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### **Mode of resistance to viral lysis affects host growth across multiple environments in the marine picoeukaryote *Ostreococcus tauri***

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1 Mode of resistance to viral lysis affects host growth across multiple environments in  
2 the marine picoeukaryote *Ostreococcus tauri*

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4 Sarah E. Heath & Sinead Collins

5

6 Institute of Evolutionary Biology, School of Biological Sciences, University of  
7 Edinburgh, Ashworth Laboratories, The King's Buildings, West Mains Road,  
8 Edinburgh EH9 3FL, UK.

9

10 Corresponding author: Sarah E. Heath.

11 Institute of Evolutionary Biology, School of Biological Sciences, University of  
12 Edinburgh, Ashworth Laboratories, The King's Buildings, West Mains Road,  
13 Edinburgh EH9 3FL, UK.

14 +44(0)131 650 8657

15 s.heath-2@sms.ed.ac.uk

16

17 Running title: *O. tauri* virus resistance in multiple environments

## 18 SUMMARY

19

20 Viruses play important roles in population dynamics and as drivers of evolution in  
21 single-celled marine phytoplankton. Viral infection of *Ostreococcus tauri* often  
22 causes cell lysis, but two spontaneously arising resistance mechanisms occur: resistant  
23 cells that cannot become infected and resistant producer cells that are infected but not  
24 lysed, and which may slowly release viruses. As of yet, little is known about how  
25 consistent the effects of viruses on their hosts are across different environments. To  
26 measure the effect of host resistance on host growth, and to determine whether this  
27 effect is environmentally dependent, we compared the growth and survival of  
28 susceptible, resistant and resistant producer *O. tauri* cells under five environmental  
29 conditions with and without exposure to *O. tauri* virus. While the effects of exposure  
30 to virus on growth rates did not show a consistent pattern in populations of resistant  
31 cells, there were several cases where exposure to virus affected growth in resistant  
32 hosts, sometimes positively. In the absence of virus, there was no detectable cost of  
33 resistance in any environment, as measured by growth rate. In fact, the opposite was  
34 the case, with populations of resistant producer cells having the highest growth rates  
35 across four of the five environments.

36

## INTRODUCTION

Marine viruses play a large role in nutrient and energy cycling in the oceans. Viral lysis of single celled organisms releases large quantities of organic matter into the environment, making nutrients available for use by bacteria and algae. This process has been termed the viral shunt (Wilhelm and Suttle, 1999). Studies on marine viruses typically focus on the importance of viruses in nutrient cycling and the release of organic matter through cell lysis. Despite the important role of marine viruses in ecosystem function across many environments, from nutrient rich coastal waters to more oligotrophic regions of the open ocean (Bruussard, 2004), host-virus interactions are typically studied in single environments. Here, we use the *Ostreococcus tauri*/*Ostreococcus tauri* virus model system to investigate variation in host-virus interactions across environments to understand (1) whether susceptibility/resistance to viruses changes with environmental change, and (2) whether the growth effect of host resistance depends on environmental context or resistance type.

We explore the relationship between host responses to environmental change and the resistance strategies of those hosts using the marine picoeukaryote *Ostreococcus tauri* (order Mamiellales). *O. tauri* is commonly isolated from Mediterranean lagoons that are connected to the open ocean via narrow channels (Clerissi *et al.*, 2014). These channels limit the exchange of seawater between the lagoon and ocean, making variations in the environmental salinity, pH, temperature and nutrients more extreme than in the open ocean (Bellec *et al.*, 2010; Clerissi *et al.*, 2014). *Ostreococcus tauri* viruses (OtVs) have been sampled frequently in water samples collected from lagoon and coastal waters where *O. tauri* is found. OtVs have strict host specificity (Clerissi

62 *et al.*, 2012), and the three OtVs sequenced to date have all been described as lytic  
63 viruses (Derelle *et al.*, 2008; Weynberg *et al.*, 2009, 2011). Virus infection of *O. tauri*  
64 usually causes cell lysis in susceptible (S) cells, though two mechanisms of resistance  
65 have been observed (Thomas *et al.*, 2011). In the first case, viruses are unable to  
66 infect the host, and these cells are referred to here as resistant (R). In the second case,  
67 hosts are tolerant to viral infection and are able to slowly release them without  
68 damage to the host cell. These cells are termed resistant producers (RP). In this paper,  
69 we refer to the three cell types as resistance types.

71 Resistance type could have consequences for growth and other cell properties, such as  
72 size and chlorophyll content. For example, a trade-off of acquiring resistance to viral  
73 lysis may come as a fitness cost. This often occurs as reduced competitive ability  
74 (Lenski, 1988; Bohannan *et al.*, 2002) and sometimes reduced growth rate (Lennon *et*  
75 *al.*, 2007; Frickel *et al.*, 2016). A modification in cell surface receptors to limit virus  
76 attachment could also result in a loss of the original function of the protein, such as  
77 metabolism or being able to target the host immune system. In several bacteria  
78 species, loss of a bacteriophage receptor results in lower virulence of the bacteria in  
79 its host, thereby lowering the fitness of resistant compared to non-resistant strains  
80 (Seed *et al.*, 2012; León and Bastías, 2015). Lastly, strong resistance to one specific  
81 virus strain may lead to increased susceptibility to lysis by other strains, as has been  
82 observed in *O. tauri* (Clerissi *et al.*, 2012) and cyanobacteria (Marston *et al.*, 2012;  
83 Avrani and Lindell, 2015).

85 The group of viruses that infects phytoplankton is the Phycodnaviruses. These viruses  
86 have been studied under environmental conditions that differ from a benign control

environment in a single driver, such as increases in temperature (Nagasaki and Yamaguchi, 1998; Wells and Deming, 2006), nutrient (Bratbak *et al.*, 1993, 1998; Bellec *et al.*, 2010; Clerissi *et al.*, 2014), light (Bratbak *et al.*, 1998; Weinbauer, 2004), UV (Jacquet and Bratbak, 2003), CO<sub>2</sub> (Larsen *et al.*, 2007; Chen *et al.*, 2014; Maat *et al.*, 2014) and pH levels (Weinbauer, 2004). When environmental conditions are stressful, one consequence can be inactivation of the virus particle. This affects host-virus interactions by preventing infection through structural degradation, the inability of the virus to inject its genome into the host or the inability of the virus to replicate (Børsheim, 1993; Jacquet and Bratbak, 2003). Additionally, since viral replication and life cycle are often closely linked to host metabolism, environmental changes such as increased temperature or nutrients will often have an indirect effect on responses to viral attack (Weinbauer, 2004; Danovaro *et al.*, 2011). Understanding the role of viruses in marine communities requires investigating their activity across environments. Here, we focus on the environmental changes of increased temperature, decreased nutrients, decreased light and decreased salinity levels.

Previous studies of resistance in *O. tauri* found that when each resistance type was maintained separately there was no significant difference in growth rates, such that a cost of resistance was too low to be detected by differences in growth alone. However, when resistant types were competed against each other, a competitive hierarchy was observed in which S had the fastest growth rate, followed by R and then by RP (Thomas *et al.*, 2011). Since the three resistance types share the same starting genotype, it is possible to make direct comparisons between them. In this study, an experiment was performed in which three populations of each *O. tauri* resistance type (S, R and RP) derived from a common ancestor were grown for one

week in the following environments in both the absence and presence of OtV5: high temperature, low light, low phosphate, and low salt. These environments were selected to represent relatively small variations from the control environment in which the populations are normally maintained in the laboratory, so that the cells responded, but were still able to grow at a rate that was measureable. The average number of cell divisions per day over a single transfer cycle (7 days), cell size and cell chlorophyll content were measured in the novel environments in the absence of OtV5. Offspring production over a fixed period of time is a proxy for fitness in single celled organisms in batch culture experiments (Brennan and Collins, 2015). Cell size and chlorophyll content were measured as additional phenotypes, to examine effects on organismal function other than cell division rates, since only small differences in growth were detected previously (Thomas *et al.*, 2011). After one week of growth in the novel environment, all populations were inoculated with OtV5 and cell densities were measured three days after inoculation to test for susceptibility to viral lysis.

## RESULTS

*The effect of viral exposure on cell division rates depends on resistance type*

After one week of growth in a novel environment, all populations were inoculated with OtV5 and cell densities were measured three days later. Supporting Information Tables S1 and S2 provide all statistical outputs in this study. Susceptibility of *O. tauri* to OtV5 was driven by resistance type, as expected (ANOVA, resistance type  $\times$  virus treatment,  $F_{2,234} = 360.14$ ,  $p < 0.0001$ ). After inoculation of *O. tauri* with OtV5, all R and RP cells remained resistant to lysis and S cells remained susceptible (Figure 1). Thus, OtV5 inoculation had a significant effect on cell density (ANOVA effect of virus treatment on growth,  $F_{1,234} = 361.62$ ,  $p < 0.0001$ ), since populations of S cells fell to almost zero (Figure 1). No difference was observed in resistance between R and RP populations ( $t=0.46$ ,  $p=0.66$ ).

Counter to our expectation, the effect of virus inoculation did not vary with environment (ANOVA, environment  $\times$  virus treatment,  $F_{4,234} = 0.89$ ,  $p < 0.46$ ). However, environment alone had a significant effect on growth ( $F_{4,234} = 26.01$ ,  $p < 0.0001$ ), because of the S cell lysis in all environments. Additionally, an interaction was identified between resistance type and environment (ANOVA, environment  $\times$  resistance type,  $F_{8,234} = 6.09$ ,  $p < 0.0001$ ). For both R and RP cells, there were cases where virus inoculation resulted in higher growth rates than the non-inoculated controls (Figure 1). Cell densities were repeatedly higher in one inoculated population (NG'13) than the control in the low salt environment in R cells and in the low light environment for one population (NG27) in RP cells. This indicates that cell growth



can increase in response to viruses in resistant populations. This occurs consistently in all replicates of a given population when it happens, but does not occur in all populations of a resistance type. We also see cases where lysis in some populations of S cells is incomplete, notably in the low light (NG'2) and low salt (NG'3) environments. Again, this does not occur in all populations, but it occurs reliably in replicates of the same population. While these effects of environment on lysis are not statistically significant because they do not occur over all populations within a resistance type, it could have evolutionary and ecological effects on the occasions when it does occur, which we discuss below.

#### *Growth rate varied across environments regardless of resistance type*

All populations were grown in a novel environment in the absence of OtV5 for one week, over which growth rate was measured. The response of *O. tauri* growth to the environment depended on resistance type (effect of environment  $\times$  resistance type,  $F_{8,114}=4.45$ ,  $p=0.0001$ ). Additionally, regardless of resistance type, population growth rates differed between environments (effect of environment on growth  $F_{4,114}=231.39$ ,  $P<0.0001$ ) (Figure 2). Growth rates were higher in the control environment except for a single RP population, NG'10, which divided rapidly in the low salt environment (Figure 2). Populations grown in the low phosphate environment all had reduced growth rates and showed less variation in growth than in all other environments.

#### *The effect of resistance type on growth depends on environment*

Resistance type alone did not significantly affect the growth rate of *O. tauri* ( $F_{2,6}=2.88$ ,  $p=0.1328$ ). This is because S and R cells had similar population growth rates in all environments (Figure 2). In contrast, some populations of RP had different growth rates than both R and S cells. There was variation in growth rates between replicate populations of RP cells, with some populations consistently showing elevated growth rates. Two out of the three RP populations (NG'10 (shown by circle in Fig 2) and NG'16 (shown by cross in Fig 2) had higher growth rates than S and R cells in four out of the five environments ( $F_{3,5}=17.19$ ,  $p=0.046$ ). The single exception was the low phosphate environment, where all resistance types had similar low growth rates. These data indicate that there is either no cost or an undetectable cost of resistance in terms of growth to either infection or lysis over a range of environments, and that there can be a growth benefit of being resistant to lysis in some environments, as evidenced by the rapid growth of some RP populations. The low or absent cost of resistance is consistent with previous studies in single environments, which have reported costs of resistance detectable in competitions, but too low to be detectable by comparing growth rates (Thomas *et al.*, 2011).

#### *Populations resistant to lysis can have a growth advantage in some environments*

In order to assess whether the S, R, and RP resistance types responded similarly to the different environments, environments were ranked from best to worst, based on population growth rates. All resistance types displayed highest growth rates in the control environment (See Supporting Information Table S3). R cells had the same rank order of environments as the S cells. Since the growth rates of the RP cells were highly variable relative to the other resistance types, containing two populations that

grew quickly, the RP populations were grouped into fast growing (NG'10 and NG'16) and normal growing (NG27). RP cells showed the same rank order of environments for both the fast and normal growing populations, except in low salt for the fast growing populations. This was due to one population (NG'10) displaying exceptionally high growth. Growth rate was the same in the low salt and low light environments for the normal growing RP population. Fast growing RP cells had higher cell growth in all environments except low phosphate.

To measure how sensitive growth rates were to environmental change, the slopes of the ranked environments were compared (Figure 3). The two fast growing RP populations had a higher intercept (ANOVA effect of rank on growth,  $F_{1,125}=1112.56$ ,  $p < 0.0001$ ), demonstrating the increase in growth rate compared to the other populations. These data show that faster growing populations had a stronger preference for environments in which they can grow more quickly, however in the lowest ranking environment (which was low phosphate for all resistance types), these populations grew equally badly.

*Size and chlorophyll content vary between cells with different resistance types in response to environment*

After one week of growth in a novel environment without viruses, cell size and relative cell chlorophyll content were measured. Response of resistance type on cell size depended on environment (effect of environment  $\times$  resistance type,  $F_{8,114}=5.48$ ,  $p < 0.0001$ ). Regardless of resistance type, environment had a significant effect on cell size ( $F_{4,114}=77.93$ ,  $p < 0.0001$ ). Cells were larger under low light ( $t=3.83$ ,  $p=0.0002$ )

and low phosphate conditions ( $t=7.49$ ,  $p<0.0001$ ), compared to the control environment (Figure 4). No significant effect of resistance type was observed on cell size ( $F_{2,6}=0.01$ ,  $p=0.9945$ ). However, under low phosphate, there was a large variation in cell size between the fast and normal growing RP populations.

The two fast-growing RP populations had smaller cells than the normal growing RP population in the low phosphate environment. The RP population with normal growth had cells that were similar in size to the S populations (Figure 4). To examine whether fast growing RP populations had different cell sizes than did populations with normal growth rates, *post hoc* models were used to analyse the two fast growing populations separately. Overall, no significant effect of resistance type was observed on cell size when normal and fast growing RP populations were analysed separately (ANOVA  $F_{2,6}=0.22$ ,  $p=0.8812$ ). Additionally, a model examining growth rate as a fixed effect was also performed. This showed a significant effect of growth rate ( $F_{1,99}=54.23$ ,  $p<0.0001$ ) and an interaction between resistance type and growth rate ( $F_{2,99}=4.64$ ,  $p=0.01$ ), although no effect of resistance type alone was detected ( $F_{2,6}=0.001$ ,  $p=0.99$ ). However, the statistical power in this data set, which contained only one population of normal growing RP cells and two populations of fast growing RP cells, was low, such that the chances of detecting an effect of resistance type on cell size is unlikely here even if one exists (power=0.142).

The effect of resistance type on chlorophyll content per cell volume depended on environment (effect of environment  $\times$  resistance type,  $F_{8,114}=10.68$ ,  $p <0.0001$ ). In addition, environment alone had a significant effect on relative chlorophyll per cell volume ( $F_{4,114}=120.45$ ,  $p <0.0001$ ), however resistance type alone did not ( $F_{2,6}=1.61$ ,

$p = 0.2757$ ). Under low light, chlorophyll varied little between the three resistance types. In the other environments, S and R strategies usually displayed similar chlorophyll content levels with RP displaying lower chlorophyll levels in all environments except low phosphate.

By inspection, we see that the fast growing RP populations have less chlorophyll per cell volume than the normal growing RP population in all environments except low phosphate (Figure 5). We used a *post hoc* model with growth rate as a fixed effect to investigate whether the fast growing RP populations also had different chlorophyll contents. Growth rate had a significant effect on chlorophyll content ( $F_{1,99}=57.86$ ,  $p < 0.0001$ ), with fast growing RP populations having lower chlorophyll content, and the effect of growth rate was dependent on environment ( $F_{4,99}=3.85$ ,  $p=0.01$ ) and resistance type ( $F_{2,99}=6.27$ ,  $p=0.003$ ). Furthermore, when growth rate was considered in the model, resistance type alone had a significant effect on chlorophyll content ( $F_{2,6}=5.49$ ,  $p=0.04$ ), suggesting that the growth rate of the fast growing RP populations reduced chlorophyll content.

## DISCUSSION

### *Effect of environment on host resistance*

We observed no differences in susceptibility of any of the populations to OtV5 over the environments tested. While the ability of the virus to lyse host cells did not depend on the environment, R and RP cells had different growth responses to viral exposure. There were two cases in which a resistant population repeatedly had a higher cell

density after exposure to OtV5 than its paired control culture that was not inoculated. We speculate that this may be a response to the virus, which causes the phytoplankton cells to divide more rapidly. This would be advantageous if, for example, a population that was made up of mixed susceptible and resistant cells were exposed to virus – any resistant cell lineages that could increase their growth rate would then take over the population by overgrowing any remaining resistant cells whose growth rate was unaffected by exposure to virus.

We did not detect a growth cost of resistance when R and RP populations were grown in the absence or presence of OtV5 after exposure to a novel environment. We expect to see a trade-off for being resistant to viral infection, because if there were no cost there should be a strong selection pressure for all cells to become resistant, yet we still find susceptible populations both in the laboratory and in the ocean (Thomas *et al.*, 2011; Clerissi *et al.*, 2012). Previous work shows that susceptible cells can have a competitive advantage against resistant cells (Lenski, 1988). Additionally, we speculate that resistance to one virus strain could make these cells susceptible to other OtVs. Clerissi *et al.*, (2012) showed that OtVs are mainly intraspecies-specific and that hosts that are the most resistant to infection can often be infected by more generalist viruses. This specificity could be caused by proteins involved in adaptive behavior (Clerissi *et al.*, 2012). Thus, we suggest that in addition to the abiotic environment, biotic environment could play a large role in *O. tauri* resistance strategy.

Since viruses are responsible for a large proportion of microbial death, there is strong selection on hosts for resistance or tolerance to viral infection. There are several

suggestions to explain the paradox of how susceptible algal cells and their viruses are able to co-exist in marine environments without extinction of the host. One theory as to how viruses and their hosts are able to coexist is that there must be a cost to being resistant to infection. This is often expected to be a reduction in growth (Weinbauer, 2004), as has been observed in *Synechococcus*, in which there was a 20% reduction in fitness compared to the ancestor in resistant strains (Lennon *et al.*, 2007). Thus in the absence of viruses, resistant cells often have a lower fitness. This could lead to decreased numbers in the absence of viruses. An evolutionary “arms race” may occur when viruses and their hosts adapt reciprocally to overcome resistance and infection, respectively. We find little evidence for a cost of resistance in our study, but this may be because the laboratory environments used are missing a key aspect of the natural environment that, if present, results in a cost of resistance in *O. tauri*. Alternatively, although deviating from the standard control environment, none of the environments in this study were severely stressful, with even the low phosphate environment allowing reasonable growth. Thus, it is possible that we did not detect a growth cost because the changes to the environments used were relatively modest.

Various strategies for virus resistance have been reported in algae, including activation of programmed cell death (Bidle *et al.*, 2007), absence of metacaspase (caspase orthologues) protein expression (Bidle *et al.*, 2007), stage of the life cycle (Frada *et al.*, 2008), changes to cell surface receptor proteins (Tarutani *et al.*, 2006), colony formation (Brussaard *et al.*, 2007) and genetic mutations (Stoddard *et al.*, 2007). However, it is still unknown how *O. tauri* cells acquire their two resistance strategies. We found that short-term exposure to novel environments does not affect

resistance type and we did not observe any cost of resistance leading to cells losing their resistance to OtV5.

#### *Effect of resistance type on population growth and other phenotypic traits*

We found that after one week in a novel environment, growth rate of *O. tauri*, as measured by the average number of cell divisions per day over seven days, varied across environments for all resistance types. RP populations had the fastest average rates of cell division in most environments. All resistance types showed the same environmental preferences, with average cell division rates highest in the control environment. The only exception was one RP population that divided rapidly in the low salt environment. The lowest growth rates were observed in the low phosphate environment, which was expected since these cells were deprived of a key nutrient.

Two of the three RP populations divided more rapidly than all of the S and R populations. These populations were fast growing in many environments, including the control environment, suggesting that the rapid growth is a general character of these two RP populations, rather than a response to stress or novelty. This faster growth rate in RP populations relative to S and R populations is in contrast with previous studies on *O. tauri*. Thomas *et al* (2011) detected no difference in growth rate between S, R and RP cells, although competition experiments revealed a small reduction of fitness in RP compared to R, and R compared to S. Our results suggest that the opposite can be true. Similarly to Thomas *et al.* (2011), we did not observe a fitness cost in terms of growth rate for the remaining populations since S, R and normal growing RP populations had similar growth rates across environments. This



351 was expected, at least in the control environment, where previous studies have only  
352 been able to detect a minimal cost of resistance by using direct competitions.  
353 Surprisingly, the two fast growing RP populations could not be detected as having  
354 more rapid growth under low phosphate, however these populations responded  
355 differently in their size and chlorophyll contents.

356  
357 Reduced growth rate is often observed as a cost of resistance in microbes and has  
358 been measured in several species (Lennon *et al.*, 2007; Haaber and Middelboe, 2009).  
359 Ecologically, a cost of resistance is part of “kill the winner” dynamics, where, it is  
360 hypothesized that viruses kill the faster growing (susceptible) cells, and thus provide  
361 an opportunity for slower growing (resistant) cells (Mojica and Brussaard, 2014). This  
362 role for viruses requires that there be a cost of resistance. However, here we did not  
363 detect a cost of resistance in terms of growth rate, since there was no environment in  
364 which resistant cell types grew at slower rates than S cells. In fact, we observed the  
365 opposite in two out of the three RP populations, where resistant cells grew faster than  
366 the S populations across all environments except low phosphate. In cases where  
367 resistant cells (R or RP populations) did not divide faster than susceptible ones, they  
368 divided at the same rate. Taken together, this suggests that the cost of resistance to  
369 OtVs is likely to be small or absent, and may not play into kill the winner dynamics.  
370 This opens the question of how the appearance of resistance to OtVs affects both host  
371 and viral ecology.

372  
373 Environment affected cell size, whereas generally, resistance type did not. However,  
374 under low phosphate, the two fast growing RP populations were smaller than the  
375 normal growing RP populations, suggesting that under nutrient limitation these cells

376 were able to divide at a smaller cell size. Smaller phytoplankton cell size is often  
377 selected for in nutrient limited environments since smaller cells have a larger surface  
378 area to volume ratio and a thinner diffusion boundary layer, thus facilitating nutrient  
379 uptake (Finkel *et al.*, 2010; Peter and Sommer, 2015). Although fast growing RP  
380 populations in this selection environment were smaller than the normal growing RP  
381 population, their cell size was not different from the fast growing RP populations in  
382 the other environments. The control was the only environment in which fast growing  
383 RP populations were larger than the normal growing populations, indicating that there  
384 may be a (direct or indirect) fitness benefit associated with the increased size of the  
385 RP type under control conditions.

386  
387 In contrast to previous studies, all populations in the low phosphate environment,  
388 except fast growing RP, increased in cell size. Cell division of larger phytoplankton  
389 cells requires greater nutrient concentrations, which can decrease the division rate.  
390 Since cells in the low phosphate environment had a reduced growth rate in terms of  
391 cell divisions, this could have resulted in cells that reached a larger volume even  
392 though the environment was phosphate-poor. It has previously been suggested that  
393 increasing algal cell size, and thus the volume to surface area ratio, can facilitate  
394 reduced phosphorus uptake under phosphate-limited conditions, and that this  
395 adaptation response may be more favourable than decreasing cell size (Šupraha *et al.*,  
396 2015). A common response of coccolithophores to phosphate limitation is reduced  
397 growth rate and increased cell size (Šupraha *et al.*, 2015).

398  
399 Smaller phytoplankton cells have often been observed growing at higher temperatures  
400 in natural environments, which is thought to arise from the temperature-size rule (e.g.

Atkinson *et al.*, 2003; Morán *et al.*, 2010). These studies used large temperature ranges, but there was no effect of the modest increase in temperature on cell size in this study. Smaller cells have also been reported to cope better with both light limitation and light saturation compared to larger cells due to a reduction in internal shading (Geider *et al.*, 1986; Raven, 1998; Finkel *et al.*, 2010). We found no significant difference in cell size under low light, although there was a non-significant trend for cells to be slightly larger in low than under control light.

Environment was found to have a significant effect on chlorophyll content per cell volume, whereas resistance type alone had no effect. We observed lower chlorophyll per cell volume in all environments compared to the control except high temperature. Although resistance type alone did not have an effect, growth rate had a significant effect on chlorophyll content per cell volume when included in the model and normal growing S, R and RP cells in the control and high temperature environments had the highest chlorophyll levels across all environments. In contrast, fast growing RP populations showed no significant difference in chlorophyll content per cell volume across all five environments. All populations had their lowest growth rates in the low phosphate environment and cells in this environment had the lowest chlorophyll content, except for fast growing RP populations. Fast growing RP populations had lower chlorophyll content than the normal growing RP population in all environments except for low phosphate.

One experiment using cultures of different phytoplankton groups found that chlorophyll content was lower during both nitrogen and phosphorus depletion (Riemann *et al.*, 1989). Additionally, phytoplankton cells grown under low nutrients

have been observed to decrease their photosynthesis rates (Litchman *et al.*, 2003; Spilling *et al.*, 2015). This may be due to the cells allocating resources to synthesizing chloroplasts under nutrient limitation. In our study the control environment was the preferred one, and it is possible that cells were unable to synthesise large quantities of chlorophyll in the other (less permissive) environments since their energy was allocated to growth. It is possible that under elevated temperature, the metabolism of *O. tauri* was increased, leading the cells to synthesise more chlorophyll. Temperature did not affect chlorophyll a content in diatoms (Sigaud and Aidar, 1993). Salinity appears to affect different phytoplankton species differently, with some species showing no change in chlorophyll content across a range of salinities, and others having higher chlorophyll contents at the optimum salinity for growth (McLachlan, 1961; Sigaud and Aidar, 1993).

#### *Concluding remarks*

Resistance of microbes to virus infection often comes at a cost, with one common observation being a reduction in growth compared to susceptible cells in the population. In this study, our aim was to measure resistance to viruses in *O. tauri* across different environments and to determine whether the magnitude of a cost of resistance depends on environmental context. We did not observe a cost of resistance as measured by cell divisions, cell size or chlorophyll content in the present study. Growth rates of *O. tauri* were reduced when grown in low phosphate, however this did not affect the ability of OtV5 to lyse susceptible cells in this environment. Additionally, although growth rates were lower than the controls in high temperature, low light and low salinity, OtV5 still caused cell lysis of susceptible cells. Indeed,

451 some populations that were tolerant to infection (RP populations) had evolved high  
452 growth rates, and some RP populations also increased their growth rates after  
453 exposure to viruses. Both observations suggest that resistance strategy could have  
454 interesting ecological consequences by changing the relative fitness of different  
455 populations.

## EXPERIMENTAL PROCEDURES

### *Susceptible and resistant populations used in this experiment*

*O. tauri* populations were obtained from N. Grimsley, Observatoire Océanologique, Banyuls-sur-Mer. Three susceptible populations (NG'2, NG'3 and NG'4), three resistant producer populations (NG'10, NG'16 and NG27) and three resistant populations (NG5, NG'13 and NG26) were used in this study. We used three biological replicates for each population in each environment. All populations were derived from a single clone of *O. tauri* (RCC 4221) and therefore had the same starting genotype (see Thomas *et al.*, 2011). All populations have since been maintained separately. All RP populations were tested for viral production prior to the start of the experiment (See Supporting Information Figure S1).

### *Culturing conditions*

Populations were grown in batch culture. Culture medium was prepared using 0.22  $\mu\text{m}$  filtered Instant Ocean artificial seawater (salinity 30 ppt) aerated with 400 ppm  $\text{CO}_2$  and supplemented with Keller and f/2 vitamins. Control cultures were maintained in a 14:10 light:dark cycle at  $85 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  and at a constant temperature of  $18^\circ\text{C}$  (Table 1).

For the selection experiments, *O. tauri* populations were grown without exposure to viruses in the control environment and four selection environments. The selection regimes used were high temperature, low light, low phosphate and low salinity (Table

1). Cultures were acclimated in each selection environment for one week, followed by one week of growth in each environment.

Environment	Control	Treatment
Phosphate ( $\mu\text{M}$ )	10	5
Salinity (ppt)	30	25
Light ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	85	60
Temperature ( $^{\circ}\text{C}$ )	18	20

For the low phosphate environment, phosphate was reduced by preparing Keller media with only half the amount of  $\beta$ -glycerophosphate that would normally be used. Although the phosphate concentration in the low phosphate environment is not low compared to natural seawater ( $0.01\text{-}2.99 \mu\text{mol l}^{-1}$  in the Leucate lagoon where *O. tauri* and OtV5 inhabit (Clerissi *et al.*, 2014)), it is low compared to the control media in which the populations had been maintained prior to the experiment. For culture medium with a lower salinity than the control, Instant Ocean was added to reach a salinity of 25 ppt. For the low light condition, culture flasks were wrapped in 0.15 neutral density foil to give a light intensity of 1000 lux. Cultures in the high temperature condition were maintained on a heat mat (Exo Terra Heat Wave substrate heat mat) set at  $20^{\circ}\text{C}$ .

#### *The effect of viral exposure on cell division rates*

Following one week of growth in the selection environment, each sample was inoculated with a fresh suspension of OtV5 particles to test whether it was susceptible

or resistant to the virus. Samples were tested by inoculating 1 ml cell culture at a density of  $10^5$  with 10  $\mu$ l OtV5 in 48-well plates with three replicates for each sample. Controls that were not inoculated with viruses were used as a control for cell growth. Cell density was measured using a FACSCanto flow cytometer 3 days after inoculation.

### *Population growth of susceptible and resistant populations across different environments*

Following the acclimation period, average cell densities per day of all cultures were measured over one week of growth in each environment. Cells were counted using a BD FACSCanto II (BD Biosciences) flow cytometer before the first transfer and after seven days of growth. Each population was counted in triplicate. The cell counts were converted to cells per millilitre and the number of divisions per day was calculated using equation (1).

$$(1) \quad \mu (d^{-1}) = \frac{\log_2 \left( \frac{N_t}{N_0} \right)}{t - t_0}$$

where  $N_t$  and  $N_0$  are the cell densities (cells  $ml^{-1}$ ) at times  $t_1$  and  $t_0$  (days). This measures the average number of cell divisions per ancestor over a single growth cycle and allows a comparison of offspring production between environments (Brennan and Collins, 2015). This is useful if different environments produce different growth curves since populations with different growth strategies can be compared. This calculation is also not sensitive to small differences in  $N_0$ , which is important if the



population size reached during the acclimation period differs between environments or resistance types.

#### *Cell size and chlorophyll content of populations with different resistance types across environments*

Cell size and relative chlorophyll content per cell volume were determined using a FACSCanto flow cytometer. Cell size was inferred from FSC (forward scatter), which was calibrated using beads of known sizes (1µm, 3µm and 6.6µm). Chlorophyll fluorescence was inferred by measuring PerCP-Cy5.5 emission with excitation at 488nm. Relative chlorophyll was analysed by taking the average chlorophyll fluorescence for all susceptible populations in the control environment and setting this to a value of 1, with chlorophyll measurements of all populations relative to this value.

#### *Statistical analysis*

Data were analysed with linear mixed effects models using the statistical package nlme in R (version 3.2.0) to identify differences in growth rates between the different environments after one week of growth and after virus inoculation. Environment and resistance type were fixed effects when analyzing growth under different environments, and environment, resistance type and treatment were fixed effects when analyzing virus inoculation under different environments. Population was a random effect in both models.

*Post hoc* mixed effects models were used to examine whether growth rate had an effect on cell size and chlorophyll content in cells. Environment, resistance type and growth rate (cells divisions per day) were set as fixed effects with populations as the only random effect.

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## TABLE AND FIGURE LEGENDS

Table 1. A comparison of the control environment and the environment treatments that were used for each environmental condition in this study.

Figure 1. Mean cell densities  $\text{ml}^{-1}$  of resistant (R), resistant producer (RP) and susceptible (S) *O. tauri* cells. Inoculated = cells inoculated with OtV5, Not inoculated = control cultures that were grown for the same amount of time, but not inoculated with OtV5. The dashed line represents the starting densities of the cultures at  $10^5$  cells  $\text{ml}^{-1}$ . There were three biological replicates for each populations. Boxes represent the interquartile range with the median indicated as the thick black line inside the box, and whiskers extend to the highest and lowest values within  $1.5 \times$  the inter-quartile range from the edge of the box. Outlier data beyond the end of the whiskers are plotted as points.

Figure 2. Mean growth rates, measured as average number of cell divisions per day over 7 days, of susceptible (S), resistant (R) and resistant producer (RP) *O. tauri* cells grown in five environments in the absence of OtV5. There were three populations for each resistance type, with three biological replicates for each population. Boxes represent the interquartile range with the median indicated as the thick black line inside the box, and whiskers extend to the highest and lowest values within  $1.5 \times$  the inter-quartile range from the edge of the box. Outlier data beyond the end of the whiskers are plotted as points.

Figure 3. Ranked environment by average cell divisions per day over 7 days ( $\pm$  SEM) for susceptible (S), resistant (R) and resistant producer (RP and RPfast) cells. Environments were ranked in order from best to worst for each resistance type based



on growth rate in the absence of OtV5, where 1 is the environment with the highest growth rate. Fast and normal growing resistant producers have been plotted separately for visual purposes.

Figure 4. Mean cell size for susceptible (S), resistant (R) and resistant producer (RP) cells after seven days of growth in the absence of viruses in five environments. There were three populations for each resistance type, with three biological replicates for each populations. Boxes represent the interquartile range with the median indicated as the thick black line inside the box, and whiskers extend to the highest and lowest values within  $1.5 \times$  the inter-quartile range from the edge of the box. Outlier data beyond the end of the whiskers are plotted as points.

Figure 5. Mean relative chlorophyll to cell size for susceptible (S), resistant (R) and resistant producer (RP) cells after seven days of growth in the absence of viruses in five environments. There were three populations for each resistance type, with three biological replicates for each population. Boxes represent the interquartile range with the median indicated as the thick black line inside the box, and whiskers extend to the highest and lowest values within  $1.5 \times$  the inter-quartile range from the edge of the box. Outlier data beyond the end of the whiskers are plotted as points.

Table S1. ANOVA results of a linear mixed effects model to analyse interaction effects of environment, resistance type and treatment (with or without OtV5 inoculation) on *O. tauri* cell density. Population was a random effect.

Table S2. ANOVA results of a linear mixed effects model to analyse interaction effects of environment, resistance type, treatment (with or without OtV5 inoculation) and growth rate (fast RP or normal) on *O. tauri* growth rate, as measured by cell divisions per day. Population was set as a random effect in all models.

Table S3. Ranked environments by fitness as measured by cell divisions per day for each resistance type. Environments were ranked in order from best to worst, where 1 is the environment with the highest growth rate. Fast and normal growing resistant producers were ranked separately to compare slopes.

Figure S1. Mean cell densities  $\text{ml}^{-1}$  ( $\pm\text{SEM}$ ) of *O. tauri* strain RCC4221 three days after inoculation with supernatant from Resistant Producing populations (NG'10, NG'16 and NG27). To ensure that the RP populations being used in this experiment were producing infectious viruses and releasing them to their external surroundings, we used the supernatant of these strains to infect susceptible *O. tauri* cells. Populations NG'10, NG'16 and NG'27 were aliquoted into 2ml Eppendorf tubes and centrifuged at  $8000 \times g$  for 15 min. Next, 400  $\mu\text{l}$  of supernatant was carefully removed without drawing up any cells from the pellet at the bottom of the tube, and used to inoculate 1 ml of susceptible *O. tauri* strain RCC4221. Eight replicates were performed. A positive control was performed using known OtV5, and a negative control was performed by adding Keller media. Controls were performed in quadruplicate. Cells were left to grow for 3 days after which their densities were measured using a FACSCanto flow cytometer. We observed cell lysis resulting from inoculation with supernatant from all three RP populations, showing that there was active virus in the media taken from these cultures.