Collection, isolation and culturing strategies for *Oxyrrhis marina*

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The heterotrophic marine flagellate *Oxyrrhis marina* is a popular and tractable model organism, being common in the intertidal environment and relatively straightforward to maintain in the laboratory. In this report, based on our experience of collecting and culturing ~400 isolates, we provide a quantification of how “easy” *O. marina* is to locate and isolate from the environment and subsequently maintain in culture. In addition, we provide a brief review of the literature pertaining to *O. marina* culturing, stressing in particular the broad physiological tolerance and a wide diet range of this organism. Finally, in the light of the increasing interest in the genetics and genomics of *O. marina*, and heterotrophic protists more generally, we highlight existing strategies for culturing heterotrophs for “-omic” applications and summarize our specific culturing approaches for the genomic and proteomic studies of *O. marina*. In this context, we outline a simplified version of Droop’s medium for axenic culturing and offer details on the use of heat-killed *Escherichia coli* as a simple and robust food source for culturing *O. marina* in the absence of other eukaryotes and live prokaryotes.

KEYWORDS: model protist; axenic culturing; intraspecific variation

INTRODUCTION

Protist experimental models should be robust and, therefore, easy to culture. Furthermore, they should be abundant and easy to isolate from the environment, such that experimental observations can be compared between replicate isolates. Finally, model organisms should be interesting and relevant tools to address fundamental biological, ecological and evolutionary questions (Montagnes et al., 2011).

The heterotrophic marine flagellate, *Oxyrrhis marina*, has proved to be a popular model organism, as demonstrated by the broad scope of the literature concerning this protist (e.g. Flynn and Davidson, 1993; Buskey et al., 1998; Salazarriaga et al., 2003; Lowe et al., 2005, 2010; Slamovits et al., 2007). It is also apparent that *O. marina* is a tractable “lab rat”. Indeed, two commonly stated anecdotes about this species are that it is easy to grow and it is abundant in the environment. Striking features from the literature are that *O. marina* has broad environmental tolerances (e.g. Droop, 1959a, b; Lowe et al., 2005), and an exceptional range of prey types support its growth (see Supplementary Table S1 and references therein). Nevertheless, despite the common use of *O. marina* in laboratory studies, explicit reports of culturing strategies and growth requirements are scarce, with the exception of Droop’s studies (Droop, 1959a, b; Droop and Doyle, 1966; Droop and Pennock, 1971) and other brief reports scattered throughout the literature. A reassessment and synthesis of methods associated with *O. marina* culturing is, therefore, timely.

The second observation that *O. marina* is abundant and easy to locate in the environment is certainly anecdotal, as explicit studies examining its abundance and...
distribution are rare. Oxyrrhis marina is undoubtedly widespread: ~50–80 isolates are reported in the literature from a broad range of locations (Lowe et al., 2010). However, estimates of the effort required to obtain isolates from the environment are absent from the literature. Given that recent studies on O. marina indicate extensive genetic and phenotypic diversity, which may be spatially structured (Lowe et al., 2005, 2010), the isolation and characterization of further strains is likely to be an important component of studies concerning this model protist. Indeed, it is now clear that there is a conspicuous lack of understanding of population level demographic processes for free-living protists in general (Martiny et al., 2006) and that in a broader microbial context free-living protists remain relatively poorly described, particularly in terms of population genetic and genomic structure (Caron et al., 2009). In this context, sampling guidelines to aid researchers to collect new O. marina strains (e.g. to allow intraspecific comparative studies) will be useful.

Here, we examine the literature on O. marina pertaining to isolating, culturing and maintaining isolates. First, we outline our experiences of isolating and establishing O. marina cultures; based on the collection of >1000 environmental water samples, we provide estimates of the rate of recovery of O. marina from the environment and provide a guide to how readily cultures can be established from such samples. We then highlight common culturing strategies for the maintenance of O. marina and emphasize its wide physiological tolerances and the breadth of prey items that support growth. Finally, in the light of the increasing interest in the genomics and proteomics of free-living protists in general (Caron et al., 2009), including specific targeted studies of O. marina (e.g. Slamovits et al., 2007; Slamovits and Keeling, 2008), we consider the culturing requirements of such applications and outline three strategies for generating axenic cultures and “clean” material for de novo genomic and proteomic analyses. In particular, we revisit Droop’s work on osmotrophy and provide a simplified version of his fully derived medium (Droop and Pennock, 1971) for axenic culturing of O. marina.

COLLECTION AND ISOLATION OF O. MARINA FROM THE ENVIRONMENT

Recovery of O. marina from coastal habitats

Oxyrrhis marina is commonly described as cosmopolitan and abundant in coastal habitats; the interested reader is directed to Watts et al. (2011) for an analysis of O. marina distributions. Most reports describe O. marina from coastal environments, particularly supra-littoral tide pools on rocky shores (e.g. Droop, 1953; Jonsson, 1994; Johnson, 2000), salt marshes, estuaries (Johnson et al., 2003) and coastal embayments (Begun et al., 2004); though some of the earliest reviews of the taxon described O. marina as most commonly occurring in saltwater aquaria (Kofoid and Swezy, 1921). These observations are for the most part anecdotal; indeed explicit studies on the abundance and distribution of O. marina are relatively rare and limited in scope (Johnson, 2000; Lowe et al., 2010). Thus, whether our current understanding of distributions and abundance is driven by sampling bias or is a genuine reflection of O. marina distribution in the environment is difficult to determine.

Nevertheless, the frequency with which O. marina has been collected from coastal habitats certainly suggests abundance: ~40 isolates are described in the literature (Lowe et al., 2011), and a further 38 were detailed in a recent study by Lowe et al. (2010)—all these were isolated from coastal, and almost exclusively intertidal, habitats. However, a quantification of how easy, or not, O. marina is to recover from such habitats is absent from the literature. As part of an ongoing 3-year study into the distribution of O. marina, we have undertaken an extensive sampling program, focusing primarily on northern European and Mediterranean waters, but also collecting globally. Currently, ~1000 environmental samples have been collected (Watts et al., 2011, describe the broad trends in the distribution of O. marina in these samples), and our subsequent sampling database provides a number of useful guidelines for the collection of O. marina.

First, the rate of recovery of O. marina from intertidal environments is remarkably consistent; across our whole study, O. marina is consistently recovered from ~50% of intertidal samples (Fig. 1a). Second, sampling effort has a clear affect on the recovery O. marina from a given location (Fig. 1c). Typically, we collect between one and ten 50 mL samples from a location, though up to 70 samples have been collected from a single site, and 160 samples were collected at one time from a 2-km shoreline in North Wales, UK. Where a single sample is collected from a given intertidal location (e.g. a stretch of shoreline), there is on average a ~40% chance of finding O. marina; the probability of collecting at least one positive sample increases to 90–100% if >10 samples are collected from one shore (e.g. samples from different tide pools; Fig. 1c). Of course, these figures are estimates and depend on the habitats sampled. Predominately, our samples have been collected from littoral pools on rocky shores, though we have also isolated O. marina from estuaries, salt marshes, coastal lagoons and marinas. Nevertheless, a summary of our sampling data provides a useful guideline for researchers wanting to isolate
Our recommendation is that the collection of 10–20 50-mL samples from an intertidal habitat (especially a rocky shore with littoral pools) will provide a high probability of obtaining *O. marina*. Finally, seasonality in *O. marina* abundance undoubtedly occurs. Periodic sampling of a tide pool at Port St Mary, Isle of Man revealed peaks in abundance in May–June then late September–October, interrupted by the periods of scarcity in midsummer and winter (Kimmance, 2001; but also see Fig. 4 in Watts et al., 2011). We have predominately sampled in the northern hemisphere during spring and summer; nevertheless, samples taken during the winter months (when *O. marina* abundance is relatively low) have also yielded isolates; thus, it can be obtained from the environment throughout the year (Fig. 1b).

**Isolation and establishment of clonal cultures**

The above observations support the notion that *O. marina* is relatively straightforward to locate in coastal habitats. Once collected, cultures are also simple to establish, as *O. marina* is large enough (15–40 μm long, Fig. 2) to be manipulated using finely drawn Pasteur pipettes. Common single cell isolation strategies can then be used to generate clonal cultures (e.g. Stein, 1973; Kemp et al., 1993). In our laboratory, we typically screen environmental samples using stereo dissection microscopes (Fig. 2); for a description of the gross morphology of *O. marina*, see Lowe et al. (2011). When *O. marina* is detected, individual cells are transferred to single wells in 24-well (2 mL tissue culture plates)—on average 40% of isolates result in established cultures.
be established following three or four rounds of single cell isolation over a period of 2–4 weeks. In our experience, on average, 40% of isolated cells divide successfully and generate moderately dense ($\approx 10^3$ cells mL$^{-1}$) cultures, though in a small number of cases, all single cell isolations from a sample may die (Fig. 1d).

**OXYRRHIS AS A LABORATORY ORGANISM**

Undoubtedly, *O. marina* has become a useful experimental model organism because it is relatively easy to isolate from the environment (as highlighted above) and simple to maintain in the laboratory. This robustness is a result of broad tolerances to a range of environmental variables and an exceptionally wide diet range (Supplementary Table S1)—including the ability to maintain population growth on a fully derived cell-free medium (Droop and Pennock, 1971). Below we summarize our strategy for the maintenance of *O. marina* stock cultures, as an indication of basic culturing requirements (at the time of writing, we maintain $\approx 150$ isolates in our laboratory; subcultures may be obtained by contacting the corresponding author). We then briefly examine the breadth of physiological tolerances in *O. marina* and assess its nutritional requirements.

**Routine maintenance**

Once established, *O. marina* is straightforward to maintain in the laboratory. We follow culturing methods described in much of the literature for growing temperate marine protozoa (Kemp et al., 1993). Briefly, stock cultures of autotrophic flagellates (e.g. *D. primolecta*; see the “Nutritional requirements” section below) obtained from a commercial culture collection (e.g. Culture Collection of Algae and Protozoa, Dunstaffnage, Scotland) are grown in sterilized 32 PSU artificial seawater or filtered natural seawater; both water types are enriched with f/2 medium (Guillard, 1975—commercially available from Sigma). We do not generally attempt to make our stock cultures of prey or *O. marina* bacteria-free, and undoubtedly *O. marina* augments its diet with these bacteria (Jeong et al., 2008). Both prey...
and *O. marina* cultures are exposed to 24 h irradiance, at \( \sim 50 \mu \text{mol photons \text{m}^{-2} \text{s}^{-1}} \), at 15–20°C; note though that light cycle and temperature will alter growth rates (e.g. Jakobsen and Strom, 2004; Kimmance et al., 2006). *Oxyrrhis marina* is maintained on prey flagellates at \( \sim 10^5 \text{cells mL}^{-1} \). Typically, cultures are kept in \( \sim 75 \text{mL} \) flasks and transferred every 4 weeks. Using this strategy, we have maintained a collection of 150–200 stock cultures for \( > 12 \text{months} \) with a \( \sim 5\% \) failure rate (i.e. \( 5\% \) of the stock cultures have died).

**Physiological tolerance**

*Oxyrrhis marina* is robust to a number of parameters that may change in culture collections. We briefly outline these below.

**Salinity.** Droop (1959a) suggested that *O. marina* thrives between 4 and 130 psu and indicated optimum growth at 16 psu for the Finnish strain CCAP 1133/5. He also demonstrated an affect of acclimation on cell viability following salinity shifts: cells acclimated to 8 psu suffered high mortality when transferred to high salinities (32–64 psu), but cells acclimated to 64 psu remained viable when transferred to salinities as low as 8 psu. Lowe et al. (2005) demonstrated salinity tolerances between 5 and 55 psu but showed that both tolerance and maximum growth varied between different strains and isolates. Lowe et al. (2005) also indicated two salinity responses, which appeared to correlate with habitat: intertidal strains grew best at relatively high salinities (i.e., \( > 30 \text{psu} \)); open-water strains grew best at low salinities (i.e., \( < 30 \text{psu} \)).

**Temperature.** Based on previously unpublished data for 10 isolates, *O. marina* can survive between 10 and 30°C, with optimal temperatures occurring between 20 and 25°C (Fig. 3, unpublished data). These estimates agree with data from Droop (1959a) on isolate CCAP 1133/5. He indicated an optimum temperature for growth of 22.5°C, with higher temperatures inducing rapid decreases in growth rate and mortality ensuing at temperatures \( > 30°C \).

**pH.** Only two studies have investigated the pH range that *O. marina* can tolerate (Droop, 1959a; Pedersen and Hansen, 2003). This species has a surprisingly wide pH tolerance; it can grow between pH 7.8 and 9.8. It does, however, suffer rapid mortality below pH 7.5.

**Intraspecific variation.** *Oxyrrhis marina* isolates differ genetically (Lowe et al., 2010) and physiologically; this includes variation in responses to temperature (Fig. 3) and salinity (Lowe et al., 2005). Thus, the physiological ranges indicated above are likely to exceed the tolerances of many individual strains and, as a result, should be treated as guidelines.

**Nutritional requirements**

*Oxyrrhis marina* consumes an exceptionally broad range of prey species and food items covering a wide size range (Fig. 4, Supplementary Table S1). Although *O. marina* is capable of feeding on bacteria (Jeong et al., 2008), the most common prey fed to *O. marina* during culturing are protists in the order of 10–50% its size (most commonly taxa in the genera *Isochrysis*, *Dunaliella* and *Rhodomonas* (Fig. 4, Supplementary Table S1). For a detailed description of prey and prey preference in *O. marina*, see Roberts et al. (2011).

In addition to phagotrophy, *O. marina* can be maintained on an entirely derived, cell-free medium. Extensive work, predominately by Droop in 1950–1970s (Droop, 1959a, b; Droop and Doyle, 1966; Droop and Pennock, 1971) established that *O. marina* could grow by osmotrophy (i.e. uptake of dissolved nutrients alone). Droop and Pennock (1971) described a fully soluble derived medium and highlighted the unusual nutritional mode of *O. marina*. Although Droop et al. provided an extensive understanding of the nutritional requirements of *O. marina*, the physiological mechanisms underlying its somewhat exceptional nutritional flexibility remain unresolved. Investigating such mechanisms is likely to provide novel future research directions.

**Culturing challenges for the “omic era”**

As a result of its phylogenetic position, basal to the dinoflagellates and a suite of highly unusual cytological, genetic and genomic features, *O. marina* is increasingly the target of genetic and genomic studies aimed at understanding the evolutionary development of major protist taxa (Saldarriaga et al., 2003; Cavalier-Smith and
Advances in genome sequencing technologies mean that even for poorly characterized organisms, such as *O. marina*, large-scale genome and transcriptome sequencing projects can be undertaken *de novo* (Hudson, 2008; Vera et al., 2008). There is, of course, a technical issue associated with genetic and genomic studies of heterotrophic protists: heterotrophy typically necessitates using prey that also contain DNA–RNA–protein; thus, such projects begin with a “built-in” contaminant. The extent of the problem is application-dependent; in small-scale genetic studies, a known contaminant is simple to deal with. For example, gene- or locus-specific PCR and sequencing strategies can easily be adapted to cope with contaminants by designing organism-specific PCR primers or by size selection or cloning. However, for larger scale sequencing applications, the presence of contaminating prey organisms can be problematic. For example, “next-generation” sequencing technologies allow *de novo* large-scale sequencing of organisms for which there is no prior genetic information (Hudson, 2008). In such cases, the presence of contaminating organisms in genome-scale sequencing projects absorb sequence reads (increasing cost) and, more critically, can be impossible to separate from target sequence, confounding downstream analyses. Clearly then, strategies to
generate “clean” DNA/RNA are required to take advantage of next-generation sequencing applications.

We have employed three strategies for dealing with prey contamination in *O. marina* cultures to generate “clean” material for mid- to large-scale sequencing applications. Below we summarize these and highlight some of their advantages and disadvantages for specific applications.

**Prey depletion**

The simplest strategy for overcoming the presence of contaminating genetic material is to allow prey to be depleted. In our experience, in the absence of light, *O. marina* can graze autotrophic protists (e.g. *Dunaliella* spp., *Isochrysis galbana*) functionally to extinction. The presence, or the absence, of algal cells can be determined by microscopical observations, or using PCR-based assays. For example, PCR primers designed against the 3′-end of the 18S and 5′-end of the 28S rDNA genes (to amplify the 5.8S ITS rDNA region) produce 430 and 620 bp PCR fragments for *O. marina* and *D. salina*, respectively (for primer details, see Lowe et al., 2005, 2010). Thus, the presence of algal cells can be determined by simple PCR and gel electrophoresis.

As an example of the above method, for a recent study developing a microsatellite library in *O. marina*, we generated 500 mL cultures at densities of 4.0–5.5 × 10^8 cells mL^-1. Cultures were established at 1 × 10^5 cell mL^-1 of *O. marina* and 5 × 10^5 cell mL^-1 of *D. primolecta*. After 11 days (maintained in the dark at 16–20°C), cultures appeared free of prey, but an rDNA PCR-based assay was positive for *D. primolecta*. Cultures were subsequently confirmed, by PCR, to be prey-free 17 days after the initial setup. Analysis of 64 candidate microsatellite loci produced one that appeared to originate from *D. primolecta* (i.e. produced a positive result in a *D. primolecta* control PCR assay; Lowe et al., in press). This illustrates that a simple prey depletion strategy can generate “clean” *O. marina* DNA for genome-scale analyses.

**Live and heat-killed Escherichia coli**

An alternative to the prey depletion strategy is to use a prey organism that has a known (i.e. sequenced) and small genome. The advantage is that any DNA sequence subsequently generated can be screened against a reference genome for the prey organism and removed from further analysis. *Oxyrrhis marina* will grow on cultures of naturally occurring marine bacteria (e.g. Jeong et al., 2008); though clearly, the use of an undefined (and likely complex) natural bacterial assemblage does not solve the problem of identifying contaminating sequences. Instead, the use of a well-characterized bacterium, such as *E. coli*, has proved useful in our laboratory. *Oxyrrhis marina* has been demonstrated to graze *E. coli* cells (e.g. Kato et al., 2000), and the use of heat-killed bacteria (HKB) as prey (as employed by e.g. Gurijala and Alexander, 1990; Landry et al., 1991) means that prey numbers can easily be controlled. A further advantage, for mRNA- and cDNA-based applications, is that the contribution of *E. coli* cells to subsequent RNA preparations will be minimal as bacterial cells are dead and thus inactive.

Our protocol for feeding *O. marina* with *E. coli* is, briefly, as follows. Cultures of *E. coli* (e.g. JM109, Promega) are grown in 50–500 mL of the Luria–Bertani broth at 37°C in an orbital incubator (210 rpm) for 8–12 h and culture growth monitored via optical density (following standard protocols; Sevónov et al., 2007). Cells are pelleted by centrifugation and washed twice in sterile filtered seawater (SFSW). They are then resuspended in 5–50 mL of SFSW (~10× concentration based on the initial volume of *E. coli* culture) and heat-killed at 65°C for 30 min. Cells can be ultrasonicated to reduce clumping and limit the occurrence of bacterial flock in the final culture. The final concentration of the preparation can be estimated based on optical density (we standardize our HKB stock solutions to ~2.5–5.0 × 10^8 cells mL^-1). A supply of 250 μL of HKB in 50 mL (~1.25–2.5 × 10^5 cells mL^-1) of culture every 3–4 days will maintain *O. marina* in exponential growth and generate cell densities up to ~1.5 × 10^9 *O. marina* mL^-1. Treatment with antibiotics to control naturally occurring bacteria in cultures is essential for the use of this feeding strategy; we use a penicillin and streptomycin solution (100 μg mL^-1) and treat with gentamicin (50 μg mL^-1) during routine subculturing at 1–2-week intervals.

**A simplified axenic medium**

Research by Droop et al. (Droop, 1959a, b; Droop and Doyle, 1966; Droop and Pennock, 1971) indicated that *O. marina* can be grown indefinitely on a medium containing solely water soluble factors, highlighting that *O. marina* does not rely exclusively on phagotrophy for survival. Clearly, in a research context, the ability to culture *O. marina* cells axenically is potentially useful in generating material for genetic analyses. In our laboratory, Droop’s axenic medium supports growth of a range of genetically diverse *O. marina* isolates, suggesting that most “variants” of *O. marina* can grow axenically on a soluble nutrient diet.

The commercial availability of readymade culture media and nutritional supplements has allowed us to simplify Droop’s original axenic medium (Table I). Our experiments with Droop’s Finnish strain of *O. marina*
Oxyrrhis marina has proved a useful model protist: it is abundant, relatively simple to isolate from the environment and straightforward to maintain in the laboratory. Perhaps more importantly, this practicality means that O. marina is likely to be a useful target for the study of a large range of questions in an ecological, physiological and evolutionary context. For example, the relatively large numbers of O. marina strains that can be practically isolated \((10^3 - 10^5)\) lends this organism to the study of population level processes and parameters, such as dispersal and geneflow, effective population size and adaptation—parameters and processes that are poorly characterized for free-living protists in general (Martiny et al., 2006; Caron et al., 2009).

It is notable from nutritional and ecophysiological studies that O. marina is incredibly flexible in its feeding requirements—able to consume a broad range of prey items but also to feed by osmotrophy. Thus, although the process of culturing is most often a means to an end, it is clear that for O. marina, explicit culturing studies to examine the physiological and biochemical basis of feeding mechanisms and nutrition are likely to be interesting avenues for future research (such directions are examined in more detail by Roberts et al., 2011). Oxyrrhis marina also displays broad environmental tolerances; in particular, it is eurytopic with regard to salinity, temperature and pH. However, there is some evidence for ecophysiological differences between strains; thus, the study of large numbers of isolates may prove useful to assess functional diversity and processes of environmental adaptation.

Finally, in the light of the increasing interest in the study of the genetics, genomics and proteomics of O. marina, we highlight three strategies employed in our laboratories for generating DNA–RNA–protein suitable for such applications. In particular, we highlight the use of heat-killed E. coli as a useful, robust and, above all, simple strategy for generating O. marina cultures free of other eukaryotic organisms. Beyond the technical challenges associated with culturing, however, the task of developing genomic resources for O. marina still remains; studies describing novel chromosome structure (Triemer, 1982; Gao and Li, 1986), unusual RNA editing mechanisms (Slamovits et al., 2007; Zhang and Lin, 2008) and potentially enormous genome size

### Table I: Components of a medium for the axenic culturing of O. marina

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock solutions</th>
<th>Final medium (~1 L)</th>
</tr>
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<tbody>
<tr>
<td>GF/C filtered autoclaved seawater (32 PSU)</td>
<td>–</td>
<td>750 mL</td>
</tr>
<tr>
<td>Amino acid/sodium acetate solution (pH 7.5), containing</td>
<td>300 mL</td>
<td>22.5 mL</td>
</tr>
<tr>
<td>Glycine hydrochloride (e.g. Sigma G1127)</td>
<td>5 g</td>
<td></td>
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<tr>
<td>L-valine (e.g. Sigma 94819)</td>
<td>2.5 g</td>
<td></td>
</tr>
<tr>
<td>L-proline (e.g. Sigma P5007)</td>
<td>0.4 g</td>
<td></td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>20 g</td>
<td></td>
</tr>
<tr>
<td>F/2 medium without silicate (e.g. Sigma G0164)</td>
<td>–</td>
<td>20 mL</td>
</tr>
<tr>
<td>Cholesterol solution (8 mg mL(^{-1})), containing</td>
<td>5 mL</td>
<td>5 mL</td>
</tr>
<tr>
<td>Water soluble cholesterol (e.g. Sigma C4951)</td>
<td>40 mg</td>
<td></td>
</tr>
<tr>
<td>Coenzyme Q(_{10}) solution (100 (\mu)g mL(^{-1})), containing</td>
<td>20 mL</td>
<td>200 (\mu)L</td>
</tr>
<tr>
<td>2 mg coenzyme Q(_{10}) in 1 mL of ethyl acetate added to 19 mL of 0.2% glycerol trioleate in absolute ethanol(*)</td>
<td>–</td>
<td>200 mL</td>
</tr>
<tr>
<td>1 (\times) Leibovitz L-15 medium (e.g. Gibco 21083) + 2.4% (w/v) NaCl</td>
<td></td>
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</table>

*Preparation of coenzyme Q\(_{10}\) follows the protocol of Droop and Pennock (1971).

The medium is a modification of that presented by Droop (1959b).

(CCAP 1133/5) and two other strains (44_PLY01, Lowe et al., 2010; and CCMP 1788) generated cell densities up to \(1.5 \times 10^5\) cells mL\(^{-1}\) in axenic culture using our modified recipe (Table I). Microscopical observations showed that the cells were morphologically similar to those grown on a phytoplankton diet. In addition, the axenic cells contained similar quantities of lipid and membrane protein \((200 \mu\)g of membrane protein, \(10^7\) cells\(^{-1}\)) as their phytoplankton raised counterparts (E. C. Wootton, personal observation).

However, one notable discrepancy occurred: strain CCMP 1788 lost its pink pigmentation during axenic culture and the cells became opaque and white. Despite this, the cells remained active and otherwise morphologically indistinguishable from other cultures (E. C. Wootton, personal observation). We have not pursued the causes of pigmentation loss in this strain under axenic culturing, though clearly the occurrence of differences in strain responses is notable in context with other observations of genetic and physiological diversity within O. marina (Lowe et al., 2005, 2010).

The success of growing visibly “healthy” O. marina cells under axenic conditions increases the potential utility of this protist as a valuable model organism, highlighting the organism’s robustness. Further, this procedure overcomes problems associated with DNA–RNA–protein contamination inherent in the genetic and genomic study of heterotrophic protists.
(Sano and Kato, 2009) suggest that the development of such resources is likely to be a complex and time-consuming process.

**SUPPLEMENTARY DATA**

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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**REFERENCES**


