion of individuals of *S. danae* feed on lestids because of the non-coincidence of the times of moulting.

The diet of *L. quadrimaculata* and *S. vulgatum* turned out to be the most diverse. These two species have fast flight and actively travel for large distances when chasing their prey. They feed low over herbage as well as over the crowns of small trees and bushes. Serological testing demonstrated that, in the majority of cases, their diet consisted mainly of chironomids (Tab. I). The percentage of positive reactions was, on average, 63% for *L. quadrimaculata* and 64% for *S. vulgatum*. However, from the second half of July, the diet of *L. quadrimaculata* changed significantly; 80% of the population of this species foraged on *Sympetrum* and *Lestes*, which became abundant in all biotopes. At this time in the feeding of libellulas horseflies were present as well; the percentage of positive reactions for this prey was 18%.

Two thirds of the way through July *L. quadrimaculata* is replaced by *S. vulgatum*. Due to the smaller size of the latter, it has difficulty foraging on such large prey as *S. flaveolum* and horseflies (*Hybomitra*). For this reason the complement of prey items of *S. vulgatum* is analogous to that of *L. quadrimaculata* but with some dif-

ferences in proportions. The main share consisted of chironomids (64%), whereas *Lestes, Sympetrum* and horseflies were quite rare in its diet (see Tab. I). Two thirds of the way through August the diet of *S. vulgatum* became more restricted because the majority of potential prey had finished their development. Chironomids were present in the diet of 38% of the dragonflies studied, while positive reaction with anti-Tabanidae was observed in 17%. It is possible that a substantial proportion of the diet of dragonflies consisted of other dipterans which became more abundant in the biotopes by that time.

Observations of feeding of *S. vulgatum* under natural conditions and prey withdrawal confirmed the results of the serological tests. Of 16 dragonflies captured 15 had chironomids as a prey and one had a drone fly.

An analysis of feeding of *Aeshna* was more complicated. Visual observations indicated that diverse insects could become their prey from small dipterans to dragonflies of their own genus. For example, *A. serrata* in the evening time actively foraged on swarming chironomids and, later, on mosquitoes, while in the daytime we observed from time to time that juvenile *A. mixta* were eaten. However, among large insects, *Sympetrum* most frequently became a prey of aeshnas because of the close coincidence of their flight times.

We also noted such insects as bees, tipulids, beetles, horseflies and tachines were preyed on by *Aeshna*. In the second decade of August, *A. mixta* was observed foraging Pentatomidae bugs. However, these fragmentary data allow only confirmation of the existing notion of wide polyphagy of dragonflies.

Mass serological testing of feeding of aeshnas is complicated for two reasons. First, a wide spectrum of antisera is needed; second, capture of the necessary number of individuals is quite difficult because they fly rather high and impetuously. Therefore, data concerning *Aeshna* in Table I, although collected during the entire season, do not reflect all the diversity of the diet spectrum of this group.

As can be seen from Table I, the maximum proportion of positive reactions (66%) is registered for chironomids. The other insects in the second decade of August occur much more rarely in the diet of aeshnas, 15-28% of positive reactions with mosquitoes being the most abundant.

CONCLUSIONS

During our initial investigation it was established that, during the season, the diet spectra of damselflies in western Siberia remained rather constant and consisted mainly of two components: mosquitoes and chironomids, the share of the latter being substantially higher. This is associated with large population sizes and greater accessibility of these prey items resulting from the behaviour of chironomids.

The diet spectra of the majority of species of dragonflies are rather diverse and change during the season. At the beginning of the season they feed on small dipterans. However, with the rise in population sizes of larger prey items *(Sympetrum, Lestes*

and horseflies [*Hybomitra* and *Chrysops*]) these also are included in the diet.

On the whole, an analysis of feeding of odonates, with reference to different systematic and ecological groups leads us to disagree with the suggestion that the share of potential prey items in the diet of dragonflies is proportional to their abundance in the biotopes studied (HIGASHI et al., 1979). Dragonflies demonstrate certain preferences associated, first of all, with the size of a prey item (MOKRUSHEV, 1972) and also with the peculiarities of its behaviour. The balance of energy outlay connected with the capture of small and large prey items presumably also plays a significant role in determining which prey are eaten.

The data obtained allow us to assert that, in near-aquatic biotopes of forest-steep zone not subjected to strong anthropogenic influences in western Siberia, dragonflies have a maximum effect on population sizes of chironomids and representatives of their own order. The results of the qualitative analysis of the diet of odonates performed by the serological method provide a base for quantitative estimates.

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