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Effects of starvation and macroalgae extracts on the survival and growth of quahog parasite unknown (QPX)

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Abstract

Quahog parasite unknown (QPX) is a relatively poorly characterized protistan parasite that causes QPX disease in infected hard clams (*Mercenaria mercenaria*). Most prior studies presented descriptive reports of the occurrence and the characteristics of the disease in cultured and wild clams, while several basic aspects of the biology and ecology of the parasite remain obscure. In an attempt to better understand factors affecting the persistence and growth of QPX in marine environment, we investigated the ability of QPX to survive in seawater and the potential role of macroalgae in supporting its growth. An association with macroalgae is particularly relevant because several members of the phylum to which QPX belongs are known to live in association with marine macroalgae and vascular plants. Results indicate that while QPX did not appear to grow in seawater under laboratory conditions, the parasite may be capable of long-term survival in seawater without growth. QPX was able to grow using macroalgae homogenates as a source of nutrients, although extracts from certain macroalgae species significantly inhibited the *in vitro* growth of QPX. Overall, our results suggest that macroalgae might be an important factor for the survival, growth and spread of QPX in the marine environment.

Keywords: *In vitro*; Macroalgae; Reservoir; Thraustochytrid

1. Introduction

Quahog parasite unknown (QPX) is a relatively poorly characterized protistan parasite that causes QPX disease in infected hard clams (*Mercenaria mercenaria*). Clam mortalities associated with QPX have occurred in the northeastern United States and Canada (Ford et al., 2002). Despite recent advances in its study, several basic aspects of QPX biology and ecology remain unexplored (Ragone Calvo et al., 1998; Dove et al., 2004). Researchers have yet to develop an understanding of where QPX can be found in the environment and how it is transmitted to its clam host. Specifically, there is a lack of information regarding environmental sources or reservoirs of the QPX organism. Ford et al. (2002) suggested that QPX is an opportunistic facultative parasite of the hard clam that becomes parasitic when clams are disadvantaged by "unfavorable gene–environment interactions". This implies that the QPX organism is able to grow and complete its life cycle outside of its clam host. Recent research documented the presence of QPX in association with marine aggregates (Lyons et al., 2005) and thereby supports the hypothesis

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that QPX can be found in reservoirs outside of *M. mercenaria*. Such reservoirs increase the probability of QPX survival between hosts and enhance disease outbreaks. Thus, elucidation of factors that affect the survival of QPX outside of the host and identification of potential reservoirs may help researchers understand how clams become infected and identify areas of high infection risk.

QPX is an osmoheterotrophic organism belonging to the phylum Labyrinthulomycota, which traditionally has been divided into two groups: the labyrinthulids and the thraustochytrids. Recent work suggests, however, that Labyrinthulomycota comprises three distinct lineages, adding labyrinthuloids to the above two classifications. QPX is classified as a member of the thraustochtyrid group (Maas et al., 1999; Ragan et al., 2000; Leander and Porter, 2001; Stokes et al., 2002). Members of the phylum Labyrinthulomycota are ubiquitous in marine and estuarine environments; they are found in the water column, sediment, and in association with organic detritus and plants. Labyrinthulids and thraustochytrids differ, however, in their association with marine plants. Labyrinthulids occur commonly on or in living marine vascular plants and macroalgae. They live as parasites, commensals, or mutualists (Ragan et al., 2000; Raghukumar, 2002). One of the most well-known associations is that of *Labyrinthula* sp. and seagrass, which can result in outbreaks of seagrass wasting disease (Muehlstein et al., 1988; Steele et al., 2005). Unlike labyrinthulids, thraustochytrids are found rarely in association with living marine plants and are found more typically in association with decomposing plant material and organic detritus (Miller and Jones, 1983; Ragan et al., 2000; Raghukumar, 2002).

Due to the wide range of associations of Labyrinthulomycota species with macroalgae (Raghukumar, 2002; Sharma et al., 1994; Miller and Jones, 1983; Perkins, 1973), it is reasonable to hypothesize that QPX survival and growth may be supported by macroalgae. The fact that prior reports suggested a link between QPX outbreaks and fouling of aquaculture nets (Ragone Calvo and Burreson, 2002) provides another indication of the potential role of fouling organisms, such as macroalgae, in the initiation of QPX infection. In this study, we investigated the role of the aquatic environment as a reservoir and vehicle of transmission for this pathogen. First, we examined the survival and growth of QPX in seawater to evaluate its ability to overcome starvation. We then investigated the survival and growth of the pathogen in fresh and decomposed macroalgae material, and in organic compounds leached from live macroalgae.

2. Materials and methods

2.1. QPX cultures

A New York isolate of QPX was cultured from nodules of infected hard clams collected in Raritan Bay, NY in October 2003 (Allam, unpublished). Isolation and subsequent subcultures were performed according to the methods described by Kleinschuster et al. (1998), and the identity of the isolate was confirmed using the polymerase chain reaction/sequencing methods described by Stokes et al. (2002). Standard culture conditions consisted of QPX propagated in 25-cm^2 flasks at 23 °C using the culture medium described by Kleinschuster et al. (1998), in which the exponential phase of growth is reached within 1 week of incubation. For all experiments described below, assays were performed in sterile plastic tubes containing different experimental culture media. All culture media were supplemented with penicillin/streptomycin to limit bacterial contamination, and incubation of tubes was carried out on a rotating shaker at room temperature (~21–23 °C). Biovolume of QPX in experimental tubes was measured using a semi-automated fluorometric technique according to Buggé and Allam (2005). This technique uses the dye fluorescein di-acetate to measure esterase activity of live cells. The fluorescence signals produced by this assay were used as a measure of cell biovolume to assess viability, relative proliferation, and growth of a specific QPX culture.

2.2. QPX growth and survival in seawater

In order to investigate minimal requirements for QPX survival and growth in the natural environment, QPX was incubated in natural seawater, and its growth was monitored over time. For this assay, 1.5 ml sterile-filtered (0.22 μm) natural seawater (FSW) (taken from Long Island Sound, New York) was added to a 4-ml tube (in triplicate). Two series of controls consisted of 1.5 ml filtered artificial seawater (FASW) or 1.5 ml sterile culture medium (CM) (in triplicate). One hundred μl of an exponentially growing QPX culture (1 week old) was added to each experimental tube and QPX biovolume was measured at t_0 and days 1, 4, 7, 11, and 19.

Based on the results from the previous assay, another assay investigated if QPX can survive in seawater and resume growth when returned to nutrient-rich CM. For this assay, 200 μl of an exponentially growing QPX culture was transferred to a 4-ml tube containing 1 ml FSW (in triplicate) for 2, 5, and 26 days. Control tubes contained CM instead of FSW. After the designated incubation, treatment tubes were well mixed and 300 μl QPX

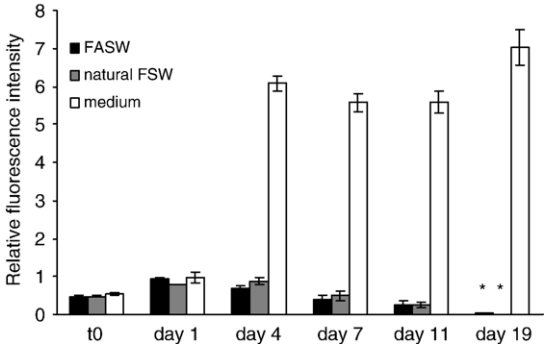

Fig. 1. QPX biovolume (as measured by fluorescence) in artificial seawater (FASW), natural seawater (natural FSW), and culture medium. There was no significant increase in fluorescence produced by QPX in natural or artificial seawater after day 1 (repeated measures ANOVA, $p>0.05$). At day 19, there was significantly lower fluorescence produced by QPX in both seawater preparations than at day 1, as indicated by asterisks (repeated measures ANOVA followed by a Holm–Sidak post-hoc test, $p<0.001$). All data are presented as Mean±SEM (3 replicates).

suspension was transferred to 1 ml CM. QPX biovolume was then measured at different time intervals after transfer.

2.3. QPX growth in macroalgae homogenates

Live specimens belonging to two macroalgae species, *Ulva lactuca* and *Ceramium* sp., were collected from West Meadow Beach, Long Island (New York). Macroalgae samples were washed to remove sediment and epiphytes. A portion of each macroalgae was weighed, homogenized with a volume of seawater equal to ten times its wet weight. Homogenates were then centrifuged (5000 *g*, 15 min) and the resulting supernatants were filter-sterilized. Fifty µl filtered supernatant was reserved for later protein analysis with a bicinchoninic acid (BCA) assay (Pierce 23235), which found both macroalgae homogenates to contain similar quantities of protein (~300 µg/ml). Tubes were filled with 1.5 ml of each filtered macroalgae homogenate (in triplicate) and inoculated with 100 µl of an exponentially growing QPX culture. QPX biovolume was measured at t_0 and days 1, 4, 7, 11, and 19. Based on the results from this assay, another experiment was designed to determine whether or not a lack of QPX growth in macroalgae homogenates was due to nutrient limitation or to the presence of anti-QPX compounds in homogenates. In this case, QPX was grown in CM that had been supplemented (v/v) with homogenates of either *U. lactuca* or *Ceramium* sp. Briefly, a culture tube containing 1 ml sterile CM supplemented with algae homogenate (v/v) was inoculated with QPX (in triplicate). Control tubes consisted of 1 ml CM diluted with 1 ml FSW. QPX biovolume was measured, as above, at t_0 and days 2, 7, 14, and 23.

A second series of experiments was designed to test the effect of a wider range of macroalgae species on QPX growth. In addition, the effect of fresh and decomposed macroalgae, as well as macroalgae leachates was investigated. Six different macroalga species, namely *Fucus* sp., *Laminaria digitata*, *Chondrus crispus*, *Grateloupia turutu*, *Ahnfeltia plicata*, and *Polysiphonia* sp., were collected from Montauk Point, Long Island during

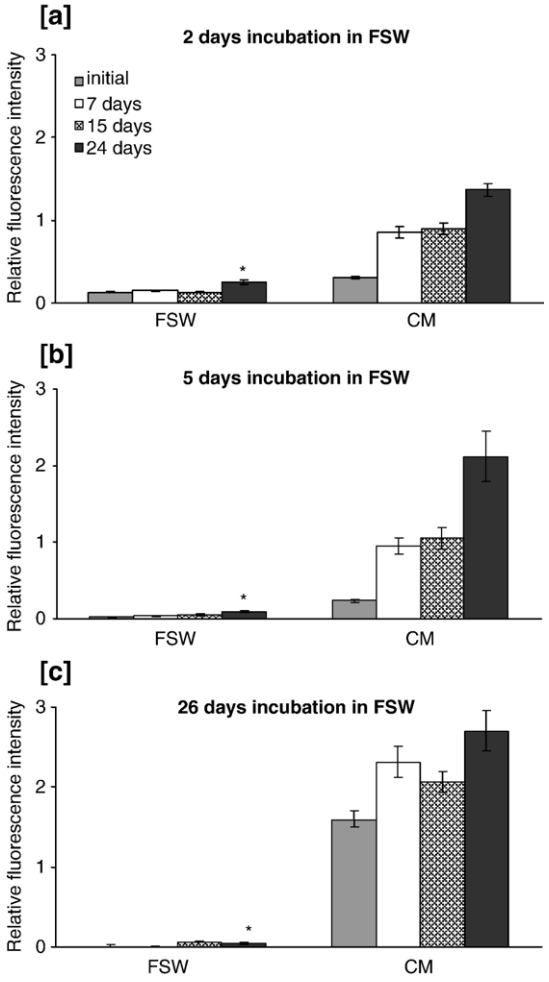

Fig. 2. QPX biovolume (as measured by fluorescence) after being transferred to culture medium following [a] 2 days, [b] 5 days, and [c] 26 days of incubation in FSW. Fluorescence was measured at approximately 1, 2 and 3 weeks after transfer. After approximately 3 weeks in culture medium, there was a slight, but significant increase in fluorescence as compared to initial measurement, indicated by asterisks (repeated measures ANOVA and Holm–Sidak post-hoc test, $p<0.001$). Fluorescence measurements of QPX that had been incubated in FSW were, however, greatly reduced as compared to controls. All data are presented as Mean±SEM (3 replicates).

low tide. Specimens belonging to each species were separated into 3 groups. The first group was homogenized as described above. Resulting homogenates were divided into 2 equal volumes and processed as follows. The first portion was centrifuged, sterile-filtered (as described above) and maintained at −80 °C until use, whereas the second portion was left to decompose at room temperature for 3 weeks before processing. After incubation, homogenates were centrifuged, filtered and frozen until use. The third group of freshly collected, non-homogenized macroalgae specimens was suspended in a volume of FSW equal to ten times its wet weight and incubated at 4 °C in the dark for three days. After incubation, macroalgae were removed and the resulting leachates were sterile-filtered (Raghukumar et al., 1992). A small volume was reserved for BCA protein analysis and the remainder frozen until use. All macroalgae homogenates were found to be in a similar protein content range (~600–1000 μg/ml) except for *Fucus* sp. Both fresh and decomposed *Fucus* sp. homogenates were diluted with FASW to adjust protein contents to 600 μg/ml. Undiluted homogenates of *Fucus* sp. were also used in the assay (~3000 μg protein/ml). Macroalgae leachates were found to contain very small amounts of protein (<100 μg/ml) except for *G. turutu* and *Polysiphonia* sp., which contained 300 and 1000 μg/ml, respectively. Homogenates and leachates of each macroalga (1.5 ml) were transferred to 4 ml tubes and CM was used as a control (in triplicate). Experimental tubes were inoculated with QPX culture as in previous assays and QPX biovolume was measured at t_0 and days 3, 7, 14, and 23. To investigate whether or not differences in QPX growth patterns were due to nutritional limitations, a second assay investigated QPX growth in CM supplemented with the same macroalgae homogenates and leachates employed above. In this case, QPX was grown in 750 μl CM that had been supplemented with 750 μl homogenates or leachates. Control tubes consisted of CM supplemented (v/v) with FSW. QPX biovolume was measured at t_0 and days 3, 7, 14, and 23.

2.4. Statistical analysis

Data were analyzed using SigmaStat (Ver. 3.11. Systat Software, Inc., San Jose, CA) statistical software. One-way analysis of variance (ANOVA) was used to analyze fluorescence signals produced by QPX at a specific incubation time and repeated measures ANOVA was used to analyze fluorescence signals produced by QPX over time in different media. ANOVA treatments that generated probability values below 0.05 were systematically followed by a Holm–Sidak post-hoc test comparing different data points (treatments or time intervals). Differences between data points were also considered statistically significant at $\alpha = 0.05$.

3. Results

3.1. QPX survival and growth in seawater

Measurements of QPX biovolume in natural seawater without medium supplementation indicate that seawater alone is not sufficient to support QPX growth. There was no significant increase in QPX biovolume in natural or artificial seawater after day 1. At day 19, there was significantly lower fluorescence than at day 1 (Fig. 1,

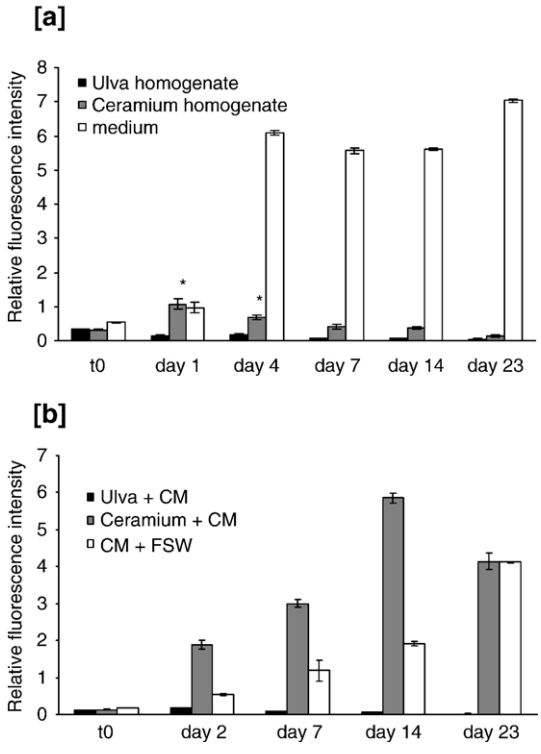

Fig. 3. [a] QPX biovolume (as measured by fluorescence) in homogenates of two species of macroalgae (*Ulva lactuca* and *Ceramium* sp.) and culture medium. There were significant increases in fluorescence up to day 4 in *Ceramium* sp. homogenates as compared to t_0, but no significant increases in fluorescence after day 4 (repeated measures ANOVA and Holm–Sidak post-hoc test, $p<0.05$). In *U. lactuca* homogenates, all fluorescent measurements were significantly lower than at t_0 (repeated measures ANOVA and Holm–Sidak post-hoc test, $p<0.001$). All data are presented as Mean±SEM (3 replicates). [b] QPX biovolume (as measured by fluorescence) in culture medium supplemented with homogenate of two species of macroalgae (*Ulva lactuca* and *Ceramium* sp.) and culture medium supplemented with FSW. Fluorescence produced by QPX was greatly increased in culture medium supplemented with *U. lactuca* homogenate and *Ceramium* sp. homogenate, as compared to QPX in culture medium diluted with FSW. All data are presented as Mean±SEM (2 replicates).

$p<0.001$). Data show that incubation of QPX in seawater for 2, 5 or 26 days results in significantly lower biovolume when QPX is returned to standard CM, as compared to QPX that had been continually maintained in CM. Although there is considerably lower fluorescence as compared to CM controls, QPX cells that had been maintained in seawater for up to 26 days and then transferred to CM showed a slight, but significant increase in fluorescent signal after 24 days in CM compared to t_0 (Fig. 2, $p<0.001$).

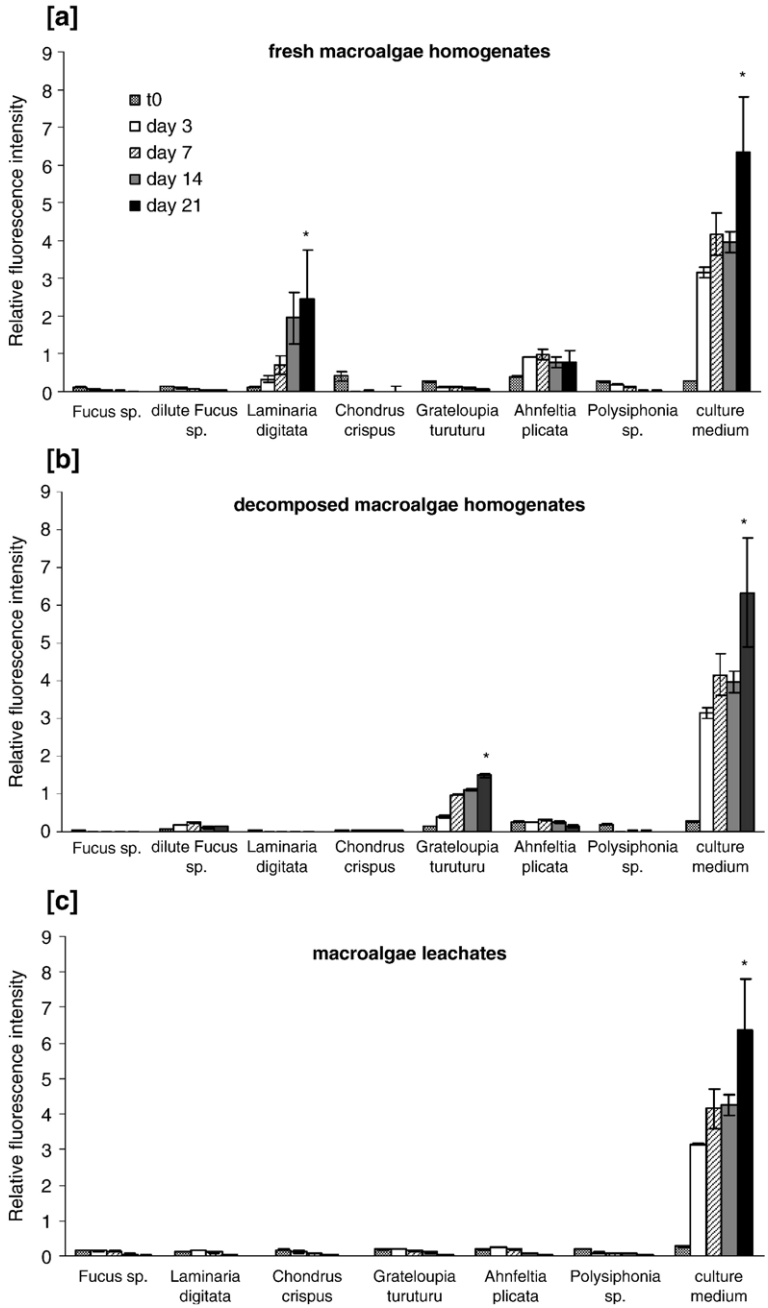

Fig. 4. QPX biovolume (as measured by fluorescence) in [a] fresh and [b] decomposed homogenates of macroalgae, and [c] macroalgae leachates. At day 21, fluorescent measurements of QPX in fresh *L. digitata* homogenate, decomposed *G. turuturu* homogenate, and culture medium, are significantly higher compared to t_0 (repeated measures ANOVA and Holm–Sidak post-hoc test, $p<0.05$). All data are presented as Mean±SEM (3 replicates).

3.2. QPX growth in macroalgae homogenates

In *Ceramium* sp. homogenate, QPX biovolume increased from t_0 to day 1 (Fig. 3a, $p<0.05$) before initiating a decrease that continued until the end of the experiment. Incubation of QPX in *U. lactuca* homogenate showed a very different trend. Every fluorescent measurement after t_0 was significantly lower than the fluorescent signal produced at t_0 ($p<0.001$). This indicated that not only did the homogenates from this

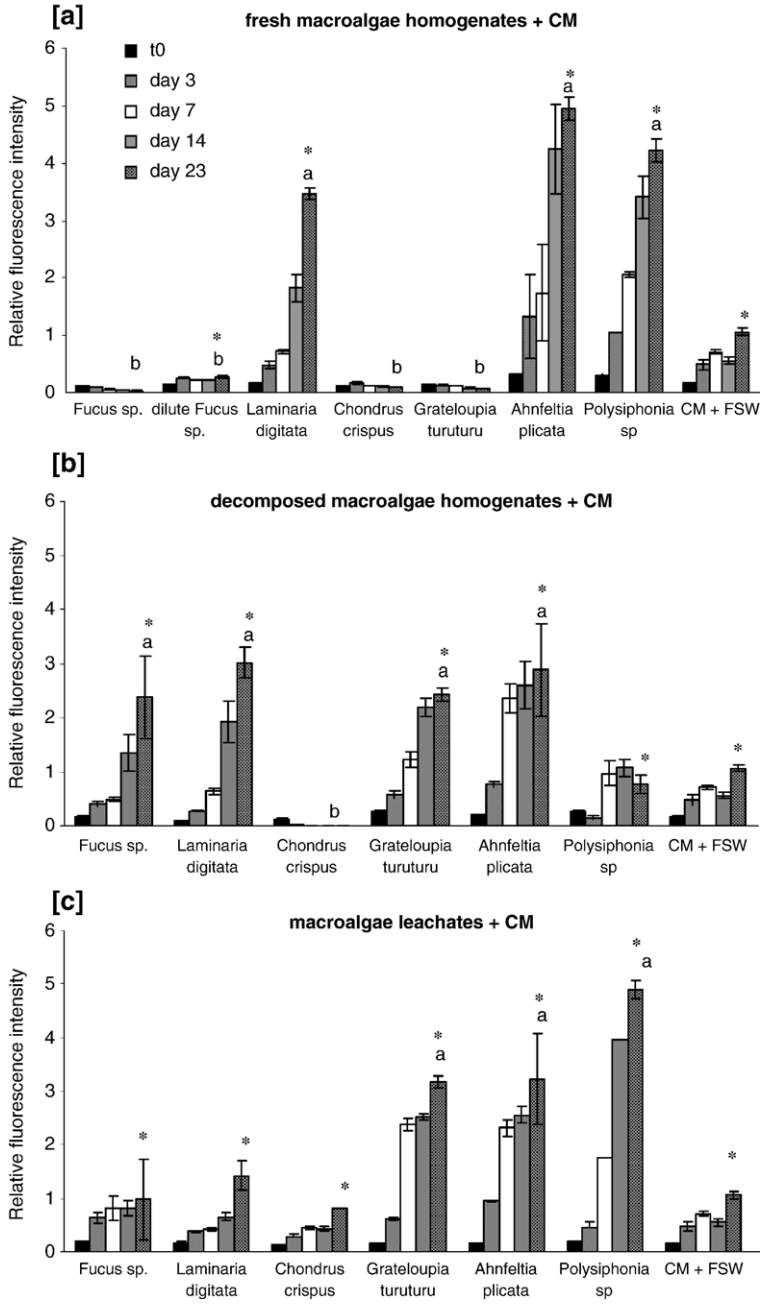

Fig. 5. QPX biovolume (as measured by fluorescence) in medium supplemented with [a] fresh and [b] decomposed macroalgae homogenates and [c] macroalgae leachates. Control tubes contained culture medium supplemented with FSW. Letters indicate significantly higher (a) or lower (b) fluorescence produced by QPX than in culture medium diluted with FSW at day 23 (ANOVA and Holm–Sidak post-hoc test, $p<0.05$). Asterisks indicate significantly higher fluorescent signals produced by cultures at day 23 than at t_0 (repeated measures ANOVA and Holm–Sidak post-hoc test, $p<0.05$). All data are presented as Mean ± SEM (3 replicates).

green macroalgae not support QPX growth *in vitro*, but there was a reduction in viability of QPX cells after initial transfer. In addition, QPX biovolume was significantly lower in culture media (CM) supplemented with homogenates from *U. lactuca* (Fig. 3b), suggesting that the failure of QPX to grow in this homogenate was not a result of trophic limitations. On the other hand, supplementation CM with *Ceramium* homogenates resulted in greater QPX biovolume when compared with non-supplemented CM.

Results of the second series of experiments demonstrated major variations in QPX biovolume depending upon the macroalgal species and the level of decomposition of the homogenates, whereas there was no noticeable increase in fluorescence produced by QPX in any of the macroalgae leachates (Fig. 4). At day 21, there were significantly higher fluorescence measurements of QPX in fresh *L. digitata* homogenates and decomposed *G. turuturu* homogenates, as compared to t_0 ($p<0.05$). There was no significant increase in fluorescence produced by QPX in fresh *G. turuturu* or in any of the other decomposed homogenates of macroalgae. When used as a supplement to CM, fresh homogenates of some macroalgae species inhibited QPX growth, whereas fresh homogenates of other species enhanced QPX growth over CM alone (Fig. 5). Fluorescence measurements of QPX in CM supplemented with fresh homogenate of *Fucus* sp., *C. crispus*, and *G. turuturu* were significantly lower than controls; QPX biovolumes measured at day 23 were similar to those measured at t_0 (Fig. 5a, $p>0.05$). In dilute *Fucus* sp., however, there was a small, but significant increase in QPX biovolume at day 23 ($p<0.05$). An inhibitory effect was not seen with decomposed homogenates of most macroalgae species. Only QPX incubated in CM supplemented with decomposed homogenates of *C. crispus* produced significantly lower fluorescence as compared to that in CM ($p<0.05$), whereas decomposed homogenates of all other species either enhanced fluorescent signals over un-supplemented CM, or produced fluorescence signals equivalent to that in non-supplemented CM (Fig. 5b). QPX incubated in CM supplemented with leachate produced either equivalent or higher fluorescence signals as compared to controls (Fig. 5c).

4. Discussion

The present study indicates that QPX has a limited ability to grow in seawater as results show that there is no significant increase in QPX biovolume when incubated in seawater. After 19 days of incubation, there appears to be a loss in viability as indicated by a loss of fluorescence. It is likely that the lag in loss of fluorescence (relative stability until day 7) is due to the culture medium that was transferred to the experimental tubes along with the QPX cells. Such a small volume of medium (100 μl of initial culture) was sufficient to support QPX for a short time, but the nutrients in the medium were quickly exhausted, resulting shortly in QPX death. However, whereas QPX does not appear to grow in seawater under laboratory conditions, the parasite may be capable of long-term survival in seawater without growth. For instance, our results show that when QPX is returned to culture medium after incubation in seawater, it resumed growth, but at much slower rate when compared to controls that were continuously incubated in culture medium (CM). It is possible that incubation in seawater causes the death of some parasite cells, but it is also likely that the transfer to such basic nutritionally poor media (seawater) induces QPX to enter a "dormant" phase with low metabolic activities. Prior reports rule out, however, the idea of dormancy in thraustochytrids, suggesting that these organisms are likely to be in a perpetually active metabolic state and are able to use their extracellular polysaccharides as an energy source during oligotrophic conditions (Jain et al., 2005). Since the technique used for QPX quantification measures esterase activity in live cells (Buggé and Allam, 2005), it is possible that the loss of fluorescence results from a reduction of cell metabolism following transfer to seawater because of nutritional limitation (dormant cells). Results from this experiment do not reveal whether or not, after longer periods, QPX would resume its typical fast growth in culture medium. These *in vitro* experiments also do not provide information to explain if the prolonged period in seawater affects the parasite's condition and ability to infect the host *M. mercenaria*. Microscopical observations of abundant cell debris clearly demonstrated that a large fraction of QPX cells exhibited cellular damage after their transfer to seawater. These microscopic observations, associated with a decrease in fluorescence signals, suggest that many QPX cells did not survive the transfer from culture media to seawater. It is noteworthy that laboratory transmission experiments using QPX cultures failed to induce the disease in clams exposed to QPX in the water column (e.g. adding QPX cultures to experimental seawater; Dahl, 2006; Dahl and Allam, submitted for publication). Clams that were inoculated with QPX via the pallial cavity or directly into the tissue, both of which reduced the time of exposure of QPX to seawater before contact with its host did develop QPX infection. The apparent limited ability of QPX to grow and maintain high level of metabolic activity in seawater, which we have observed here, may have prevented the parasite from successfully infecting clams in these experiments.

This study shows that QPX is able to survive and grow using nutritional sources derived from marine organisms other than the hard clam. In our experiments, fresh homogenates of *L. digitata* and decomposed homogenates of *G. turuturu* supported QPX growth *in vitro*. In addition, whereas QPX grew in certain fresh macroalgae homogenates, there was no growth in decomposed homogenates from the same species. This indicates that there may be nutrients present in some species of live macroalgae that QPX requires for growth and as these macroalgae decompose, they lose their nutritional value for QPX. Although marine macroalgae are known to release large amounts of organic nutrients (Raghukumar, 2002; Sieburth, 1969) that could potentially provide a source of energy for QPX, our experimental evidence indicated that QPX cannot use leachates from the tested species of live macroalgae as a nutritional source. This indicates that QPX requires nutrients from within algal tissues or leachates do not contain enough nutrients to support QPX growth under our experimental conditions.

The trend of QPX growth in homogenates made with some macroalgae species (*Ceramium* sp. for example, Fig. 3a) is similar to those seen in seawater (Fig. 1), suggesting that growth limitation in those species results from nutritional limitations. This is confirmed by an enhancement in growth when homogenates were used as a supplement to standard culture media (Figs. 3b and 5).

Despite the evidence that some macroalgae can provide nutritional resources to support or enhance QPX growth, there was also obvious inhibition of QPX growth by homogenates of several macroalgae species. Results from these experiments indicate that the effect of macroalgae homogenates and leachates is species specific. It is likely that certain species of macroalgae provide nutrients that QPX is able to use, whereas other species produce antimicrobial compounds to which QPX is sensitive. The production of antimicrobial products has been documented in many macroalgae species (Miller and Jones, 1983; Jones, 1988; Arnold and Targett, 2002). These antimicrobial substances, including phenolic compounds, likely protect macroalgae against various marine pathogens (Raghukumar, 1992, 2002). Fresh homogenates of *U. lactuca*, *Fucus* sp., *C. crispus*, and *G. turuturu* clearly inhibited QPX growth when they were added as a supplement to culture medium. The addition of dilute *Fucus* sp. homogenate to culture medium resulted in a slight increase in QPX growth. It is likely that the dilution of this macroalga reduced the amount of inhibitory factors added to the medium. The variability in QPX growth observed here is probably related to the quality and quantity of antimicrobial or promicrobial substances produced by the macroalgae. Surveys of macroalgae along the New York/New Jersey coast have found that different macroalgae species produce many different antimicrobial substances, and that species of the same order or genus did not show similar antimicrobial profiles (Lustigman et al., 1992; Lustigman and Brown, 1991). It is likely that, in some species, decomposition of the macroalgae samples over a period of three weeks is sufficient to degrade the antimicrobial properties, whereas in other species, the antimicrobial effects persist. Another factor to take into consideration is QPX resistance to algal antibiosis. Some macroalgae species may possess antimicrobial properties to which QPX is resistant. For example, the mucoid coating produced by QPX, which has been shown to protect QPX from antimicrobial factors present in clam plasma (Anderson et al., 2003), may act to protect the organism against macroalgae antibiosis in this case. Future experiments investigating the effects of macroalgae homogenates or leachates on washed versus unwashed QPX are needed to answer whether QPX mucus plays a role in protecting the organism from algal antibiosis. In addition, further testing of more greatly decomposed macroalgae may clarify whether further decomposition (and subsequent loss of antimicrobial properties) will allow QPX to use additional macroalgae species as a nutritional source.

Bacteria associated with macroalgae may have been involved in supporting or inhibiting QPX growth in decomposed homogenates. The decomposed macroalgae homogenates used in these experiments were prepared by allowing macroalgae–seawater homogenate to decompose for two weeks before being filter-sterilized. Bacteria associated with the surface of the macroalgae may have released nutritive compounds into the seawater during that decomposition period (Miller and Jones, 1983). Other than nutritional properties, epibiotic bacteria may also release antimicrobial substances that work alone or in concert with macroalgae-derived compounds (Boyd et al., 1999; Armstrong et al., 2001), leading to an inhibition of QPX growth. Based on the results presented here, it is impossible to establish whether macroalgae-associated bacteria played a role in supporting or inhibiting QPX growth in decomposed macroalgae homogenates. Other variables that may need to be taken into consideration in future studies include previously documented seasonal variation in the production of antimicrobials by macroalgae (Jones, 1988). Such variations suggest that QPX may have different relationships with macroalgae at different times during the year.

Overall, our results suggest that macroalgae might be an important factor for the survival, growth and spread of QPX in the marine environment. Thraustochytrids are typically found in association with organic detritus or

decomposing macroalgae (Raghukumar, 2002). The fact that outbreaks of QPX disease are believed to be aggravated by poor husbandry practices (Ragone Calvo and Burreson, 2002), which include the fouling by epiphytes of aquaculture nets used to protect clams from predation, reinforce the idea of a possible link between macroalgae and the infection. This information, combined with evidence that QPX can be found in association with macroalgae debris (Lyons et al., 2005), indicates that macroalgae may play a role in the life cycle of QPX. QPX may be able to survive in association with live or decomposed macroalgae, allowing the parasite to persist in the environment, regardless of the presence or absence of hard clam hosts. Results from *in vitro* experiments, however, do not provide direct evidence that concentrations of compounds that support or inhibit QPX growth *in vitro* could occur in nature (Steinberg and de Nys, 2002; Steinberg et al., 2002; Jones, 1988). A more thorough investigation of the potential role of macroalgae in harboring or supporting the survival of QPX will indicate whether monitoring of marine macroalgae–QPX associations may be a useful tool to better understand QPX outbreaks.

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References

Anderson, R.S., Kraus, B.S., McGladdery, S., Smolowitz, R., 2003. QPX, A pathogen of quahogs (hard clams), employs mucoid secretions to resist host antimicrobial agents. J. Shellfish Res. 22, 205–208.

Armstrong, E., Yan, L., Boyd, K.G., Wright, P.C., Burgess, J.G., 2001. The symbiotic role of marine microbes on living surfaces. Hydrobiologia 461, 37–40.

Arnold, T.M., Targett, N.M., 2002. Marine tannins: the importance of a mechanistic framework for predicting ecological roles. J. Chem. Ecol. 28, 1919–1934.

Boyd, K.G., Adams, D.R., Burgess, J.G., 1999. Antibacterial and repellent activities of marine bacteria associated with algal surfaces. Biofouling 14227–14236.

Buggé, D.M., Allam, B., 2005. A fluorometric technique for the in vitro measurement of growth and viability in quahog parasite unknown (QPX). J. Shellfish Res. 24, 1013–1018.

Dahl, S., 2006. Transmission of quahog parasite unknown in hard clams: interactions between different host strains and pathogen isolates. M.S. Thesis, Stony Brook University, USA.

Dahl, S., Allam, B., submitted for publication. Laboratory transmission studies of QPX disease in the hard clam: I. Development of an infection procedure. J. Shellfish Res.

Dove, A.D.M., Bowser, P.R., Cerrato, R.M., 2004. Histological analysis of an outbreak of QPX disease in hard clams, *Mercenaria mercenaria*, in New York. J. Aquat. Anim. Health 16, 246–250.

Ford, S.E., Kraeuter, J.N., Barber, R.D., Mathis, G., 2002. Aquaculture-associated factors in QPX disease of hard clams: density and seed source. Aquaculture 208, 23–38.

Jain, R., Raghukumar, S., Tharanathan, R., Bhosle1, N.B., 2005. Extracellular polysaccharide production by thraustochytrid protists. Mar. Biotechnol. 7, 184–192.

Jones, A.K., 1988. Algal extracellular products—antimicrobial substances. In: Rogers, L.J., Gallon, J.R. (Eds.), Annual Proceedings of the Phytochemical Society of Europe. Biochemistry of the Algae and Cyanobacteria. Oxford University Press, New York, pp. 257–281.

Kleinschuster, S.J., Smolowitz, R., Parent, J., 1998. *In vitro* life cycle and propagation of Quahog Parasite Unknown. J. Shellfish Res. 17, 75–78.

Leander, C.A., Porter, D., 2001. The Labyrinthulomycota is comprised of three distinct lineages. Mycologia 93, 459–464.

Lustigman, B., Brown, C., 1991. Antibiotic production by marine algae isolated from the New York/New Jersey coast. Bull. Environ. Contam. Toxicol. 46, 329–335.

Lustigman, B., Lee, L.H., Thees, N., Masucci, J., 1992. Production of antibacterial substances by macroalgae of the New York/New Jersey coast, USA. Bull. Environ. Contam. Toxicol. 49, 743–749.

Lyons, M.M., Ward, J.E., Smolowitz, R., Uhlinger, K.R., Gast, R., 2005. Lethal marine snow: pathogen of bivalve mollusc concealed in marine aggregates. Limnol. Oceanogr. 50, 1983–1988.

Maas, P.A.Y., Kleinschuster, S.J., Dykstra, M.J., Smolowitz, R., Parent, J., 1999. Molecular characterization of QPX (quahog parasite unknown), a pathogen of *Mercenaria mercenaria*. J. Shellfish Res. 18, 561–567.

Miller, J.D., Jones, E.B.G., 1983. Observations on the association of thraustochytrid marine fungi with decaying seaweed. Bot. Mar. 26, 345–351.

Muehlstein, L.K., Porter, D., Short, F.T., 1988. *Labyrinthula* sp., a marine slime mold producing the symptoms of wasting disease in eelgrass, *Zostera marina*. Mar. Biol. 99, 465–472.

Perkins, F.O., 1973. Observations of thraustochytriaceous (Phycomycetes) and labyrinthulid (Rhizopodea) ectoplasmic nets on natural and artificial substrates—an electron microscopy study. Can. J. Bot. 51, 485–491.

Ragan, M.A., MacCallum, G.S., Murphy, C.A., Cannone, J.J., Gutell, R.R., McGladdery, S., 2000. Protistan parasite QPX of hard-shell clam *Mercenaria mercenaria* is a member of Labyrinthulomycota. Dis. Aquat. Org. 42, 185–190.

Raghukumar, S., 1992. Bacterivory: a novel dual role for thraustochytrids in the sea. Mar. Biol. 113, 165–169.

Raghukumar, S., 2002. Ecology of the marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). Eur. J. Protistol. 38, 127–145.

Raghukumar, C., Nagarkar, S., Raghukumar, S., 1992. Association of thraustochytrids and fungi with living marine algae. Mycol. Res. 96542–96546.

Ragone Calvo, L.M., Burreson, E.M., 2002. QPX susceptibility in hard clams varies with geographic origin of brood stock. V. S. G. M. R. Advisory, Virginia Sea Grant Marine Advisory Program and Virginia Institute of Marine Science. No. 74 VSG-02-18, p. 7.

Ragone Calvo, L.M., Walker, J.G., Burreson, E.M., 1998. Prevalence and distribution of QPX, Quahog Parasite Unknown, in hard clams

Mercenaria mercenaria in Virginia, USA. Dis. Aquat. Org. 33, 209–219.

Sharma, S., Raghukumar, C., Sathe-Pathak, V., Chandramohan, D., 1994. Thraustochytrid and fungal component of marine detritus. II. Laboratory studies on decomposition of the brown alga *Sargassum cinereum*. J. Ag. J. Exp. Mar. Biol. Ecol. 175, 227–242.

Sieburth, J.M., 1969. Studies on algal substances in the sea. II. The production of extracellular organic matter by littoral marine algae. J. Exp. Mar. Biol. Ecol. 3, 290–309.

Steele, L., Caldwell, M., Boettcher, A., Arnold, T., 2005. Seagrass–pathogen interactions: 'pseudo-induction' of turtlegrass phenolics near wasting disease lesions. Mar. Ecol. Prog. Ser. 303, 123–131.

Steinberg, P.D., de Nys, R., 2002. Chemical mediation of colonization of seaweed surfaces. J. Phycol. 38, 621–629.

Steinberg, P.D., de Nys, R., Kjelleberg, S., 2002. Chemical cues for surface colonization. J. Chem. Ecol. 28, 1935–1951.

Stokes, N.A., Ragone Calvo, L.M., Reece, K.S., Burreson, R.E., 2002. Molecular diagnostics, field validation, and phylogenetic analysis of Quahog Parasite Unknown (QPX), a pathogen of the hard clam *Mercenaria mercenaria*. Dis. Aquat. Org. 52, 233–247.