Vol. 37: 139–144, 1999 | DISEASES OF AQUATIC ORGANISMS Dis Aquat Org | Published July 30

Attraction of *Urastoma cyprinae* (Turbellaria: Urastomidae) to the eastern oyster *Crassostrea virginica*

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ABSTRACT: *Urastoma cyprinae* Graff, 1882 has been reported on the gills of various bivalve species, including the eastern oyster *Crassostrea virginica*. While earlier workers refer to *U. cyprinae* as a commensal, recent findings reveal that the 'gill-worm' can be pathogenic to its molluscan host and may, therefore, also be considered to be parasitic. To determine whether there exists a definite attraction of *U. cyprinae* to oysters, a series of experiments were conducted using specially designed glass chambers. Results indicate that *U. cyprinae* is negatively phototactic and that it is more attracted to oysters when compared to other molluscan species such as mussels and clams. *U. cyprinae* was found to be highly attracted to isolated oyster mucus, and, to a lesser extent, to mucus-coated gill tissue. Findings confirm that the presence of *U. cyprinae* in oysters is not coincidental and that mucus plays an important role in attracting the worms to its host.

KEY WORDS: Turbellaria · *Urastoma cyprinae* · Molluscs · *Crassostrea virginica* · Gills · Oyster mucus

INTRODUCTION

Urastoma cyprinae is a ciliated turbellarian that measures between 0.4 to 0.8 mm in length. It has 2 prominent eye spots situated anteriorly (Burt & Bance 1981, Pike & Wink 1986, Tyler & Burt 1988) and an oral-genital pore located at the posterior end (Fig. 1).

Urastoma cyprinae has been reported as free-living in marine mud and on algae (Marcus 1951, Westblad 1955). It has also been described on the gills of various bivalve species, including the clams *Tridacna maxima* and *T. gigas* (Goggin & Cannon 1989), and the mussels *Mytilus edulis* (Fleming et al. 1981, Teia dos Santos & Coimbra 1995) and *M. galloprovincialis* (Noury-Sraïri et al. 1990, Murina & Solonchenko 1991, Robledo et al. 1994, Trotti et al. 1998). In Atlantic Canada, this worm (Fig. 2) has been observed in the eastern oyster *Crassostrea virginica* (Burt & Drinnan 1968, Fleming et al. 1981, Fleming 1986, Boghen et al. 1993).

The nature of the host-parasite relationship has never been well-defined. Although Burt & Drinnan (1968) and Fleming et al. (1981) consider *Urastoma cyprinae* to be a commensal in oysters, and therefore, not harmful to its host, a more recent study (Robledo et al. 1994) has shown that the worm is responsible for serious destruction of host gill tissue in the mussel *Mytilus galloprovincialis*.

Fleming (1986) suggested that, in oysters, *Urastoma cyprinae* feeds on the mucus coating the gills. This, however, has never been the subject of any scientific investigation. If it can be shown that the worms are attracted to mucus, such a finding would support this contention.

The current study attempts to determine (1) if there is a definite attraction of *Urastoma cyprinae* to oysters, (2) how this compares to other molluscan species and (3) to establish whether mucus plays a role in attracting the worm to its host.

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Fig. 1 Whole mount of *Urastoma cyprinae*. Arrows: eyes, op: oral-genital pore

MATERIAL AND METHODS

Field study. In June 1997, 300 adult oysters *Crassostrea virginica*, 300 mussels *Mytilus edulis*, and 300 clams *Mya arenaria* were obtained from the following commercial culture operations in Atlantic Canada: Cocagne Oyster Farm, Chiasson Aquaculture and Mills Sea Food Ltd. Ten bivalves of each species were dissected and carefully examined under a dissecting microscope to make certain that they were free of *U. cyprinae*. The molluscs were combined and subsequently separated into 3 heterogeneous groups, each group consisting of 100 animals of each of the 3 species. These were placed into standard Vexar bags (0.6 m × 1.2 m, 1 cm mesh) and anchored on the ocean bottom along a beach in Shippagan Bay (New Brunswick, Canada), in an area where *Urastoma cyprinae* is known to occur. The bags were held at a depth of 25 cm (below low tide), and separated from each other by distances of 4 m. Sampling was conducted every 3 to 4 wk over 5 mo, at which time 10 molluscs of each species were collected from each bag for a composite sample size of n = 90. The animals were transported in plastic bags on ice to the Université de Moncton and were examined for *U. cyprinae* under a dissecting microscope within 48 h of arrival. A total of 150 molluscs of each of the 3 species were collected and examined (see Table 1).

Laboratory study. Seven experiments were conducted between the spring of 1995 and the fall of 1997, using wild oysters infected with *Urastoma cyprinae* collected at low tide in Shippagan Bay, New Brunswick. The oysters were transported in plastic bags to the Université de Moncton at either 4°C for Expts 1 and 2 or at ambient water temperatures of 20 to 23°C for Expts 3 to 7. The oysters were opened and the worms were removed from the gills using a Pasteur pipette. They were divided into groups of 60 to 100 depending on the experiment (see below), and maintained in glass Petri dishes in filtered sea water (25‰) for 12 h. All experiments were conducted at room temperature (22 to 23°C) and in total darkness (based on our findings from Expt 1) in specifically designed glass chambers with either 2 or 4 interconnected wells (Fig. 3). The total volume capacity of the 2- and 4-well glass chambers was 3.4 ml and 7.0 ml respectively.

To determine the response of *Urastoma cyprinae* to light versus dark conditions: in Expt 1, half of the wells of the experimental glass chambers were covered with dark plastic, thus preventing light penetration on one side. Sea water (25‰), filtered twice (Whatman Qualitative filter paper, <11 µm) was added to the chambers through the central opening (Fig. 3a) to provide an aqueus medium in which the worms could move freely.

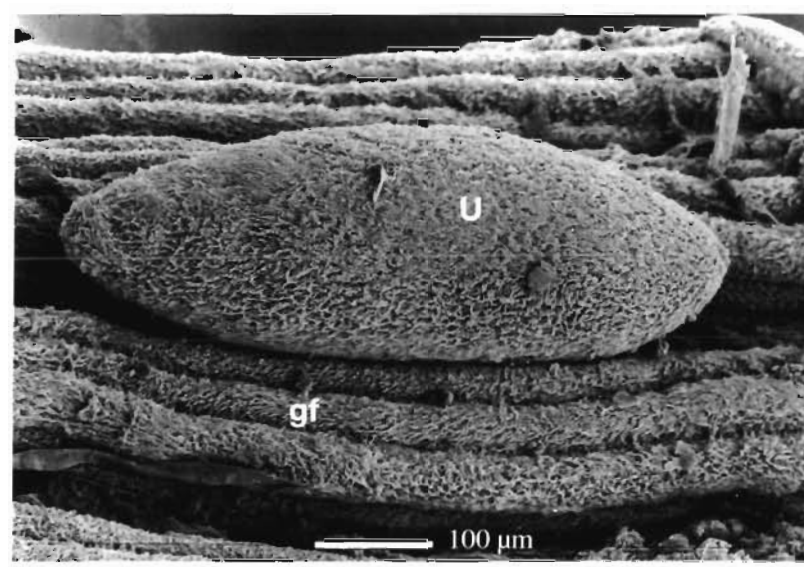

Fig. 2. *Urastoma cyprinae*. Scanning electromicrograph of gill worm on the oyster gill filaments. u: *U. cyprinae*, gf: gill filaments

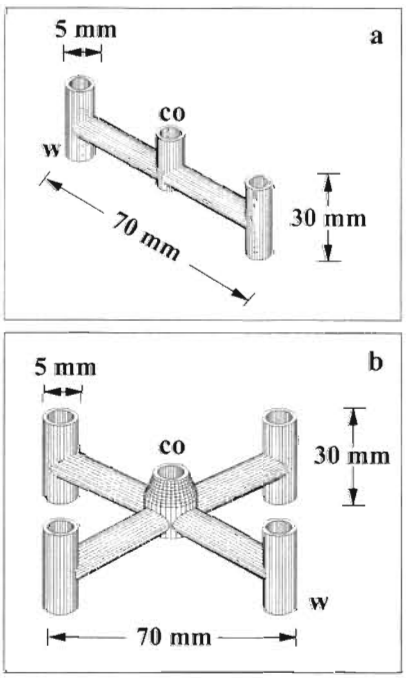

Fig. 3. Specially designed glass chambers used in experiments with *Urastoma cyprinae*. (a) 2-well glass chamber; (b) 4-well glass chamber. co: central opening, w: well

A total of 10 trials using 60 worms trial^{-1} was undertaken. *U. cyprinae* were introduced into the chambers through the central opening and after 60 min the wells were drained with a pipette and the worms were counted under a dissecting microscope.

Expts 2 to 4 explored the extent of attraction of *Urastoma cyprinae* to whole oysters versus sea water (5 trials, 60 worms trial^{-1}), whole oysters versus whole mussels (10 trials, 60 worms trial^{-1}) and whole oysters versus whole clams (10 trials, 60 worms trial^{-1}). The possible attractants were mixed with an equal amount of filtered sea water (25‰) and homogenized for 2 min, after which the homogenates were diluted with filtered sea water at 1:10 (v/v) homogenate:sea water. The mixtures were subsequently centrifuged for 5 min at 3800 rpm (2300 × *g*), and the semi-formed pellets were collected by pipette and introduced into the wells of the glass chambers. This was immediately followed by the addition of sea water and *U. cyprinae*, as previously described for Expt 1.

Expts 5 and 6 were conducted using the 2-well chambers to test the attraction of *Urastoma cyprinae* to the following potential attractants:isolated oyster mucus versus oyster body (without gills) and isolated oyster mucus versus gills. These studies were based on 10 trials (60 worms trial^{-1}). Expt 7 made use of the 4-well chambers (Fig. 3b) and explored the attraction of *U. cyprinae* to the following stimulants: isolated oyster mucus versus gills versus mucus-free gills versus sea water. Processing of all stimulants was similar to that described above, with the exception of isolated oyster mucus and mucus-free gills.

For the preparation of isolated oyster mucus, 3 ml of mucus was carefully drawn by pipette from the oyster gills, diluted with 10 parts filtered sea water (25‰) and concentrated at 3800 rpm for 5 min. Preparation of mucus-free gills involved gentle rinsing of isolated gills with filtered sea water 5 to 6 times, followed by homogenization of gill tissue and centrifugation as described above.

Student *t*-tests were employed to analyze the data based on the findings using the 2-well glass chambers. A 1-way ANOVA, followed by the Tukey multiple comparisons test was used to analyze the data based on the experiment employing the 4-well glass chambers (Zar 1984).

RESULTS

The field study demonstrates that *Urastoma cyprinae* is more attracted to oysters than to the other molluscan species (Table 1). *U. cyprinae* is consistently more prevalent in oysters (100%), compared to mussels (0 to 16.6%) and clams (3.3 to 33.3%). This tendency is also reflected by the average mean number of worms per oyster (45.3 to 346.9) compared to individual mussels (0 to 0.03) and clams (0.07 to 0.6) respectively.

The results of the *t*-tests based on the data using the 2-well glass chambers are summarized in Table 2. Expt 1 (Fig. 4a) demonstrates that *Urastoma cyprinae* is

Table 1. Prevalence, mean and range of intensity of *Urastoma cyprinae* in *Crassostrea virginica*, *Mytilus edulis* and *Mya arenaria* in Shippagan Bay during the summer of 1997

Date collected	Host	n	Prevalence (%)	Intensity Mean[a]	Intensity Range
Jun 24	*C. virginica*	30	100	45.3	1–50
	M. edulis	30	0	0	0
	M. arenaria	30	3.3	0.1	3–3
Jul 21	*C. virginica*	30	100	309.5	49–656
	M. edulis	30	13.3	0.3	1–4
	M. arenaria	30	16.6	0.2	1–2
Aug 20	*C. virginica*	30	100	346.9	42–944
	M. edulis	30	16.6	0.1	1–2
	M. arenaria	30	33.3	0.6	1–5
Sep 19	*C. virginica*	30	100	97.6	23–251
	M. edulis	30	0	0	0
	M. arenaria	30	10	0.1	1–1
Oct 7	*C. virginica*	30	100	313	18–833
	M. edulis	30	0	0	0
	M. arenaria	30	6.6	0.07	1–1

[a]Average number of parasites per infected host

negatively phototactic ($p < 0.001$). Results of Expts 2 through 4 (Fig. 4b to d) show that *U. cyprinae* is more attracted to oysters compared to sea water ($p = 0.028$), and this phenomenon is also evident when the worms are offered a choice between oysters and mussels ($p 0< 0.001$), and oysters and clams ($p = 0.020$).

Data from Expts 5 and 6 indicate that *Urastoma cyprinae* is more attracted to isolated oyster mucus ($p = 0.004$) than to the oyster body (without gills), but there is no significant difference in attraction ($p = 0.174$) when *U. cyprinae* has to choose between isolated oyster mucus and isolated gill tissue (Fig. 4e,f).

In Expt 7 (Table 3), the Tukey test confirms that *Urastoma cyprinae* is most attracted to isolated oyster mucus, followed by gills, mucus-free gills and sea water respectively (Fig. 5). Examination of the normal probability plots for each attractant show the residuals are approximately normal. The standard deviation varies from approximately 3 to 9 among the 4 possible attractants. As a consequence a non-parametric test was also carried out to confirm the ANOVA results.

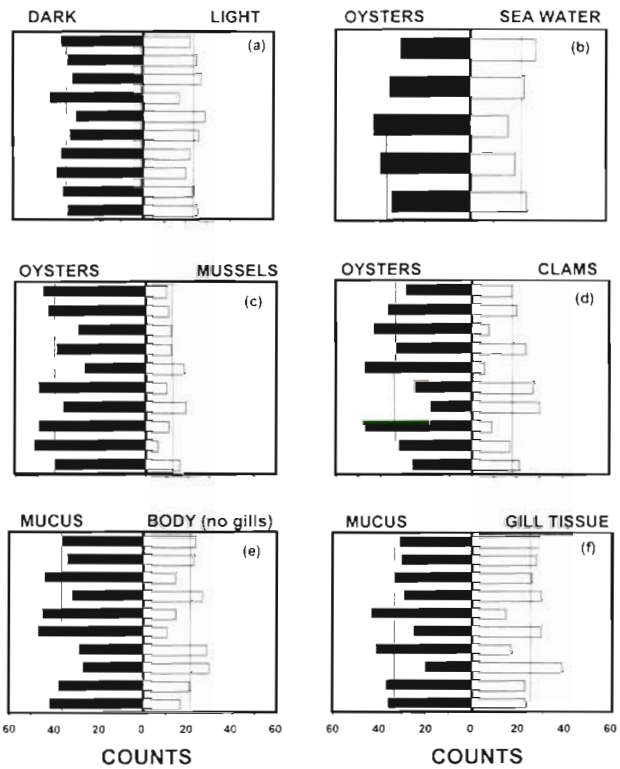

Fig. 4. *Urastoma cyprinae*. Number of gill worms recovered in each well containing the respective materials tested. Each bar represents 1 trial of the total number of *U. cyprinae* recorded. (a) Number *U. cyprinae* depicted in wells under light and dark conditions (Expt 1); (b) number of worms attracted to oyster versus sea water (Expt 2); (c) number of *U. cyprinae* attracted to oysters versus mussels (Expt 3); (d) number of worms attracted oysters versus clams (Expt 4); (e) number of *U. cyprinae* attracted to isolated oyster mucus versus oyster body with gills removed (Expt 5); (f) number of worms attracted to isolated oyster mucus versus gills (Expt 6). Vertical lines indicate the average number of *U. cyprinae* for all trials for each stimulant tested

DISCUSSION

Although *Urastoma cyprinae* occurs as a free-living organism (Marcus 1951, Westblad 1955), it has also been observed on the gills of various bivalve molluscs (Burt & Drinnan 1968, Fleming et al. 1981, Fleming 1986, Goggin & Cannon 1989, Noury-Sraïri et al. 1990, Murina & Solonchenko 1991, Robledo et al. 1994, Teia dos Santos & Coimbra 1995). Burt & Drinnan (1968) described the association between *U. cyprinae* and the eastern oyster as being commensal. More recently, certain authors (Murina & Solonchenko 1991, Teia dos Santos & Coimbra 1995) have suggested that *U. cyprinae* induces pathology in mussels *Mytilus galloprovincialis* and *M. edulis*. Robledo et al. (1994) have shown conclusively that in the case of *M. galloprovincialis*, *U. cyprinae* causes serious disruption of gill filaments and significant hemocytic infiltration in the affected areas. These authors signal that *U. cyprinae* may be a potential threat to the mussel industry in Northern Spain.

Given the economic importance of the oyster industry in Eastern Canada, valued at 2.6 million dollars in 1995, a closer examination of the nature of the host-parasite relationship between *Urastoma cyprinae* and *Crassostrea virginica* is warranted.

Our work confirms that *Urastoma cyprinae* is highly attracted to oysters and that their presence in the host is not coincidental. Furthermore, the field work and laboratory Expts 2 to 4 indicate that *U. cyprinae* shows a greater attraction to oysters than to other native molluscan species.

Table 2. Results of the Student *t*-tests for equality between the number of worms attracted to each stimulant of a tested pair

Expt	Stimulant	$X^a \pm SE^b$	Stimulant	$X^a \pm SE^b$	n	p
1	Dark	36.4 ± 1.1	Light	26.3±1.1	10	$p < 0.001$
2	Oysters	37.0±2.1	Sea water	23.0±2.1	5	$p = 0.028$
3	Oysters	42.1±2.5	Mussels	12.5±1.3	10	$p < 0.001$
4	Oysters	33.7±3.1	Clams	18.0±2.6	10	$p = 0.020$
5	Mucus	37.4±2.2	Body (no gills)	21.2±2.0	10	$p = 0.004$
6	Mucus	32.5±2.2	Gills	26.0±2.2	10	$p = 0.174$

[a]Average number of worms attracted to a given stimulant
[b]Standard error on the mean

Table 3. P-values for the Tukey multiple comparisons test based on the data using the 4-well glass chambers (Expt 7)

	Mucus	Gills	Gills (mucus removed)	Sea water
Mucus	1.000	0.187	0.002	0.000
Gills	0.187	1.000	0.149	0.001
Gills (mucus removed)	0.002	0.149	1.000	0.086
Sea water	0.000	0.001	0.086	1.000

Fleming (1986) suggests that the turbellarian may be feeding on the mucus secreted by oyster gills. While the present research did not focus on the worm's diet, our results clearly indicate a definite attraction of *Urastoma cyprinae* to isolated oyster mucus. The attraction of *U. cyprinae* to both isolated oyster mucus and oyster gills is perhaps to be expected, given the fact that gills are heavily coated in mucus, and that the latter plays an integral part in the suspension-feeding of bivalve molluscs (Ward et al. 1994, Ward 1996).

In vivo studies on suspension-feeding in bivalves using endoscopic techniques (Ward 1996) reveal that mucus present in the dorsal tracts is of low-viscosity and that particles are transported suspended in a mucus-slurry. This is qualitatively different from the high-viscosity mucus present along the ventral margins, in which material is bound in a continuous mucus string (Beninger et al. 1993). It would be an interesting challenge to establish if there is a correlation between the number of *Urastoma cyprinae* on specific regions of the oyster gills and mucus composition and flow. Such information may prove to be useful in future studies that focus on the incidence of *U. cyprinae* in relation to the rate of growth of oysters as well as the final quality of the cultured product.

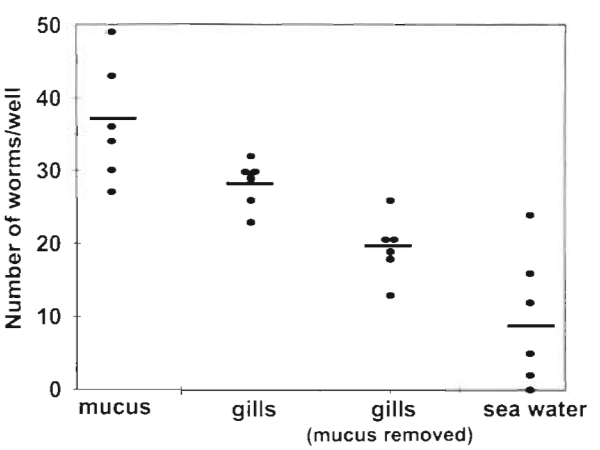

Fig. 5. *Urastoma cyprinae*. Number of gill worms (points) present in each well containing the following materials tested during each of the 6 trials (Expt 7): isolated oyster mucus, gills free of mucus and sea water. Bars represent the mean number of worms for each of the 4 substances

Acknowledgements. We would like to express our gratitude to Dr H. Rosenthal (University of Kiel, Germany), Dr M. D. B. Burt (Huntsman Marine Science Centre, St. Andrews, NB, Canada) and Dr G. Miron (Department of Biology, Université de Moncton, NB, Canada), for reviewing our manuscript and for their very helpful comments and suggestions. We are also grateful to Mr John Dalgleish (Department of Physics, Université de Moncton, NB, Canada) and Professor Charles Maillet (School of Engineering, Université de Moncton, NB, Canada) for their technical assistance with the construction and the drawings of the experimental chambers used in the present study. Financial support provided to the senior author by the Faculty of Research and Graduate Studies of the Université de Moncton is appreciated. This project is part of the Richibucto Environment and Resource Enhancement Program.

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Editorial responsibility: Albert Sparks, Seattle, Washington, USA

Submitted: October 29, 1998; Accepted: May 2, 1999
Proofs received from author(s): July 29, 1999