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THE IMPACT OF PARASITES IN ADULT POPULATIONS OF ZYGOPTERA

A. ÅBRO
Institute of Anatomy, University of Bergen,
Årstadveien 19, N-5009 Bergen, Norway

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Based on countings and mark-release-recapture experiments the effects of intestinal eugregarines and ectoparasitic water-mite larvae on zygopteran imagines were studied in habitats on the W coast of Norway. In *Enallagma cyathigerum* and *Coenagrion hastulatum*, the larval mites attach during emergence: imagines on their maiden flight revealed the highest numbers of attached mites; the figures appeared to decline in the course of adult life. Heavily loaded individuals disappeared during the prereproductive and the succeeding reproductive phase. *Pyrrhosoma nymphula* and *Lestes sponsa* on their maiden flight carried low numbers of mites; the infestation increased consequent on repetitive visits to the water for reproduction, and the number of mites tended to rise with increasing adult age. In all Zygoptera the load of gregarines increased with advancing adult age owing to recurrent ingestion of infective gregarine oocysts; the gregarines influence zygopteran longevity. No individual carrying very high numbers of both gregarines and mites was ever recorded. Parasitism by mites and gregarines influences community structure of zygopterans through selective pressure against heavily-loaded hosts. — In wet cool summers there was virtually a non-appearance of propagating stages of the gregarines. Production of infective gregarine oocysts depends on the heat budget of the zygopteran flying season: the intrahost processes leading to union in syzygy demand ambient air temperatures of 18-20° C or higher, while the extra-host development of oocysts over the subsequent 2-3 weeks requires temperatures not much below. As for damages inflicted by mites, a corresponding dependence on warm weather could not be demonstrated. Ruptures of the midgut wall by gregarine overinfection involve injuries and partial dissolution also of the zygopteran epidermis, which is recognizable in the field as a discoloration of the zygopteran abdomen. Lesions to the midgut wall by gregarines and epidermal damage by mites were most tangible during periods of fine weather; enfeeblement of the hosts is presumed to be due to desiccation.

INTRODUCTION

In western Norway, dense colonies of Zygoptera imagines associated with small isolated tarns or ponds are afflicted by two types of parasites: (1) Loads of ectoparasitic water-mite larvae, sucking the host's tissue fluids, cause injuries to the cuticle and epidermis so that hosts become prone to desiccation; damages to the epidermis appear soon after attachment of the larval mites (ÅBRO, 1982). (2) Heavy infection of intestinal gregarines inflicting injuries to the midgut wall weakens the hosts, most pronounced in individuals of somewhat advanced adult age (ÅBRO, 1974, 1987).

The present study is based on persistent observations over several years. It comprises an extension of previous investigations on parasites in Zygoptera (ÅBRO, 1982, 1987) and was undertaken to elucidate joint effects of the two kinds of parasites in zygopteran colonies of environments with endemic water-mite and gregarine parasitism. The coexistence of the two kinds of parasites on the zygopteran individual as well as on the population level of zygopterans was related to environmental factors.

MATERIAL AND METHODS

The study was undertaken in an area of heath and boggy ground with coniferous woodland near Bergen, western Norway, mainly in a habitat around a small tarn, the breeding site of large dense zygopteran colonies with endemic gregarine and water-mite parasitism. For comparison, sparse marginal populations of zygopterans associated with wide bodies of water were also surveyed. The zygopteran species most abundant within the study area were *Enallagma cyathigerum* (Charp.), *Pyrrhosoma nymphula* (Sulz.), and *Lestes sponsa* (Hansem.). Less frequent was *Coenagrion hastulatum* (Charp.). The censuses are confined to these species.

As damages to the zygopteran hosts depend upon the number of parasites present (ÅBRO, 1974, 1982), censuses of larval water-mites as well as gregarine trophozoites of the zygopteran imagines are essential. To estimate the viability of the hosts, it proves useful to know their postermergence age. To that purpose, teneral imagines captured on their maiden flight were marked and released to be recaptured in the habitat on subsequent occasions. Teneral imagines of *E. cyathigerum* and *C. hastulatum* on their maiden flight away from the water had their freshly attached water-mite larvae counted in the field under a dissecting microscope before marking and release. Larval mites on *Enallagma* and *Coenagrion* specimens, recaptured in surrounding woodland or during later visits of the zygopterans to the water for reproduction, were also counted in the field, whereupon the damselflies were brought to the laboratory for further examination. The laboratory specimens had their alimentary canal dissected free to make acetic orcein squash preparations for viewing the condition of the midgut wall and counting the gregarine trophozoites present (ÅBRO, 1974). Also imagines of *Pyrrhosoma*, with its closely synchronized emergence different from the spread emergence in *Enallagma* (ÅBRO, 1987), were screened as to mite infestation and gregarine load. In most years a succession of several fine warm days during late May ensured a synchronized emergence in *Pyrrhosoma*.

The larval water-mite produces within its host a feeding device called stylostome, a gelatinous, resilient, blind sac, which is formed at the expense of subjacent epidermal cells (ÅBRO, 1984). Feeding is accomplished via the stylostome. Under natural conditions fully formed stylostomes are

found one day after piercing of the host cuticle. Each larval mite develops only one stylostome and it remains within the host's body after the mite has dropped off as a record of past parasitic association (ÅBRO, 1982). Thus, the stylostomes belong to the host for a much longer time than do the mites themselves. The inner of the zygopteran body walls were examined regarding stylostomes. To improve the visibility of stylostomes, they could effectively be stained in alcian blue without previous chemical fixation. By counting stylostomes (functional and postfunctional) it is possible to obtain reliable figures of larval mites that have parasitized any zygopteran individual.

Faecal pellets were collected from damselflies stored overnight in cardboard boxes in a refrigerator. Histo-processing methods used were according to earlier reports (ÅBRO, 1974, 1984).

RESULTS

REMARKS ON PHENOLOGY

The climate of western Norway with incessant weather changes predominates the study area. The zygopteran imagines fly only when there is sunshine. Considering the entire flying season, weather conditions may vary notably from year to year with exceptional, stable periods of prolonged sunny weather. Surveillance over several years has revealed the duration of the zygopteran adult season to be governed largely by the prevailing weather conditions. The flying season for respective zygopteran species dwindles and ceases earlier in dry summers compared with an average summer and above all when compared with cool rainy summers.

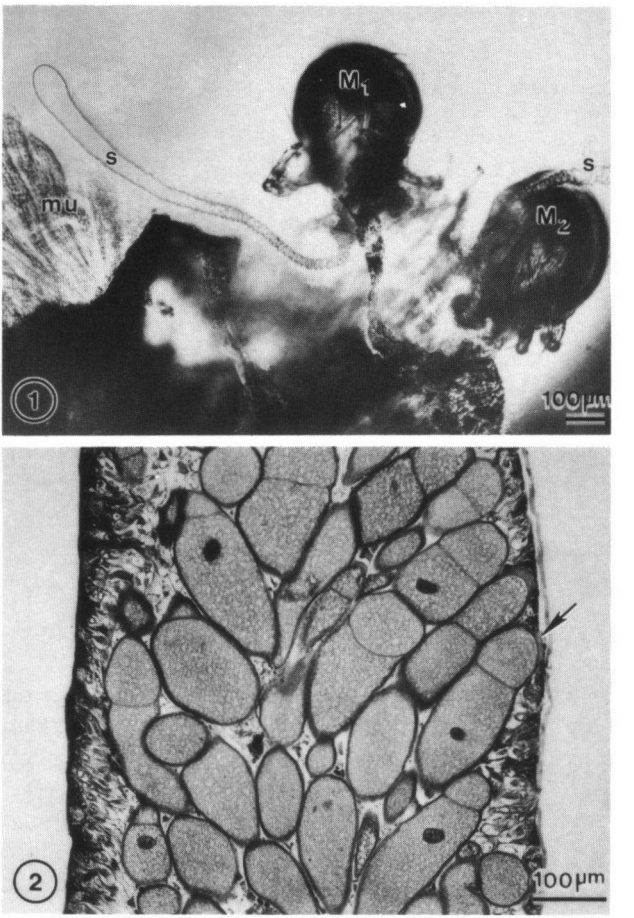
WATER-MITES

Promptly after anchoring in feeding position each larval water-mite (*Arrenurus* spp.) produces a single stylostome tubule within the host's epidermis layer. On suitable sites of the host's cuticle mite larvae may settle in close proximity, thus leading to loss of considerable patches of the epidermis (Fig. 1); the damages are only imperfectly repaired by congregating haemocytes (ÅBRO, 1984).

In the study area, *Enallagma cyathigerum* and *Coenagrion hastulatum* become infested during emergence and the larval mites engorge synchronously; fully formed stylostomes could be found on the second day of adult life. The stylostome formation has not been found to be noticeably influenced by fluctuations of ambient temperatures. *Pyrrhosoma nymphula* and *Lestes sponsa* on their maiden flights carried low numbers of mites; they become infested mostly during later visits to the water for reproduction but to a lesser extent (ÅBRO, 1982). In habitats surveyed, mite infestation of *Pyrrhosoma* and *Lestes* revealed increasing loads in the course of the reproductive phase. *Pyrrhosoma* had several mites attached to soft cuticle of the abdomen's deep pleural folds. *Pyrrhosoma* was infested with larval mites of two different colours, olive-green and bright red; the red ones were attached to the pleural folds. The other zygopterans carried olive

coloured mites only.

Water-mite larvae were counted on teneral zygopteran imagines captured on their maiden flight and so were the mites on zygopterans sampled in woodland glades during the subsequent prereproductive maturation phase spent away from



Figs 1-2. *Enallagma cyathigerum:* (1) A fragment of thoracal body wall (day 2 of adult life), with two attached ectoparasitic *Arrenurus* larvae (M_1 and M_2) freshly submersed in insect Ringer's solution and viewed unstained in transmitted light. The tissues were gently squashed beneath the coverslip to disclose the vitreous stylostome (s) adhering to the larval mouthparts. The stylostome is a long, thin, convoluted tubule terminating in a rather smooth bulb. mu — muscle tissue. — (2) Midgut, longitudinally cut, with the lumen blocked by free crowding gregarines. Day 16 of adult life. Considerable damage is inflicted to the gut epithelium. The arrow points to a site where the epithelium is worn away so the bare muscle layer remains. Paraffin section stained with haematoxylin-eosin.

the water. Mites were also recorded from samples of fully coloured and reproductively active imagines collected at the water; these imagines were taken to the laboratory for counting stylostomes. In the material are included also zygopteran individuals without any mites/stylostomes. Samples taken during the prereproductive phase demonstrated a close correspondence between the number of stylostomes and attached mites. In the reproductive phase, however, the number of stylostomes in a zygopteran individual was often higher than the number of attached engorged mites, thus indicating that several mites having established a parasitic association had detached. Based on counts of stylostomes, the distribution of parasites (with a higher average figure) appeared different from that based on attached mites only; the stylostome number reflects the parasite load most obviously. The results are presented in Figure 3. Table I displays the mite load of some marked and recaptured individual zygopterans. None of the zygopterans captured on their maiden flight with a heavy mite load and then marked have been recaptured.

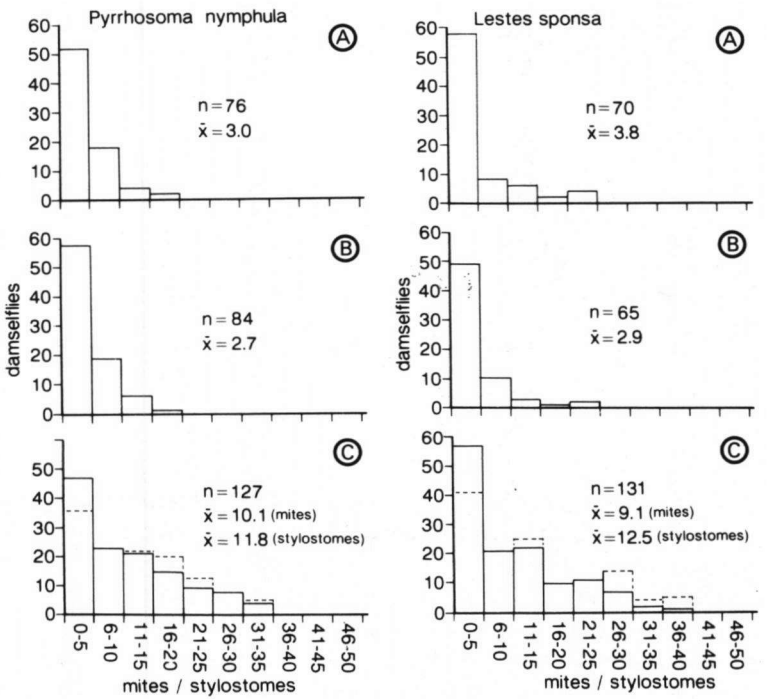


Fig. 3. The load of mites/stylostomes associated with zygopteran imagines of four species during (A) maiden flight, (B) prereproductive maturation phase, and (C) reproductive phase. Based on data collected from a habitat in three successive seasons. [Mites: continuous line; — stylostomes: broken line].

Table I
Number of larval water-mites on adult Zygoptera marked on their maiden flight and subsequently recaptured; stylostome number in parenthesis

Specimen	Maiden flight (day 1)	Prereproductive maturation phase (day 2-13)	Reproductive phase (day 14-)
Enallagma cyathigerum			
1 ♂	42	41	—
1 ♂	30	30	—
1 ♂	31	-	22(31)
1 ♂	0	0	0(0)
1 ♀	14	—	9(13)
1 ♀	11	—	8(11)
Coenagrion hastulatum			
1 ♂	29	29	—
1 ♂	24	—	13(22)
1 ♂	9	9	0(9)
1 ♀	11	11	10(10)
Pyrrhosoma nymphula			
1 ♂	0	0	3(3)
1 ♂	5	—	12(12)
1 ♂	7	—	13(14)
1 ♀	0	—	7(7)
1 ♀	6	6	—
Lestes sponsa			
1 ♂	2	—	6(7)
1 ♂	0	—	1(1)
1 ♂	0	0	4(4)
1 ♀	2	2	11(11)
2 ♀	0	0	0(0)
1 ♀	1	0	3(4)

In *Enallagma* and *Coenagrion* the average number (= arithmetic mean) of mites/stylostomes is highest on the maiden flight; the average later diminishes because of the disappearance of heavily loaded damselflies. Based on several years of surveillance in the study area, a record of thirty or more mite larvae/stylostomes on an individual zygopteran is considered a heavy infestation; thus the selected figure of 30 is an arbitrarily-established limit. The highest numbers of larval mites recorded on *Enallagma* and *Coenagrion* specimens on their maiden flight amount to 60-70; during the reproductive phase the number of mites (respectively stylostomes) did not surpass 50. At that time, extremely high individual loads did not occur, so a trend appeared toward a lower average load in the course of adult life.

GREGARINES

Recurrent infections of intestinal gregarines, belonging to the species *Hoplorhynchus oligacanthus* (Sieb.) and *Menospora polyacantha* Léger (Actinocephalidae), take place in the course of zygopteran adult life, so heavily loaded individuals were of somewhat advanced age (Fig. 2); in the study area *Hoplorhynchus* is predominating. More than one ingestion of gregarine oocysts seems to be a prerequisite to promote the reproductive processes in gregarines for eventually producing a new generation of infective oocysts (ÅBRO, 1976).

Growth and development of the gregarine trophozoites and a maturing of the free gamonts run most smoothly in periods with high ambient air temperatures during daytime, i.e. 18-20° C or higher. The salient point concerns temperatures within the abdomen of zygopteran imagines when exposed to solar radiation. The sexual processes in the gregarine gamonts with attendant pairwise union in syzygy and joint envelopment in a gametocyst appear to take place only if the temperature rises to such a favourable level. On the other hand, the gregarines appear to undergo developmental arrest with falling temperatures. In the laboratory, newly formed gametocysts isolated from zygopteran faecal pellets were found to stop developing at 15° C. After the attached gregarines have become free in the gut, their mobility clearly increases with rising temperatures. Disruptions of the midgut epithelium resulting in ulceration and subsequent ruptures of the gut wall are brought about by the movements of crowding, free gregarine gamonts prior to association in syzygy. On warm calm sunny days severe intestinal damage in zygopterans becomes recognizable in the field as feeble imagines with spots of discoloration on their abdomen; such individuals appeared sick and could readily be captured. The discolorations were found to comprise partial necrosis or dissolution of the epidermis layer owing to deposition of gut contents on the internal body wall. Such discolorations did not appear in predominantly cool rainy summers.

The flying season 1986 was wet and cool from beginning to end; no gametocysts were recorded in samples of zygopteran imagines collected during this summer. On the other hand, the year 1982 and especially 1988 had longlasting periods of fine sunny weather with a clear sky and continual suitable temperatures during the flying season; in these years huge numbers of fully formed gametocysts were recorded. In several years a continual 2-3 week period of warm sunny weather in June around midsummer appeared to provide an opportunity for gregarine reproduction. In the years 1982 and 1988 as in other years with much fine weather, the flying season for respective zygopteran species came to an early end. Observations for several years have revealed the gregarine germ production, manifested primarily as findings of gametocysts in zygopteran faeces, to depend on the weather conditions and heat budget of the entire flying season. The stage of gregarine life cycle found to be most temperature-sensitive is that

prior to syzygy, which in *Hoplorhynchus* and *Menospora* occurs just before gametocyst formation.

Individual zygopterans combining high figures for both mites/stylostomes and intestinal gregarines have not been recorded. Generally, imagines harbouring heavy loads of gregarines carried low or moderate numbers of mites. Severe winters were not found to influence the occurrence of gregarines or arrenurid mites.

DISCUSSION

The parasite burden is presumed to have debilitating effects on zygopteran imagines so that they are less likely to survive when exposed to adverse environmental conditions (ÅBRO, 1987). Mites are believed to drain considerable quantities of body fluids from their hosts. Mite-infested zygopterans may suffer desiccation because of injuries to cuticle/epidermis (ÅBRO, 1982), most tangibly in periods of fine weather with rather high ambient air temperatures. For full development of their gonads zygopterans need to take in water in addition to food. From the first day of adult life till reproductive maturation zygopteran imagines were found doubling their weight or more (measured on a microbalance, unpublished observations). Flying imagines could be seen snapping water from surfaces and also swallowing drops of dew at dawn. In addition, zygopterans are supposed to take up water when roosting during rain.

The host-parasite interaction of larval water-mites on insects has been summarized by SMITH (1988). Despite the present study's sparse material certain trends can be outlined. It seems probable that different site preferences exist for different zygopteran hosts and water-mite species. Deleterious effects owing to water-mite (*Arrenurus* spp.) infestation soon become manifest in *Enallagma cyathigerum* (ÅBRO, 1984), recognizable already during the prereproductive maturation phase spent away from the water as the disappearance of those individuals most heavily loaded. The lower maximum counts and averages as to mites/stylostomes in *Enallagma* and *Coenagrion* specimens during their reproductive phase compared with records from their maiden flight and even from the prereproductive phase could be interpreted as a sign that heavily-parasitized individuals succumb early in adult life. Larval mites attach firmly to their hosts to develop a stylostome and do not detach easily during this phase of active parasitism; only very few mites become abortive (ÅBRO, 1979, 1984). A damselfly population, heavily parasitized by larval water-mites (*Hydrachna* spp.) and studied using mark-recapture techniques, revealed that the survival rate of mature females decreased with increasing parasitism (ROBINSON, 1983). A mite-induced mortality in zygopteran communities seems to exist. Gerrids and corixids (Hemiptera-Heteroptera) heavily infested by larval water-mites (*Limnochares* spp.) were found to vanish early (FERNANDO & GALBRAITH, 1970).

A joint heavy burden of water-mites and gregarines as well as an overload of either of the parasites seem to enfeeble the host to a similar measure; deleterious effects appear most clearly when it happens to coincide with lasting periods of fine weather and favourable temperatures; at least, it is most readily observed under such circumstances. In *Enallagma* and *Coenagrion* afflicted with mite infestation early during adult life, the gregarine infection manifests itself during later adult life, thus influencing longevity. That no zygopteran imagines with a very high number of both gregarines and mites have been taken in the field indicates that specimens thus heavily-burdened might not survive for long, or individuals carrying a high number of mites do not live long enough to become overinfected with gregarines.

The pattern of water-mite infestation appears fairly similar at all kinds of zygopteran breeding sites within the study area, while the gregarine infections have been found considerably more heavy, with several overinfected individuals, in tight zygopteran colonies associated with small solitary tarns and ponds of ancient standing, in contrast to the slight gregarine infection recognized in marginal habitats (ÅBRO, 1987). Since harm due to gregarines becomes discernible in somewhat aged imagines (ÅBRO, 1971), it remains obscure whether gregarines influence the ability of the zygopterans to survive the pre-reproductive phase or to what extent the infection interferes with zygopteran reproductive rate.

Injuries to the midgut epithelium by gregarines are supposed to diminish the uptake of nutrients (ÅBRO, 1987). Ambient air temperatures seem to have a substantial effect on the gregarine-induced detriments to the gut, as massive deteriorations of the gut wall related to bending movements of detached, crowding gregarine gamonts about to couple in syzygy (ÅBRO, 1974) are observed mostly in periods of warm weather, when free gamonts move most vigorously. Sexual/reproductive processes leading to the subsequent oocyst formation in gregarines do not occur if the temperature within damselfly hosts falls below certain levels. Also, the extrahost development of the gregarines requires rather high temperatures in the habitat. It might well be that gregarines scarcely complete reproduction in exceptionally wet and cool summers with only a few records of gametocysts or none at all. It should be emphasized that gametogony, zygote formation and subsequent development of infective gregarine germs (= oocysts) take place within the extrahost gametocyst (ÅBRO, 1976). After release from the gametocyst, which takes place by simple rupture, oocysts, remaining in a cryptobiotic state, are apparently scattered over the habitat ground by wind and splashing rain-water. The oocysts have a highly resistant capsule. It has been shown, however, that a considerable number of fully formed oocysts are non-infective because of unsuccessful development (ÅBRO, 1976). Oocysts are ingested along with food; thus parasitism appears to be mostly accidental. In the course of adult life, zygopterans consume considerable

amounts of small-sized insects (with chironomids and ceratopogonids predominating); it has been demonstrated that these insects may serve as carriers of *Hoplorhynchus* oocysts, which were suspended on tarsal bristles of the midges (ÅBRO, 1976). Accordingly, this might be one mode of infection but presumably not the only one.

The weather appears as the primary determinant of gregarine development. It is generally believed that harsh weather conditions stress zygopterans but so does apparently also warm sunny weather, because high local temperatures, intensified by direct solar irradiation, seem to promote the activity and development of parasites, thus bringing adverse effects to bear on their hosts. The dark pigmented abdominal cuticle of the damselflies absorbs solar radiation that is transformed into heat. Weather and parasites interact complexly in the population dynamics of damselflies.

Dormant extrahost gregarine oocysts spending the winter in the habitat seem to retain their infective potential for two or more years: The infection pattern of gregarines in dense damselfly colonies with endemic gregarine parasitism does not exhibit interseasonal variation (ÅBRO, 1987) despite intervening poor years as to gregarine reproduction and oocyst formation, and a high level of infection persists.

Unlike findings in zygopterans from other climatic regions (SCHNEIDER, 1875; FOERSTER, 1938a, 1938b; GEUS, 1969) zygopteran larvae from waters in the coastal districts of western Norway have never been found to harbour any gregarines (ÅBRO, 1974, 1987). The absence of gregarines in zygopteran larvae at these latitudes might be explained by the prolonged larval development of zygopterans in water of rather low temperatures which may be unsuitable for gregarine development and propagation.

GEUS (1969) states *Actinocephalus sieboldi* (Kölliker) to be a genuine eugregarine species different from *Hoplorhynchus oligacanthus* (Sieb.); however, LEVINE (1988) considers *A. sieboldi* a synonym for *H. oligacanthus*. Previously, *A. sieboldi* has been announced as a gregarine species occurring in *Lestes sponsa* and *Sympetrum danae* from western Norway, both odonates flying in late summer and early autumn; the stages of syzygy, gametocyst formation or oocysts of that gregarine were never recorded (ÅBRO, 1974). According to GEUS (1969) the gametocyst and oocyst of *A. sieboldi* are unknown. It should be kept in mind that attached gregarine trophozoites as well as the free gamonts exhibit a large amount of individual variation as to size and shape. In the late flying *Sympetrum danae, A. sieboldi* were found to be the only gregarine present. It may well be that *A. sieboldi* ought to be considered a seasonal form of *H. oligacanthus,* generated owing to continual moderate and low temperatures or other environmental factors of a fading summer and early autumn in western Norway. In the habitats studied, the gregarine parasites may reproduce in specimens of *Enallagma, Pyrrhosoma, Coenagrion,* and occasionally *Lestes* at the height of the season

around midsummer, thus providing infective oocysts also for late flying *Lestes* and *Sympetrum*. Records of aberrant and unsuccessful gregarine gametocysts in response to the time of the year prevail during early or late season (ÅBRO, 1976). Norwegian odonates live near the edge of their range of distribution, which might be reflected in their gregarine fauna differing from that of continental populations of the same odonate species.

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