A novel association between an endemic stickleback and a parasitic dinoflagellate. 1. Seasonal cycle and host response

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We report the first case of a dinoflagellate infection of stickleback (Gasterosteidae), discovered on an endemic population of *Gasterosteus* that inhabits an acidic bog lake on the Queen Charlotte Islands, western Canada. A major difference between this and other dinoflagellate infections is that the autotrophic vegetative cyst rather than the parasitic trophont is the predominant stage on the fish. During peak infection in July, 99% of the fish were infected, and cysts often covered the entire fish including the eyes. Density of cysts was highest on the dorsal surface of the fish (to 68/mm); this is possibly associated with the photosynthetic ability of the cysts. There were no consistent differences in the infection among sizes classes of fish or between the sexes. Salmonids (*Oncorhynchus kisutch* and *Salvelinus malma*), which are uncommon in the lake, also harboured cysts, but at very low densities. Host reponse to the initial infection included extensive epithelial hyperplasia, producing a layer of cells over the entire fish that enclosed the dinoflagellates. Subsequent infections were covered by additional layers of epithelium, resulting in a thick gelatinous coating. Even in cases of extreme infection, the fish exhibited no obvious behavioral indicators of pathological responses to the infection.

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Cet article présente le premier cas connu d'infection d'épinoches (Gasterosteidae) par des dinoflagellés; les épinoches provenaient d'une population endémique de *Gasterosteus* d'un lac de tourbière dans les îles de la Reine-Charlotte, dans l'Ouest canadien. La principale différence entre ce cas d'infection et les autres infections de dinoflagellés réside dans le fait que c'est le kyste végétatif autotrophe plutôt que le trophonte parasite qui constitue le stade prédominant chez les poissons. Au moment où l'infection a atteint son point culminant, en juillet, 99% des poissons étaient infectés et les kystes recouvraient alors entièrement le poisson, y compris les yeux. La densité des kystes était maximale sur la surface dorsale des poissons (jusqu'à 68/mm), probablement à cause de la capacité de photosynthèse des parasites. Il n'y avait pas de différence significative entre les classes d'âge ou entre mâles et femelles quant à la gravité des infections. Des salmonidés (*Oncorhynchus kisutch* et *Salvelinus malma*), rares dans le lac, portaient aussi des kystes, mais en très petits nombres. Les poissons réagissaient à l'infection initiale par une importante hyperplasie épithéliale qui se manifestait par l'apparition d'une couche de cellules recouvrant tout le poisson et enfermant les dinoflagellés. Les infections subséquentes déclenchaient la formation de couches additionnelles d'épithélium donnant lieu à un épais recouvrement gélatineux. Même dans les cas très graves, les poissons n'ont pas manifesté de comportements particuliers indicatifs de réactions pathologiques à l'infection.

[Traduit par la revue]

Introduction

Protozoan parasites of freshwater stickleback include a diversity of ciliate, sporozoan, and flagellate taxa (Wootton 1976). Among the groups not represented on this host are the dinoflagellates, which are infrequent parasites of freshwater fish (Jacobs 1946; Petrushevski and Shulman 1958; Cachon and Cachon 1987). During surveys of stickleback (*Gasterosteus*) on the Queen Charlotte Islands, western Canada, an endemic population in which the fish were covered with dinoflagellates (Fig. 1) was discovered in an acidic pond (Reimchen 1984). The occurrence in highly acidic conditions (pH 4.1) was unexpected, since most freshwater dinoflagellates, including all of those parasitic on fish, are alkalinophilic (Taylor and Pollingher 1987). Furthermore, the dinoflagellate exhibited life cycle and morphological features that have not been previously described in general parasitological studies of freshwater fish in British Columbia (Bangham and Adams 1954) and throughout Canada (Margolis and Arthur 1979).

We describe here the general features of this novel association, including seasonal occurrence, distribution on the host, and host responses to the dinoflagellate. A description of the life cycle and a preliminary classification of the dinoflagellate will be presented separately (Buckland-Nicks et al. 1990).

Material and methods

Rouge Lake is located in a *Sphagnum* bog approximately 2 km from open marine coastline in the northeastern region of the Queen Charlotte Islands, British Columbia. General characteristics of the habitat and fish are described by Reimchen (1984). In brief, the pond (1.5 ha) is shallow (ca. 1.5 m depth) and highly acidic (pH 4.1–4.5), and has low calcium levels (0.36–1.20 ppm), low magnesium levels (0.03–0.91 ppm), and low overall conductivity (70 μS^{-1}). The pond contains threespine stickleback (*Gasterosteus aculeatus*), small numbers of Dolly Varden (*Salvelinus malma*), and occasional juvenile coho salmon (*Oncorhynchus kisutch*).

Rouge Lake was sampled on 12 occasions from 1976 to 1987 and samples were routinely preserved in 10% formaldehyde. Samples obtained in July 1987 were fixed in 2.0% glutaraldehyde in 0.1 M cacodylate buffer (for detailed methodology see Buckland-Nicks et al. 1990).

Heavily infected stickleback were sent to a number of parasitologists for identification of the parasite. F. J. R. Taylor (Department of Oceanography, University of British Columbia) tentatively identified the cysts as dinoflagellate although with unknown affinities. Our subsequent observations confirmed this identification and showed a suite of characters, including a dinokaryotic nucleus and chloroplasts, that supported a dinoflagellate classification (Buckland-Nicks et al. 1990).

Samples contained stickleback ranging from 20 to 75 mm standard

length (SL). As a preliminary method of estimating numbers of dinoflagellate cysts per fish and to obtain a general measure of the regional distribution of parasites on the fish, we counted total numbers of dinoflagellates on the following regions which were chosen to provide a representative sample of the fish surface. (*i*) Mouth: midline on roof of buccal cavity (total area 2.8 mm^2). (*ii*) Lateral head: behind and level with the eye but anterior to the pre-opercular bone (total area 2.8 mm^2). (*iii*) Dorsal head: frontal bone midway between the eyes (total area 2.8 mm^2). (*iv*) Dorsal trunk: midline between first and second dorsal spines (total area 2.8 mm^2) (*v*) Ventral trunk: junction between base of pelvic spine and ascending process of the pelvic girdle (total area 2.8 mm^2). (*vi*) Anal fin: membrane between third and fourth rays of anal fin (total area ca. 4 mm^2). (*vii*) Gills: third gill arch and filaments (total area ca. 40 mm^2). Total number of cysts was recorded under a dissecting microscope (×60), with the area delimited on an ocular grid. The third gill arch was removed and all cysts were counted. These counts were made on six fish, which on visual examination showed the heaviest gelatinous coating in the sample, from each of the 12 samples. All the remaining fish were scored only for the dorsal head region. Differences in density of cysts among seasons and size classes and between the sexes of fish were analyzed with ANOVA.

Several coho fry and Dolly Varden were also captured and examined for cysts.

Results

Dinoflagellate frequency on host stickleback

In heavily infected stickleback, dinoflagellate cysts covered most of the body (Figs. 1–3) and the gill filaments (up to 230 cysts on the third arch). Mean densities were greatest on top of the head (9.3 dinoflagellates/mm^2, maximum 68/mm^2) and lowest on the roof of the mouth and gill filaments (2.04/mm^2; ANOVA, $F = 10.32$; $P < 0.001$; Table 1). Densities on top of the head and on the side of the head differed significantly from each other and both differed from those at all other sites (Student–Newman–Keuls test, Table 1). There were no differences in density between the ventral trunk, fins, and buccal cavity.

Since differences in dinoflagellate densities between samples were significantly positively correlated among the six sites on the stickleback ($P < 0.001$ for all comparisons), we used the dorsal head region as a representative site for comparing dinoflagellate abundance among samples. There were marked seasonal differences in abundance: dinoflagellates were absent in winter, rare in spring, and most abundant in midsummer (Fig. 8; ANOVA, $F = 52.8$; $P < 0.001$). The relative proportions of infected fish were 0% in January, 15% in May, 85% in June, 99% in July, and 65% in September. We compared males and females for dinoflagellate density but found no significant difference ($\bar{x} = 12.7$ and 14.8, respectively; $F = 0.65$; $P = 0.42$; ANOVA).

Among different length classes (20–75 mm) there was a marginal but nonsignificant tendency for small-bodied stickleback to have the lowest densities of dinoflagellates ($P = 0.053$, ANOVA). Partitioning the data into month groups confirms this trend for June and September but in July, during peak infection, both the smallest and the largest stickleback had lower infection rates than intermediate-sized fish (Fig. 9).

TABLE 1. Dinoflagellate densities (per square millimetre) on representative regions of stickleback ($N = 78$)

	Mean	SD	Student–Newman–Keuls test						
			1	2	3	4	5	6	7
1. Dorsal head	9.23	14.9		*	*	*	*	*	*
2. Lateral head	6.63	10.8			*	*	*	*	*
3. Dorsal trunk	3.15	6.7							
4. Ventral trunk	2.82	5.3							
5. Anal region	2.58	6.3							
6. Mouth	2.04	2.8							
7. Gills	0.85	1.6							

*$P < 0.05$; all other comparisons are nonsignificant.

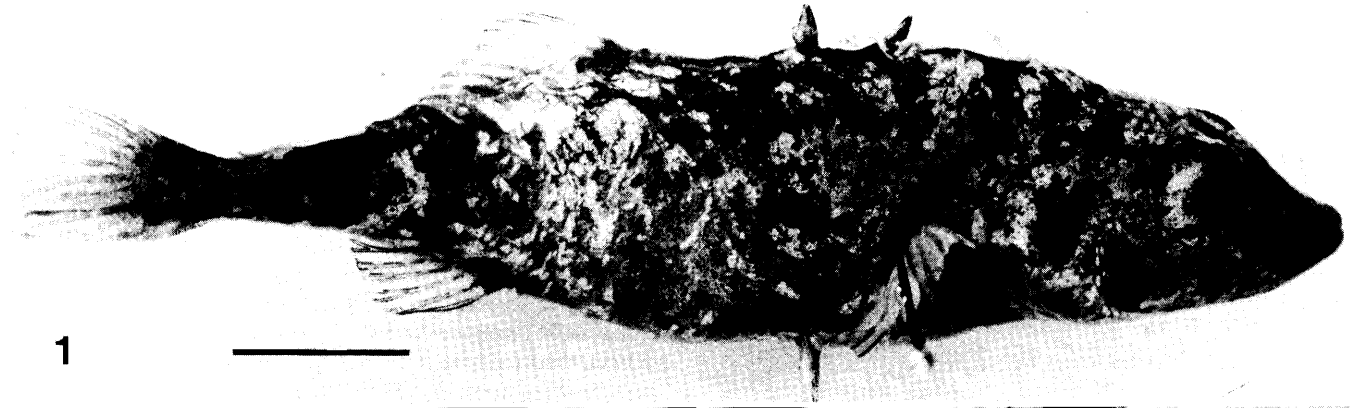
Most salmonids occurred in the samples from January and May, and like the stickleback during these months, lacked dinoflagellates. However, a single coho fry in the June samples and a single Dolly Varden in the September samples had a few dinoflagellates on the head and trunk. No salmonids were captured during July, the period of greatest cyst density on stickleback.

Host response to dinoflagellates

Dinoflagellate infection induces hyperplasia in the host stickleback, during which superficial epithelial cells migrate onto the surface of the fish (Fig. 4) and gradually form a layer that overgrows and encloses individual dinoflagellates (Figs. 5, 6, and 7). Contact between migrating epithelial cells is maintained by pseudopodia and by desmosomes (Fig. 5). The pseudopodia contain numerous filaments, notably tonofilaments, which enable frequent shape changes to occur during migration (Fig. 4). The epithelial cell envelope can become quite thick (ca. 0.5 mm) and has a gelatinous consistency. Several other cell types also migrate into this layer, including macrophages and mucus-secreting goblet cells (Fig. 4). Concentrations of mucus-secreting goblet cells around dinoflagellate cysts may be partly responsible for the slimy, gelatinous texture of the epithelial envelope.

Early in the year (January–April) no epithelial hyperplasia is evident; in May cysts can occasionally be found in the gill filaments, and by June hyperplasia begins to appear on the snout and head as a thin whitish film. This film can be easily scraped off, and examination shows cysts in all stages of development from the smallest (ca. 20 μm) to the largest (150 μm). By July

FIG. 1. Threespine stickleback from Rouge Lake with numerous dinoflagellate cysts embedded in gelatinous coating (visible as white areas on eye and body). Scale bar = 1 cm. FIG. 2. Portion of eye of lightly infected threespine stickleback, showing embedded cysts (arrowheads). Dark areas represent cavities where cysts have fallen out during handling. Scale bar = 0.3 mm. FIG. 3. Light micrograph of 1 μm thick section of gelatinous coating removed from an infected threespine stickleback and viewed with transmitted light to show embedded dinoflagellate cysts of different sizes. Three of the cysts have undergone fission. Scale bar = 75 μm. FIG. 4. Light micrograph of gelatinous coating comprising superficial epithelial cells (SE), macrophages (M), and mucus-secreting goblet cells (G) which have overgrown surface of fish. Scale bar = 3 μm. FIG. 5. Transmission electron micrograph of squamous epithelial cells that form gelatinous coating. Numerous filaments in cytoskeletal matrix (arrowheads) permit migration of cells via pseudopodia (P). Adjacent cells maintain contact via desmosomes (D). Scale bar = 0.4 μm. FIG. 6. Scanning electron micrograph of superficial epithelial cells of fish that have overgrown a dinoflagellate cyst (C). Note that during processing, the epithelial cell layer cracked, revealing the cyst beneath. Scale bar = 10 μm. FIG. 7. Transmission electron micrograph through edge of dinoflagellate cyst (C) that has been overgrown by superficial epithelial cells. Scale bar = 1 μm.


1


2


3


M
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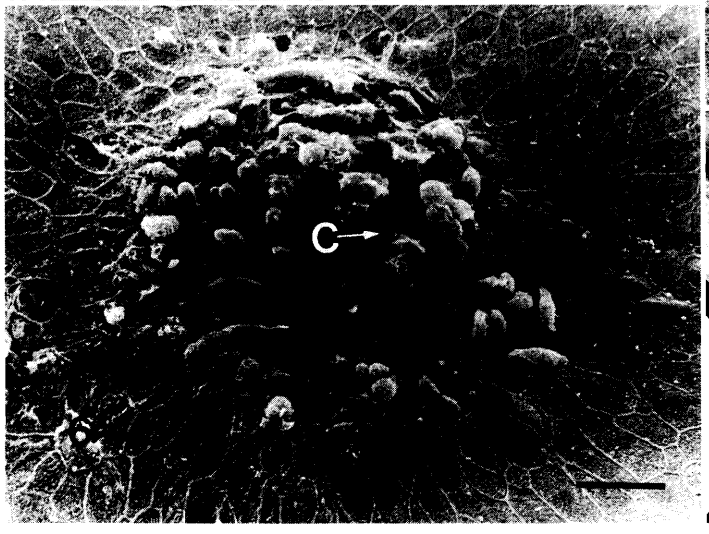
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7

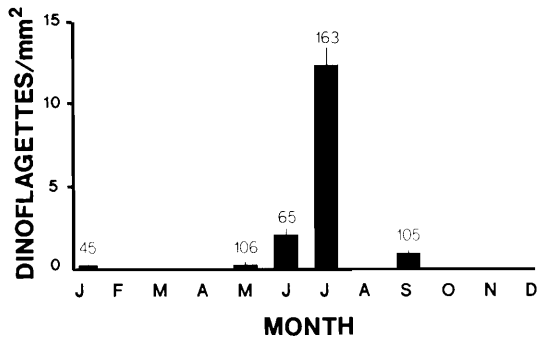

FIG. 8. Seasonal differences in mean cyst density (+1 SE) on dorsal head region of threespine stickleback. Numbers above histograms show number of stickleback analyzed.

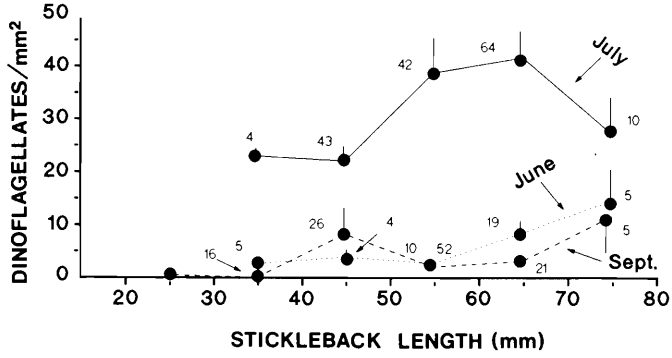

FIG. 9. Relationship between mean cyst density (±1 SE) and length of threespine stickleback. Numbers beside data points show number of stickleback in sample.

the entire fish is covered with a thick gelatinous coating and by fall the coating and embedded cysts have disappeared. In brief observations of live fish we saw no apparent adverse responses to the infection. The presence of territorial males and gravid females with extensive coating indicates reproductive potential.

Discussion

These results constitute the first reported evidence of dinoflagellate infection in Gasterosteidae (see review by Wootton 1976). Stickleback have been sampled throughout their distribution and it seems unlikely that such an association would have been overlooked if it was geographically widespread. The general similarity in appearance between the infected stickleback and those infected with the common ciliate *Ichthyophthirius multifiliis* introduces a potential for misclassification, but even in studies focussing on protozoan parasites (Lester 1974) dinoflagellates have not been observed.

A common substrate for ectoparasitic protozoans is the gills (Noble et al. 1963; Lawler 1967; Lom 1970; Rogers and Gaines 1975). Although a large number of cysts were found on the gills in our study, densities were highest on the head and lowest on the gills, mouth, and ventral regions of the trunk, suggesting that the dorsal surface is a preferred substratum of the settling dinoflagellates.

A typical life cycle of a dinoflagellate parasite such as *Piscinoodinium* begins with a free-swimming flagellated swarmer which transforms into a trophont after attachment to the fish. The trophont derives nutrition from the host through embedded rhizoids. After a period of growth, the trophont drops off the fish, encysts, and falls into the sediment (Jacobs 1946; Lom and Schubert 1983). The dinoflagellate in the present study spends most of the life cycle in the encysted state on the fish. Numerous cysts have been examined in the size range 20–150 μm and none have been found with any evidence of rhizoids; we suspect that the swarmer must encyst very quickly after attachment to the fish (Buckland-Nicks et al. 1990). All cysts examined contained chloroplasts, and therefore, the presumed source of nutrition is photosynthesis. These ideas are in keeping with our observations that the highest frequencies of cysts occurred on the dorsal surface of the host, which is subject to the greatest illumination.

Epithelial hyperplasia is a recognized response to many ectoparasitic infections of fish (Rogers and Gaines 1975) but has not been reported for a dinoflagellate infection. The timing of the initial host response is important because if it precedes the peak infestation, the majority of dinoflagellates would attach to the external layer of epithelial cels and thereby be excluded from the sensitive host tissues. Subsequent overgrowth of new epithelial cells would enclose the most recently attached dinoflagellates. This would explain why dinoflagellate cysts are found at all levels in the gelatinous coating, which is often many cells thick. Furthermore, the gelatinous coating may act as a barrier to other types of ectoparasites, such as the trematode *Gyrodactylus* sp., that would normally penetrate sensitive host tissues (Lester 1974; Lester and Adams 1974; J. Buckland-Nicks, personal observations).

Protozoans ectoparasitic on fish, such as *Coatia* and *Ichthyophthirius* (Petrushevski and Shulman 1958) and *Piscinoodinium* (Lom and Schubert 1983), are commonly pathological to their hosts and result in extensive mortality in the populations. Juvenile freshwater fish infected with the dinoflagellate *Piscinoodinium limneticum* may succumb within several weeks of initial infection, but heavily infected adult fish are able to survive for extended periods without clear evidence of stress (Jacobs 1946). Dinoflagellate densities on the smallest stickleback might therefore be relatively low because heavily infected individuals had already succumbed. The coating on infected Rouge Lake stickleback, which covered the trunk, eyes, and gill filaments, would reduce lateral line and visual sensitivity as well as restrict respiration. In our brief visits to the locality, we did not observe any shoreline mortality of infected stickleback. Heavily infected stickleback collected in traps exhibited no adverse behavioral traits, such as gulping air, flashing, or other signs of agitation, that would indicate a pathological effect of the dinoflagellate on the fish. Furthermore, although 99% of the fish were infected in summer, by January all fish observed had lost the gelatinous coating and appeared healthy. It would seem probable that this dinoflagellate does not cause a typical parasitic infection. In some respects, the dinoflagellate is uncharacteristic of the parasitic order Blastodiniales, not only because it is an acidophile but also because the major phase on the host is an autotrophic cyst rather than a parasitic trophont. There are several instances of nonparasitic symbioses between dinoflagellates and metazoans (Trench 1987).

The dinoflagellate–stickleback association may not be widely distributed. Over 30 ponds and lakes in the vicinity of Rouge Lake were surveyed (Reimchen et al. 1985; T. E. Reimchen, unpublished data), and cursory examination of these samples has failed to show any other instances of this association. Consequently, it may be limited to Rouge Lake. Morphological endemism of the stickleback in this locality indicates that the population has been isolated from other populations for extended periods (Reimchen 1984). Analyses of dinoflagellate cysts in sediment cores could provide a chronology of the

association, since a pond adjacent to Rouge Lake shows a continuous depositional sequence during the last 9000 years (Warner 1984). Assuming that the dinoflagellate association is indeed restricted to this small geographical region, it represents a further example of the endemism that characterizes a diversity of biota on the Queen Charlotte Islands (Calder and Taylor 1968; Foster 1965; Moodie and Reimchen 1976). The present study, in conjunction with previous observations that the cestode *Cyathocephalus* is common in some of these stickleback populations yet is rare elsewhere in western Canada (Reimchen 1982), indicates that a more extensive examination of fish parasites in the archipelago would be warranted.

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