VIRGINIA FISHERY RESOURCE GRANT PROGRAM

FRG 2022 Final Report

Title: Problems with Peritrichs

Project Investigator: Mike Congrove, Oyster Seed Holdings, Inc

Signature of Principal Investigator

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Date

Introduction

Stalked ciliates are a type of protozoa that have branched or unbranched stalks containing inverted bell-shaped bodies. The presence of cilia, or hair-like structures, on the oral region of the organism are their defining characteristic. Stalked ciliates mostly feed on small bacteria and debris, but some also consume other ciliates. Their life cycle contains a free-swimming stage and a sedentary stage. These organisms are common in freshwater, brackish, and marine environments, especially where bacterial populations are high, and they are commonly found as epibionts (colonizing surfaces of living organisms). Epibiosis was previously understood as a commensal relationship between organisms (an epibiont and "basibiont"or host), however, studies have shown that epibionts can cause damaging effects to their host organisms (Overstreet 1987; Shinn et al. 2015; Mahasri et al. 2021).

There are several privileges that epibiont pests enjoy while being attached to their host: ease of transport, protection from predators, availability of nutrients, and expansion of their range. They may occasionally provide benefits to their host by masking chemical scents from predators (Fernandez-Leborans & Gabilondo 2007). However, this relationship is not always commensal, if they share the same niche, both the epibiont and their host may end up competing for resources. In some instances, the epibiont may decrease growth and reproduction of the host, and impede normal physiological functions of the host. Solitary stalked ciliate epibionts may have little to no effect on their hosts, but colonial stalked ciliates could have a significant impact.

The detrimental effects of stalked ciliate infestations are documented for commercially important crustaceans (primarily shrimp and copepods), but not bivalves. In crustaceans, stalked ciliates primarily infest the gills and cause mechanical obstruction and physical damage, which deprives the host of oxygen. However, other adverse effects of epibiont relationships with crustaceans include decreased fecundity, interference with feeding and locomotion, and increased sensitivity to contaminants. Our oyster hatchery has dealt with both light infestations of these pests and heavy infestations that result in slowed growth and mortality of oyster seed. From our own observations, it appears that these organisms attach themselves to the bill of the oyster, and get pulled into the mantle with the feeding current. It is documented that attaching to surfaces that experience increased water flow can enhance stalked ciliate feeding rates, which could be why they are attaching to the oyster bill. When the oyster seed is disturbed by the stalked ciliates entering the mantle, it snaps shut. Therefore, the stalked ciliates are inhibiting their feeding process. Based on our communication with other local hatcheries and nurseries, some are also experiencing these infestations with the same deleterious effects.

Objectives

This project had two overall main objectives:

1. Identify and quantify specific stalked ciliates that are causing these infestations of oyster seed.
2. Identify the seasonality of stalked ciliate infestations.

Methods

We sampled these stalked ciliates opportunistically at our hatchery and other sampling locations as infections occurred from March 2022 - August 2022. Frozen, fixed, and live samples were collected for analyses conducted by Dr. Richard Snyder. For DNA analyses, a sample of small, colonized seed was placed in a microfuge tube and stored in the freezer until transported. Fixed samples contained a small sample of seed placed in a vial of Lugol's iodine and 14 ml of seawater. Live samples were collected if immediate transport (within 24 hrs) to the VIMS Eastern Shore Laboratory was feasible. These samples were kept in a vial of seawater at room temperature during transport. Dr. Richard Snyder at Virginia Institute of Marine Science Eastern Shore Laboratory quantified and identified stalked ciliates from our samples to the genus level.

Results

Results from samples dated March 11 and March 28, 2022 indicated that the most abundant stalked ciliates in the bottle system are a Suctorian species (Fig. 1) in the genus *Acineta* (Table 1 & 2). These organisms contain two bundles of tentacles and a stalk that coils when it contracts. It is unknown if the toxin in their tentacles reacts with oyster tissue. Free-swimming Scutio ciliates, branched peritrichs in the genus *Zoothamnium* (Fig. 2), and single-stalked peritrichs in the genus *Vorticella* were also observed, but appeared to be rare compared to Suctorians. It was determined that the heavy organic load in the bottle culture system supports all the stalked and free-swimming ciliates and the bacteria they feed on.

During June 2022, our hatchery also observed the first detrimental infestation of stalked ciliates on oyster seed in our outdoor upwellers. This sample was considerably different from the bottle system samples. The quantity of suctorians were relatively the same and may have been carried over from the bottle system. However, there were more stalked ciliates in larger colonies (Table 3) which appeared to be a different species compared to previous samples. A *Folliculina* (Fig. 3) species was also observed in this sample, and are commonly found on adult oysters in Nandua Creek on the Eastern Shore of Virginia. Benthic diatoms and detritus common to surface

waters were also identified. Overall, the stalked ciliates in this sample, other than suctorians, appear to be inocula from surface waters.

Our other hatchery sampling location, Cherrystone, and one of the nursery sampling locations, Rappahannock River Oysters, did not communicate any observation of an infestation this season. However, Little Wicomico Oyster Company did reach out to us with concerns of an infestation and slow growth of seed, and we received a sample of the infested 3 mm seed on 6/23/22. Identification results from this sample determined that the same suctorian genus *Acineta* found in OSH bottle systems was present as well as another suctorian genus *Ephelota*, which was not observed in our systems.

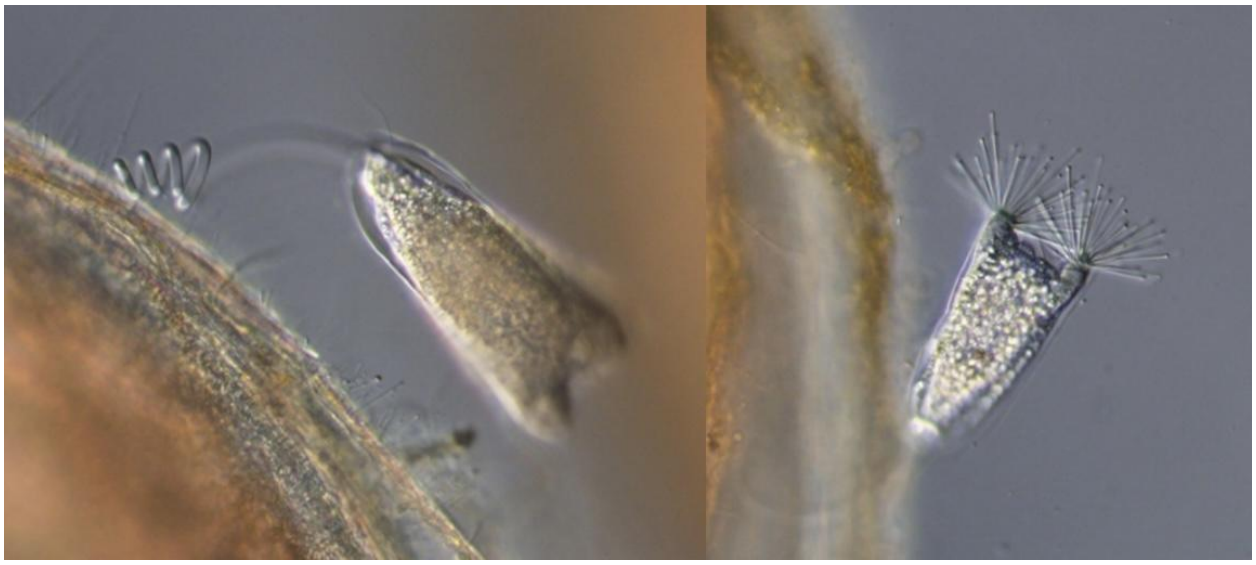

Figure 1. Sessile ciliates in the order Suctoria, genus: *Acineta*. Note the two clusters of stinging tentacles.

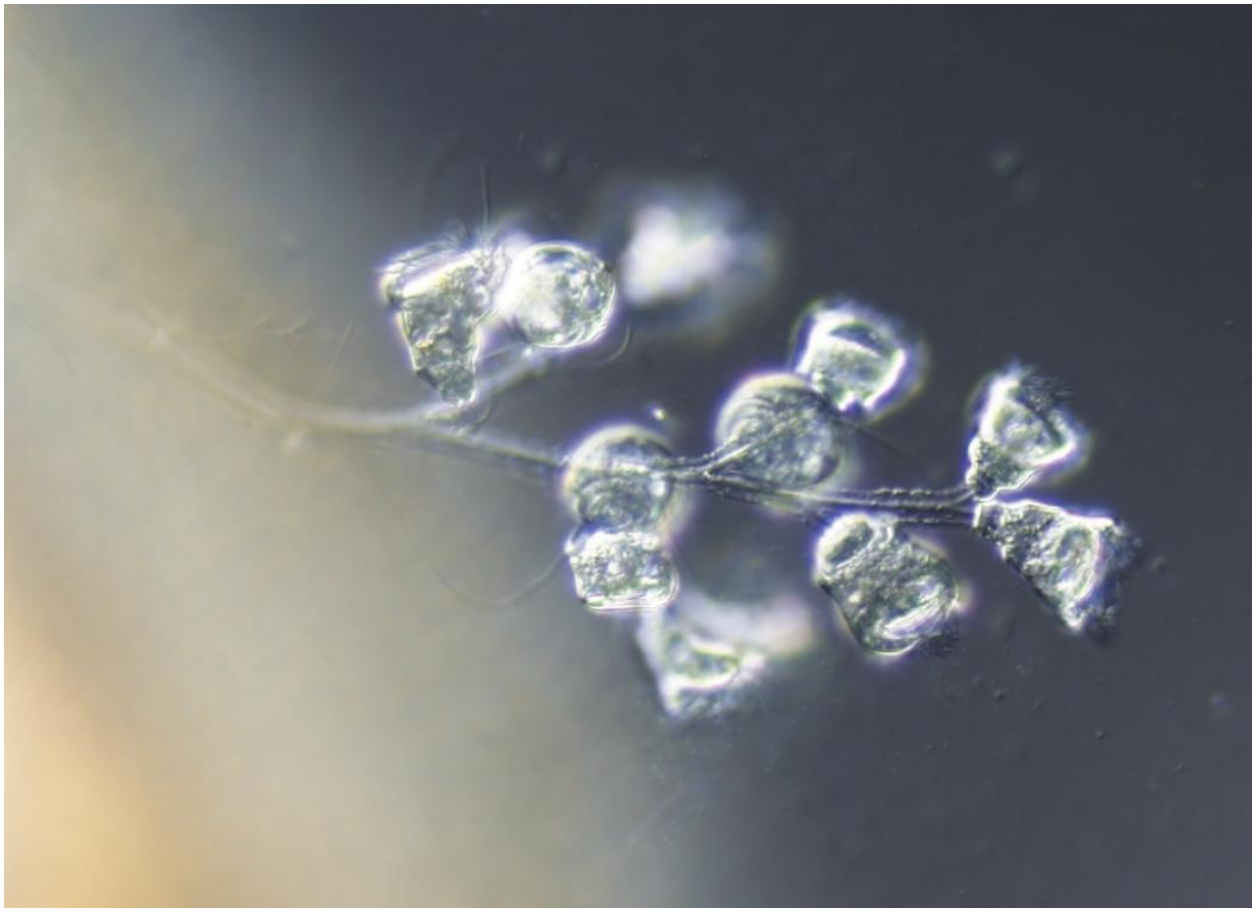

Figure 2. Sessile ciliate *Zoothamnium sp.* Note the branching stalks.

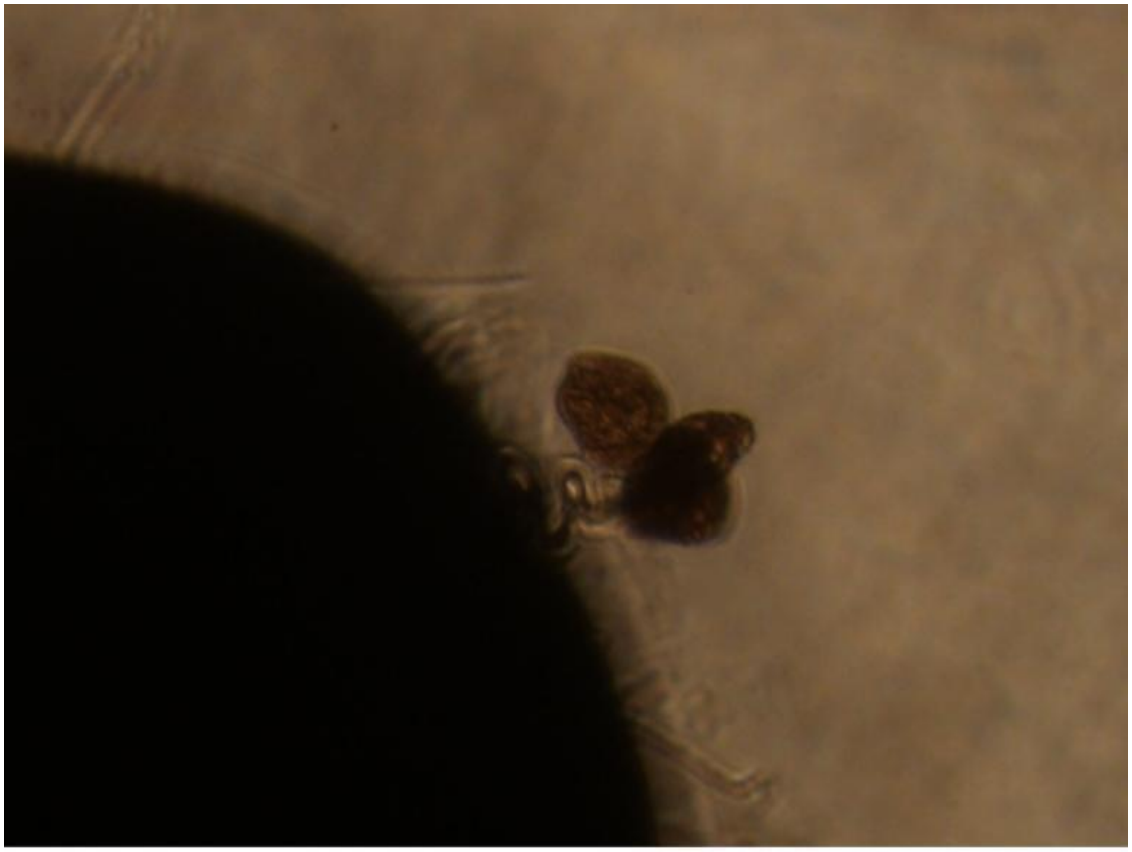

Figure 3. Sessile ciliate in the family *Folliculina.*

Oyster #	# of *Acineta*	#*Zoothamnium*	# *Zoothamnium* zooids	# *Vorticella*
1	1	1	4	0
2	12	0	0	0
3	7	0	0	0
4	9	0	0	2
5	6	0	0	0
6	6	2	12, 2	0
7	1	0	0	0
8	4	0	0	0
9	7	0	0	0
10	3	0	0	0
11	8	0	0	0
12	3	0	0	3
13	1	0	0	0
14	3	0	0	1
15	5	0	0	1
16	1	0	0	0
17	8	0	0	0
18	8	0	0	0
19	9	0	0	0
20	3	1	1	0
21	2	0	0	0
22	0	0	0	0
23	3	0	0	0
24	5	0	0	1
25	3	0	0	0
Average	**4.7**	**0.2**	**6.0**	**0.3**
Stdev	**3.2**	**0.5**	**5.0**	**0.7**
min	**0**	**0**	**0**	**0**
max	**12**	**2**	**12**	**3**

Table 1. Quantification of attached ciliates from samples dated March 11, 2022.

Oyster #	# of *Acineta*	# of *Zoothamnium*	# of *Zoothamnium* zooids	# of *Vorticella*
1	9	3	89	0
2	2	0	0	0
3	5	0	0	0
4	7	1	4	0
5	0	0	0	0
6	5	0	0	0
7	0	0	0	0
8	2	0	0	0
9	0	1	11	0
10	2	0	0	0
11	1	0	0	0
12	6	1	4	0
13	0	0	0	0
14	1	0	0	0
15	1	0	0	0
16	0	0	0	0
17	1	0	0	0
18	0	0	0	0
19	2	0	0	0
20	9	0	0	0
Average	**2.65**	**0.3**	**5.4**	**0**
Stdev	**3.05**	**0.73**	**19.86**	**0.00**
min	**0**	**0**	**0**	**0**
max	**9**	**3**	**89**	**0**

Table 2. Quantification of attached ciliates from samples dated March 28, 2022.

Oyster #	# of *Acineta*	# of *Zoothamnium*	# of *Zoothamnium* zooids	# of *Vorticella*
1	4	1	3	0
2	0	0	0	0
3	1	7	62	0
4	2	1	3	0
5	0	4	19	0
6	1	3	11	0
7	2	1	2	0
8	6	4	12	0
9	2	2	17	0
10	0	4	6	0
Average	**1.8**	**2.7**	**13.5**	**0**
Stdev	**1.93**	**2.11**	**18.24**	**0.00**
min	**0**	**0**	**0**	**0**
max	**6**	**7**	**62**	**0**

Table 3. Quantification of attached ciliates from samples dated June 15, 2022.

Conclusion

Overall, this project informed us that we are dealing with various stalked ciliate species inhabiting our bottle and nursery systems throughout the hatchery season. All samples (frozen, fixed, and live) were collected opportunistically as infestations occurred. We previously thought *Vorticella sp.* was dominating infestations in our bottle system, but it was actually a Suctorian (*Acineta*). Additionally, we documented our first outbreak in our upweller nurseries this season. The outbreak in the upwellers primarily consisted of stalked ciliates in larger colonies (*Zoothamnium sp.*), but also contained Suctorians thought to be carried over from our bottle systems. Little Wicomico Oyster Company provided an infested sample from their nursery which contained two species of suctorians: *Acineta* and *Ephelota*. Now that we know who the problem pests are, it is time to find ways to prevent or better manage these infestations. Our Research and Development Manager Samantha Glover will be presenting results and leading a discussion about this project at the National Shellfisheries Association meeting in 2023. In phase II of this project, we plan to run experiments to test various different treatment approaches to eradicating stalked ciliate pests.

Literature Cited

Fernandez-Leborans, G., Gabilondo, R. (2007) Invertebrate and protozoan epibionts on the velvet swimming crab *Liocarcinus puber* (Linnaeus, 1767) from Scotland. *Acta Zoologica*, 89(1), 1-17.

Mahasri, G., Mukti, A. T., Nisa, M., Prakosa, G. C., & Satyantini, W. H. (2021, February). Ectoparasite infestation and survival rate of pacific white shrimp (Litopenaeus vannamei) that immunized with crude protein Zoothamnium penaei in intensive ponds. In *IOP Conference Series: Earth and Environmental Science* (Vol. 679, No. 1, p. 012019). IOP Publishing.

Overstreet, R. M. (1987). Solving parasite-related problems in cultured crustacea. *International Journal for Parasitology*, 17(2), 309-318.

Shinn, A. P., Pratoomyot, J., Bron, J. E., Paladini, G., Brooker, E. E., & Brooker, A. J. (2015). Economic costs of protistan and metazoan parasites to global mariculture. *Parasitology*, 142(1), 196-270.