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Assessing alternative grazing-tolerant algae for nursery culture of abalone, *Haliotis iris*

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ABSTRACT

Feeding during the nursery stage of abalone aquaculture, from larval settlement to 10 mm in shell length (SL), is a major challenge in the intensification of abalone aquaculture. Commercial abalone nurseries typically settle larvae onto vertical plastic plates coated with algae that act as a settlement cue and an important post-settlement food source. It becomes increasingly difficult to maintain an adequate algal food supply as the abalones grow and for this purpose many nurseries use the alga, *Ulvella lens*, as it is a very effective settlement cue as well as offering a resilient food source for the developing abalones. However, juvenile abalones can deplete a film of diatoms on the plates before they are capable of ingesting *U. lens*, and once abalone reach ~5 mm SL the high grazing pressure may exceed even what *U. lens* can support, requiring nurseries to provide additional cultured algae or prematurely wean the juveniles onto manufactured diets. Three prospective microfilamentous algal species isolated from the tanks of a commercial abalone facility, were selected as potentially suitable nursery algae as they demonstrated resistance to intensive juvenile grazing pressure, displayed rapid vegetative growth, and could be triggered to mass sporulate as a means of rapidly coating nursery tanks. The current study involved two experiments and assessed the suitability of each species as a live algal feed for juvenile abalones compared to *U. lens*. These experiments measured: (1) ingestibility and grazing resistance of each alga against juvenile abalone 1–8 mm SL, and (2) the ability of each species to recover once grazers had been removed. Two algae (Ulvophyceae sp. 1 and 3) demonstrated the desirable properties of being easily grazed by abalones of a smaller size (average SL 2.33 mm and 2.20 mm respectively) than *U. lens* (2.89 mm) yet provide an algal film that is likely to be resilient and durable under grazing pressure. These algae also displayed significantly greater rates of recovery following grazing than *U. lens* ($p<0.01$), an important attribute for restoring algal films in nursery systems between grazing events or between batches of juvenile abalones. The results of this study indicate that these novel algae have a combination of desirable characteristics for use in commercial abalone nurseries. Further experiments in commercial scale nursery systems should be pursued to assess the food value and performance of these species as a feed for juvenile abalones.

1. Introduction

Intensive nursery culture of abalone suffers from a lack of suitable artificial feeds and from difficulties in maintaining a film of algae on nursery tanks against the grazing pressure of juvenile abalones of 1.5–10 mm shell length (Daume and Ryan, 2004; Krsinich et al., 2000; Strain et al., 2006). Abalone nurseries seek alternative algal species that are sufficiently grazing tolerant and nutritionally adequate to support intensive grazing by juveniles whilst also providing high rates of growth and survival of abalones (Parker et al., 2007). The ability of juvenile abalones to ingest algae depends on the size of the abalone in relation to the algal cell size, the strength of the surface attachment of the algae, and the strength of the algal cell wall or the frustule in the case of diatoms (Kawamura et al., 1998). The transition some marine organisms undergo from newly-settled post-larvae through to adult form is typically accompanied with marked changes in behaviour, morphology and digestive capacity (Van Alstyne et al., 1999). For abalones, there is pronounced ontogenetic development in the morphology of feeding apparatus, as well as the types and activities of digestive enzymes (Johnston et al., 2005). As the abalones grow, their ability to ingest and digest food changes with their increasing mouth size (Fleming et al., 1996), the efficiency of the radula (Daume and Ryan, 2004; Johnston et al., 2005), and the morphology of the digestive tract (Johnston et al., 2005). These developmental changes create difficulties for intensive abalone nurseries to maintain a supply of readily ingestible and digestible food in the face of rapidly increasing grazing pressure from growing juveniles with increasing feeding capability (Kawamura et al., 1998). For abalone nurseries this

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challenge is best met by using a succession of increasingly larger and more grazing resistant live food sources to match the developing feeding capability of the juvenile abalones (Parker et al., 2007). Easily removed food, such as loosely attached benthic diatoms, is suitable for early post-settlement juveniles. However, the ease of the removal of diatoms means they can be quickly depleted and are therefore unable to re-establish whilst under intensive grazing pressure (Roberts et al., 1999). Maintaining the diatom film can be challenging and time consuming. Settlement density is often variable amongst nursery plates typically used in abalone aquaculture, so excessive grazing pressure from juvenile abalones can quickly clear the diatom film from the plates. Populations of benthic copepods living on abalone nursery plates typically rise exponentially in the 1–2 months after settlement, and the copepods compete directly with abalones for benthic diatoms. Copepod numbers can be reduced temporarily by physical removal (e.g., washing of plates), but this is a labour intensive process. Crustaceanicides, such as cypermethrin (Excis), would likely be effective for copepod control but are generally not registered for use in abalone aquaculture or are avoided because of the desire to use chemical-free methods. Grazing-resistant green macroalgae, such as *Ulvella lens*, offer a more resilient food source than diatoms and consequently commercial abalone nurseries in many countries now use *U. lens* because of its benefits as a larval settlement cue and juvenile food. *U. lens* is often overlaid with a film of benthic diatoms to provide a succession of nursery feeds for the developing early juvenile abalones (Daume and Ryan, 2004; Daume et al., 2000, 2004).

Although suitable as a feed for larger juveniles, *U. lens* produces poor growth rates and survival for abalones <3 mm SL, and is thought to be inaccessible to feeding abalones of this size class possibly due to its high strength of attachment to the surface of culture tanks(Daume et al., 2000; Huggett et al., 2005). It can be challenging to maintain adequate diatom film until abalones reach this size. Even in systems employing *U. lens* and diatoms, the rapid depletion of diatom food by abalones and copepods can lead to mortality of juvenile abalones, particularly in the 1.5 to 2.5 mm SL size range before the abalones are capable of readily ingesting *U. lens*. Algae that were more grazing resistant than diatoms, but were able to be ingested by abalones at a smaller size than *U. lens* would help ensure a continuous supply of food for early juvenile abalones.

Once abalones are approximately 5 mm SL the intense grazing pressure from the abalone exceeds what *U. lens* is capable of supporting (Daume and Ryan, 2004), requiring nurseries to add formulated feed. The current lack of an effective alternative live feed for abalones of >5 mm SL make the use of formulated feed common, despite being a less suitable food source for abalones of this size. Although examples of successful early weaning of juvenile abalones exist (e.g., Dyck et al. (2010)) the use of the formulated feed at this stage of abalone culture typically results in slower growth and higher mortalities than live algal feeds. For example, the daily growth rate of juvenile abalones on formulated feed can be 15% less and mortality rates 200% higher than for juveniles maintained with sufficient *U. lens* (Daume, 2003; Daume and Ryan, 2004). There is a need to develop a more effective live algal feed for this later stage of the nursery culture that would allow farmers to retain their abalones on the nursery plates longer and thereby reduce the high weaning mortality that is currently associated with the early introduction of artificial feeds (Daume, 2006).

Despite indications that the early growth history of abalones may determine their later performance, very little emphasis has been placed on improving growth rates of early juvenile abalones (Daume and Ryan, 2004). Therefore, the aim of this study is to examine the potential for finding alternative algal species that will help to ensure a continuous supply of food for cultured abalones in the 1 to 10 mm SL range, after which weaning onto formulated feed becomes more successful (Daume, 2003). This study examines the potential of three microfilamentous algal species as alternatives to *U. lens* as a nursery feed. The three algal species were isolated, cultured, and molecular genetic techniques were used to confirm that they were separate taxa within the class Ulvophyceae. These three species were selected as potentially suitable for use in abalone nurseries because they had rapid vegetative growth, and could be triggered to mass sporulate resulting in rapid coating of abalone nursery tanks and they demonstrated resistance to intensive grazing pressure of juveniles of New Zealand's commercial abalone species, *Haliotis iris*. Therefore, the aim of this study was to assess the suitability of these species as a live algal feed for juvenile abalones compared with *U. lens*, the alga currently considered the best feed for juvenile abalones of 3–10 mm SL (Daume, 2006).

1.1. Materials and methods

1.1.1. Isolation and cultivation of algae

Three epilithic microfilamentous algal species that appeared partially resistant to intensive grazing by juvenile abalones (*H. iris*) were isolated from commercial abalone rearing tanks at OceaNZ Blue Limited (OBL), Ruakaka, New Zealand. Pure cultures of the three microfilamentous algal species were isolated by taking scrapings of algae from nursery abalone tanks, separating out individual thalli and repeatedly sub-culturing until monoalgal cultures were established. Clones were made repeatedly by pipetting individual germlings or vegetative fragments into separate Petri dishes or 24 well plates containing sterile growth media. Molecular genetic techniques were used to identify the cultures as three separate species within the Ulvophyceae and are therefore referred to in this study as Ulvophyceae sp. 1–3 (Fig. 1) (Dyck, 2009). Further taxonomic identification to a species level proved problematic with either morphological or molecular techniques (Dyck, 2009). Cultures of the three species were held until required for experimentation, along with a monoalgal culture of *U. lens* isolated from Cawthron's Glenhaven Aquaculture Centre in, Nelson, New Zealand. The algae were held for up to several months in 24 well culture plates or 50 ml plastic tissue culture flasks containing Conwy growth medium (Walne, 1974). These stock cultures were kept in a controlled environment room at 18 ° C under fluorescent illumination (220 μmol s^{-1} m^{-2}, 2× Philips cool white bulb model TLD 18 W/840, 12:12-h light: dark photoperiod). Once sufficient quantities of each alga were achieved for experimental use, stocks were kept in 50 ml plastic tissue culture flasks with opaque lids and with reduced lighting to slow growth and nutrient uptake.

1.2. Experiment 1: grazing resistance

1.2.1. Establishing algal covering on the plates

Eight 24 well tissue culture plates (BD Falcon, well volume 3.5 ml, floor area 2.0 cm^2) were used in this experiment, with each plate having six wells containing each of the four algal species. In these wells, an algal film of the desired species was simultaneously established in every well by triggering zoospore release from a small amount of starting algal cultures. Zoospore release was triggered in each of the four algal species by keeping starting cultures in 50 ml culture flasks in total darkness for a period of two weeks. From these dark-stored starter cultures a small amount of algal material (about 1 mm^2) was pipetted into individual wells of the eight 24 well culture plates. The four species were randomly assigned to a well in each of the six columns of wells on the culture plate. Conwy growth medium was added to each well and the plates transferred to 24:0 (L:D) photoperiod and constant temperature (18 ° C). Depending on the species, zoospore release occurred 3–10 d after exposure to light. Three weeks later when zoospores had germinated and developed into plants of around 80–120 μm in diameter, the grazing experiment was ready to commence and the 1 mm^2 starting culture removed.

1.2.2. Preparing the plates for grazing

Plates were emptied of liquid and any algal tissue growing on the vertical walls of each well was removed using a pipette and a soft bladed spatula to encourage juvenile abalones to graze only the floor of the well during the experiment. Following cleaning, natural seawater (10 μm