Journal of Applied Phycology (2022) 34:2135–2144
https://doi.org/10.1007/s10811-022-02763-6

Asexual propagation of *Asparagopsis armata* gametophytes: fragmentation, regrowth and attachment mechanisms for sea-based cultivation

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Received: 25 November 2021 / Revised and accepted: 29 April 2022 / Published online: 28 May 2022

Abstract

The red algal genus *Asparagopsis* produces secondary metabolites that when fed to ruminants reduce methane production by up to 98%. However, cultivation methods for *Asparagopsis* are nascent and fundamental information on reproduction, which is essential for large-scale cultivation, is lacking. In this study we examined asexual propagation in *Asparagopsis armata*, the regrowth of fragments and mechanisms of attachment to assess the potential for fragments to be used in sea-based cultivation. *Asparagopsis armata* gametophytes grow specialised structures, barbs, that hook fragments onto substrata. Surveys revealed barbs were abundant occurring at ~ 1 barb every 3–4 cm on gametophyte branches. Barbs did not regrow, but fronds did, either when attached to a barb or on their own. In contrast, fronds doubled in size with most developing barbs within 4 weeks. Barbs were, however, critical for the reattachment of fragments: barbs attached to substrata at four times the rate of frond fragments without barbs and they also attached in higher proportions to mussel rope than polypropylene rope, and two types of net. Utilising fragmentation for the propagation of *A. armata* gametophytes in sea-based cultivation requires that fragments can attach to a substratum and regrow once attached. We have shown that *A. armata* fragments in Tasmania require barbs for attachment and frond tissue for growth, which has implications for cultivation. Optimising fragmentation, attachment and out-planting methods are important future steps in establishing fragmentation as a method for sea-based cultivation in *A. armata.*

Keywords *Asparagopsis* · Rhodophyta · Asexual reproduction · Fragments · Red algae · Seaweed aquaculture

Introduction

Many seaweeds reproduce both sexually and asexually (Santelices 1990; Collado-Vides 2001) and asexually derived propagules can be the main source of recruitment into some seaweed populations (Ceccherelli and Cinelli 1999; Walters et al. 2002; Herren et al. 2006). Asexually derived propagules often have high post-recruitment survivorship (Walters et al. 2002; Herren et al. 2006), allowing dense aggregations to form due to a feedback between growth of existing thalli and recruitment of asexual fragments (Wright and Davis 2006). Moreover, propagation of vegetative fragments is also important in seaweed aquaculture with several of the world's largest seaweed industries dependent on asexual reproduction (FAO 2020).

There are different mechanisms of asexual reproduction in seaweed. In many green (Walters et al. 2002; Wright and Davis 2006; Khou et al. 2007) and red (Smith et al. 2004; Conklin and Smith 2005; Geoffroy et al. 2012; Herren et al. 2013) algae any part of the thallus can fragment and regrow although certain combinations of traits make fragments more successful (Bulleri et al. 2020). In addition, many red algae also produce specialised multicellular propagules that grow vegetatively from the parental thallus, detach, and go on to form new individuals (reviewed in Cecere et al. 2011). Although these specialised propagules are common, most of our understanding of the regrowth of

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asexual seaweed propagules comes from non-specialised fragments.

Three red algal species (*Eucheuma, Kappaphycus* and *Gracilaria/Gracilariopsis*) which make up ~ 45% of the global seaweed production by biomass (FAO 2020), are grown via propagation of vegetative fragments (Oliveira et al. 2000; Hurtado et al. 2015; Hayashi et al. 2017; Kim et al. 2017). Generally, for these species, small vegetative fragments are created and attached to ropes, frames or baskets for grow-out in open water, ponds or tanks (Azanza and Ask 2017; Zubia et al. 2020). Fragmentation offers advantages for cultivation over sexual reproduction as fragments can be easily created in the hatchery, they often grow quickly, have high survivorship and desirable traits can be selected and maintained (Kim et al. 2017).

The red algal genus *Asparagopsis* (family Bonnemaisoniaceae) has recently been identified for mass cultivation due to it being a rich source of biologically active halogenated secondary metabolites which, when fed in small amounts to ruminants, reduces methane production caused by enteric fermentation (Black et al. 2021; Glasson et al. 2022). Given methane production by ruminants is a significant contributor to greenhouse gas emissions globally (~ 14% of global GHG emissions, Gerber et al. 2013), supplementing the diet of livestock with a small amount of *Asparagopsis* (< 1%) can reduce global methane production (Black et al. 2021; Glasson et al. 2022). However, cultivation methods to supply *Asparagopsis* require development, and fundamental information on its reproduction, critical for large-scale cultivation, is limited.

The two recognised *Asparagopsis* species, *A. armata and A. taxiformis*, are native to the southern hemisphere and have been introduced into the northern hemisphere (Bonin and Hawkes 1987; Andreakis et al. 2007; Zanolla et al. 2022). *Asparagopsis armata* gametophytes produce specialised multicellular vegetative structures (barbs with spines) that grow from branches and enable attachment (Bonin and Hawkes 1987; Zanolla et al. 2022; Fig. 1). In the northern hemisphere, barbs from *A. armata* regrow fronds in natural seawater and under certain artificial culture conditions (Codomier et al. 1979, 1981; Haslin and Pellegrini 2001). Consequently, regrowth from barbs has been utilised for ocean-based farming in France where gametophytes are fragmented to 1- 2 cm pieces, attached to a monofilament net which is then deployed in the ocean (Seguin et al. 1995; Moigne 1998; Kraan and Barrington 2005). Thus, although the regrowth of barbs appears critical for ocean-based farming of *A. armata* gametophytes in France, a greater understanding of the basic biology of these barbs and their attachment potential in other regions is required to allow the production of *Asparagopsis* at scale.

This study has two broad aims. First, to investigate natural history aspects of native *A. armata* gametophyte barbs which included determining: 1) the number of barbs per gram and per length of gametophyte, 2) traits of barbs including their size and relationships between barbs and spines and, 3) regrowth of different types of vegetative fragments

Fig. 1 Photograph of *Asparagopsis armata* gametophyte showing barb (arrow)

(barbs, fronds, fronds with barbs). Second, to determine the potential for barbs to be used in sea-based cultivation by assessing: 4) the attachment of barb vs. frond fragments on substrata for out-planting and, 5) the attachment of barb with frond fragments and their attachment strength over time.

Materials and methods

Asaparagopsis armata life-cycle and collections

Asparagopsis armata Harvey (Bonnemaisoniales, Rhodophyta) is native to temperate Australia, New Zealand and sub-Antarctic islands (Bonin and Hawkes 1987; Womersley 1996). Isomorphic gametophytes grow to ~ 200 mm in height while tetrasporophytes occur as spherical clumps ~ 10 mm in diameter (Bonin and Hawkes 1987; Womersley 1996; Zanolla et al. 2022). Both can occur epiphytically on other seaweed or attached to the bottom and gametophytes are typically observed in spring and early summer with reproductive structures visible throughout that time (Bonin and Hawkes 1987).

We collected gametophytes of *A. armata* from several sites on the east coast of Tasmania in southern Australia. Gametophytes were transported to the laboratory at the Institute for Marine and Antarctic Studies (IMAS) in coolers with seawater where all experiments were done. Once gametophytes were at IMAS they were held at 11 °C in filtered (0.2 µm), UV sterilised F/2 seawater media with aeration under ~ 40–60 µmol photons m^{-2} s^{-1} on a 12:12 light:dark cycle.

Abundance of barbs on gametophytes

We quantified the abundance of barbs per thalli in gametophytes collected from Lomas Point, Dover (43°21′00.7"S, 147°03′19.7"E) at two times: October and November 2020. Each time, 12 gametophyte samples were collected. *A. armata* gametophytes at Lomas Point grow in small clumps, typically attached epiphytically to other algae (mostly *Sargassum* spp.). Anecdotally, the habitat and morphology of gametophytes at Lomas Point was the same as gametophytes collected at other sites that were used to examine the regrowth and attachment of fragments. These clumps are often attached to their host in dense aggregations and likely include multiple individuals. Approximately 30 g (wet weight) of gametophyte tissue were collected by hand from a clump and placed into a ziplock bag, making sure that each clump was at least 1 m apart. It was not possible to identify and sample individual thalli in the field. Gametophytes were transported back to the lab at IMAS and the abundance of barbs per cm of gametophyte tissue was determined by arranging all the tissue from each sample on a 1 cm grid on a flat surface and taking a photograph. The number of barbs and length of each piece in the sample were measured. After barbs were counted, gametophyte tissue was spun in a salad spinner and weighed to calculate the abundance of barbs g^{-1}.

Barb traits and barb – spine relationships

Barbs contain spines that hook onto substrata and the length of the barb, number of spines and size of spines might influence the success and strength of attachment. To measure barb traits, we isolated 134 barbs from approximately 50 thalli collected from Lomas Point by removing them at the base where they attach to the thallus. A photograph of each barb was taken and barb length, spine number and spine length (base to the tip of the spine) and width (at the base) determined. Barb and spine sizes were measured using Image J.

Regrowth of gametophyte fragments

Gametophytes were collected from Triabunna (42°32′19.5"S, 147°55′31.9"E) and transported to IMAS. Twenty of each of three fragment types (frond, barb and frond with barb, Supplementary Fig. 1) were removed from the gametophytes using tweezers, photographed and placed individually into 250 mL jars filled with 50 mL of pre-sterilised F/2 seawater media with aeration. The initial lengths (mean ± SE) of these fragments were 23.2 ± 0.5 mm (barbs), 26.0 ± 0.6 mm (fronds) and 47.9 ± 1.6 mm (frond with barb combined) while initial surface areas were 96.3 ± 6.3 mm^2 (fronds) and 133.9 ± 9.6 mm^2 (frond with barb combined). Photographs were then taken after 2, 3 and 4 weeks, but only the initial and week 4 photos were used to determine absolute growth rate based on an increase in length and surface area of fragments, analysed using ImageJ. The number of new barbs produced after 4 weeks was also compared among fragment types. Contamination of fragments by epiphytes at week 4 was determined by estimating the percentage cover of fouling (largely filamentous macroalgae e. g. *Hincksia sandriana* but also *Ulva* spp.). Fragments were then determined as being in one of five contamination classes: 1 = 1–25% of surface area contaminated, 2 = 26–50% contaminated, 3 = 51–75% contaminated, 4 = 76–99% contaminated, 5 = 100% contaminated.

Attachment of barb and frond fragments on four substrata

Fragments of gametophytes used to determine attachment success were also collected from Triabunna on 11 August 2020. Once in the laboratory, gametophytes were fragmented into two types, barbs or fronds (with no barbs) which were

held for two days to ensure they were viable before being placed into experimental jars.

We tested attachment success of fragments on four types of substrata which may be suitable for commercial scale sea-based gametophyte cultivation: polypropylene 'mussel spat collection and grow out' rope (20 mm diameter: no. M-102-B, Donaghys Ltd), polypropylene rope (20 mm diameter: no. ROS2002, Donaghys Ltd), nylon mono-filament net (2 mm strand, 40 mm mesh) and nylon multi-filament net (1 mm strand, 25 mm mesh; Supplementary Fig. S2). For this experiment, one of each substratum type (adjusted to be of similar dimensions: 90 mm length x approximately 20 mm diameter) was placed in a 2 L jar filled with 1.5 L of filtered seawater. The substrata were suspended in the water from above using string. Twenty fragments of one fragment type were dropped into each jar (n = 10 jars per substrate type) with consistent aeration (strong enough to make fragments circulate in the jar). After 24 h the number of fragments attached to the substratum was counted. For barbs, photos were taken of attached and non-attached fragments to determine if size influenced attachment. Lengths of barbs used in the experiment ranged from 5.7 – 28.0 mm (16.1 ± 0.1 mm, mean ± SE) and were randomly allocated to treatments.

Attachment success and strength of frond with barb fragments to mussel rope

Gametophytes were collected from Brother and Sister Islands (43°06′39.6"S, 147°43′40.8"E) in September 2020 and 300 frond fragments with a single barb were prepared. If a fragment had more than one barb, the largest barb was selected, and the others removed. A photograph of each fragment was taken before 10 fragments were placed in 2 L jars each filled with 1.5 L filtered seawater and with a 90 mm long piece of mussel rope (N = 30 jars) with sufficient aeration to ensure circulation of fragments in the jars. After 24 h the number of fragments attached to rope was determined for each jar. Ten jars were then randomly selected and the attachment strength of five attached fragments per jar was determined using a force gauge. This was done by removing each rope from the jar, attaching a clip onto each fragment, and pulling with the force gauge until the fragment detached from the rope. Attachment strength was measured in the same way after one and two weeks (N = 10 jars at each time).

Analyses

Differences in number of barbs per gram and cm for each clump of gametophytes were compared between October and November using t-tests. Relationships between barb length, and spine number, spine length and spine width were examined using linear regressions. Differences in the absolute growth (both length and surface area) over 4 weeks between the frond, and frond with barb, fragments were compared with an Analyses of Covariance (ANCOVA) with initial length or area as the covariate. Similarly, the growth of new barbs over 4 weeks was compared between the frond, and frond with barb, fragments with ANCOVA using surface area as the covariate. Because barb fragments did not grow, they were not included in these analyses. Differences in the percentage attachment success was compared using a 2-factor ANOVA (Rope treatment x Fragment type, log + 5 transformed) while differences in the length of barb fragments was compared between attached and unattached fragments and among rope treatments with a 2-factor ANOVA. The attachment strength data was not analysed as many replicates recorded zero using the force gauge. ANOVA assumptions were tested using diagnostic plots, model residuals, and data were transformed as required based on the maximum log-likelihood λ value from Box-Cox plots. Where significant effects occurred, differences between means were tested with Tukey's post-hoc tests. All analyses were conducted using R studio (ver. 1.0.136) and R (ver. 1.68).

Results

Abundance of barbs on gametophytes

Barbs typically grew near the base of branches and the average number of barbs per gram of gametophyte ranged from 1.5 – 7.4 and did not differ between months (t_{20} = -1.336, P = 0.198). The average number of barbs per cm was higher in October compared to November (t_{20} = 3.642, P = 0.002; Fig. 2).

Barb traits and barb – spine relationships

The length of barbs ranged from 5 – 27 mm, however, it was not a strong predictor of the number of spines on a barb (R^2 = 0.025, P = 0.065, N = 134, Fig. 3). For example, a barb that was 17 mm long could have between 6 – 34 spines. Alternatively, both spine length (R^2 = 0.213, P < 0.001, N = 350) and width (R^2 = 0.267, P < 0.001, N = 350) were positively correlated with barb length indicating that spines increased in size as barbs became longer (Fig. 3).

Regrowth of gametophyte fragments

Barbs did not regenerate either when on their own or when attached to fronds (Fig. 4A). In contrast, nearly all frond fragments grew (both fronds on their own and fronds with barbs), increasing in length and doubling in area in four weeks (Fig. 4). There was no difference in growth based

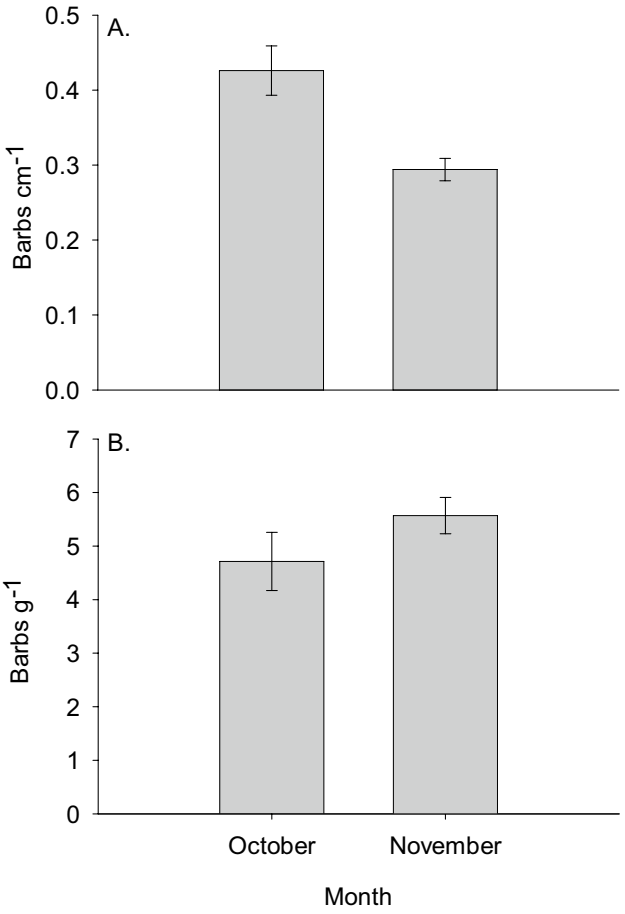

Fig. 2 Mean (±SE) barb abundance per cm (A) and per gram of gametophyte over four months (B), $n = 12$

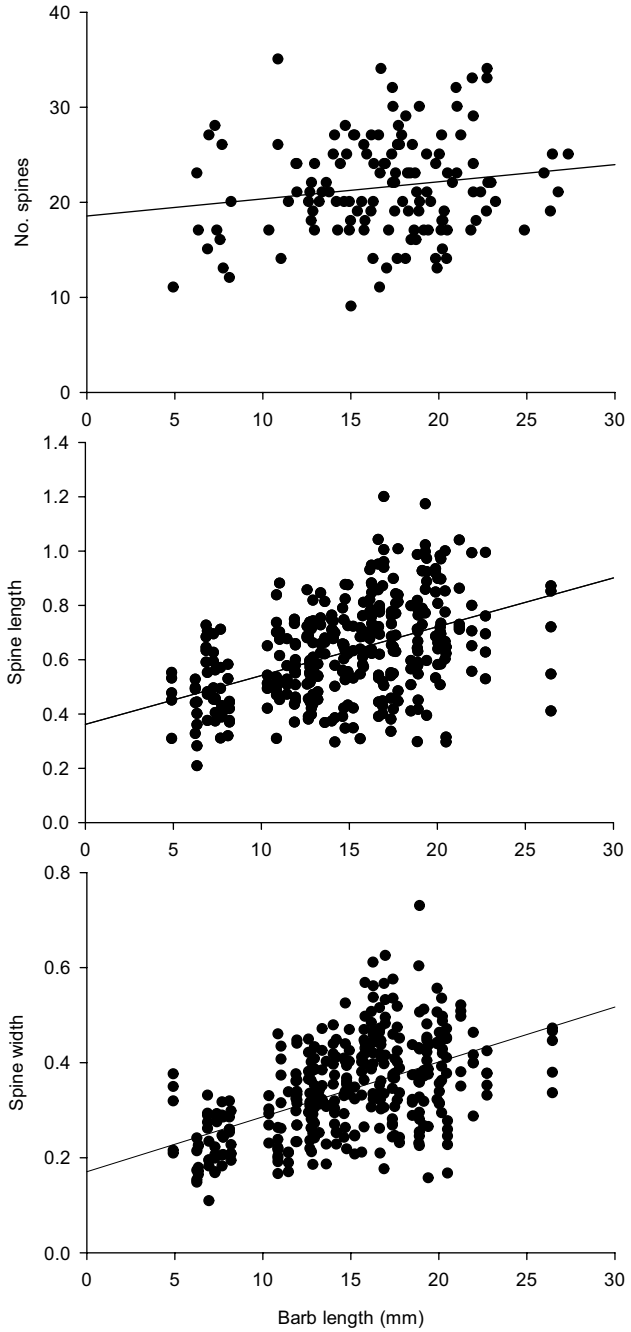

Fig. 3 A) The relationship between barb length and total number of spines, B) the relationship between barb length and spine length, and C) the relationship between barb length and spine width. Significant relationships were observed in B) and C)

on length between fragment type (Fig. 4A, $F_{1, 37} = 0.194$, $P = 0.662$) but initial length of fragments was a significant predictor of growth with smaller fragments increasing more in length ($F_{1, 37} = 18.469$, $P < 0.001$). In contrast, for surface area, growth differed between fragment type ($F_{1, 35} = 10.679$, $P = 0.002$) with fronds without barbs growing ~ 30% more than fronds with barbs (Fig. 4B). For frond fragments, growth was positively correlated with initial surface area, but no such relationship occurred for fronds with barb fragments (i. e. significant fragment type x initial surface area interaction: $F_{1,35} = 5.603$, $P = 0.002$). Barb fragments decreased in length on average by ~ 5% but their surface could not be accurately determined after four weeks due to high contamination. Visually, there was no increase in surface area of barb fragments. Moreover, both frond fragments and fronds with barbs fragments grew 1–2 new barbs over four weeks (Fig. 4C) and this did not differ between fragment type ($F_{1,36} = 2.454$, $P = 0.126$) or depend on initial surface area ($F_{1,35} = 1.659$, $P = 0.206$). These two fragment types also experienced much lower contamination compared to barb fragments which were all heavily (~ 100%) contaminated (Fig. 4D). The barbs attached to fronds had no or very little fouling, estimated at less than 5%.

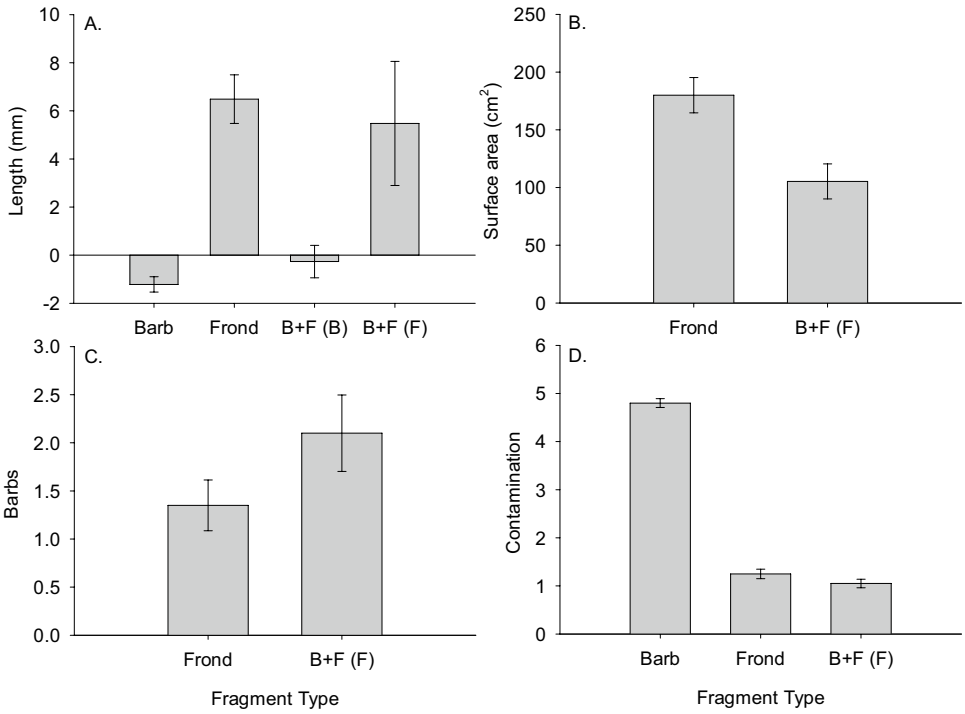

Fig. 4 Mean (±SE) regrowth and contamination of *Asparagopsis armata* fragments after 4 weeks ($n = 20$): A) the increase in length for barb, frond and frond with barb fragments (barb, B + F (B) and frond, B + F (F) presented separately); B) the increase in surface area of frond and frond with barb (B + F (F)) fragments; C) the number of newly formed barbs on frond and frond with barb fragments and; D) contamination of barb, frond, and frond with barb fragments based on a scale of 1–5 where 0 = no contamination, 1 = 1–25% of tissue contaminated, 2 = 26–50% contaminated, 3 = 51–75% contaminated, 4 = 76–99% contaminated, 5 = 100% contaminated

Attachment of barb and frond fragments on four substrata

Barbs attached at significantly higher levels than fronds (49% vs ~ 13% averaged across substrata, $F_{1, 72} = 78.927$, $P < 0.001$, Fig. 5A). Moreover, although fronds without barbs were scored as attached, they were mostly entangled within the net or rope or unattached on their surface and many (37% of fronds scored as attached, averaged across treatments) were easily detached by minor water motion. There was also a significant difference in attachment rates among substrata ($F_{3, 72} = 8.139$, $P < 0.001$) and barbs attached to mussel ropes at significantly higher levels than the polypropylene rope and multi-filament net (75% vs. 37%, Tukey's test, $P < 0.05$) but not the mono-filament net (50%, Fig. 5A). Barbs that attached to the substrata were slightly shorter than those that did not attach (15.68 ± 0.25 mm, $N = 206$ vs. 16.30 ± 0.15 mm, $N = 322$, mean ± SE, $F_{1,781} = 4.725$, $P = 0.030$) and of the barbs that attached, they were smaller when attached to polypropylene rope (15.61 ± 0.24 mm, $N = 30$) compared to mono-filament net (16.37 ± 0.27 mm, $N = 34$, mean ± SE, $F_{3, 781} = 2.733$, $P = 0.043$, Tukey's test, $P < 0.05$) but no other substrata differed ($P > 0.05$, Tukey's test, Fig. 5B).

Attachment success and strength of frond with barb fragments to mussel rope

Attachment success of frond with barb fragments to mussel rope after 24 h was similar than that recorded for barb fragments (76%). Overall, the attachment strength was low and did not increase over time (Fig. 6). Most fragments required less than 10 g of force to remove them from the rope with only 1–2 fragments requiring more than 10 g force.

Discussion

We have shown that specialised structures, barbs, occur every ~ 3–4 cm along branches on *A. armata* gametophytes although they are most abundant near the base of branches (Bonin and Hawkes 1987). These barbs provide a mechanism of attachment for *A. armata* gametophyte fragments and attach more frequently to specific substrates, in this case mussel rope (75% attachment rate), compared to other ropes or nets (37 – 50% attachment rate) for in-sea cultivation. Attachment strength after two weeks on the preferred substratum did not increase over time, highlighting the significance of substrate materials for cultivation. There is a clear differentiation in the response of different tissue types to fragmentation. Barbs did not regrow and rapidly became epiphytized by algae, while fronds with barbs grew rapidly, remaining free of epiphytes and doubling in surface area in four weeks. Importantly, fronds fragments that were initially without barbs grew barbs over the four-week period of cultivation, improving their potential for later attachment to substrates for in-sea cultivation. Therefore, asexually derived fragments with both barbs and fronds will best support attachment and regrowth in *A. armata* and natural recruitment into populations, where they attach epiphytically to other macroalgae and other biogenic

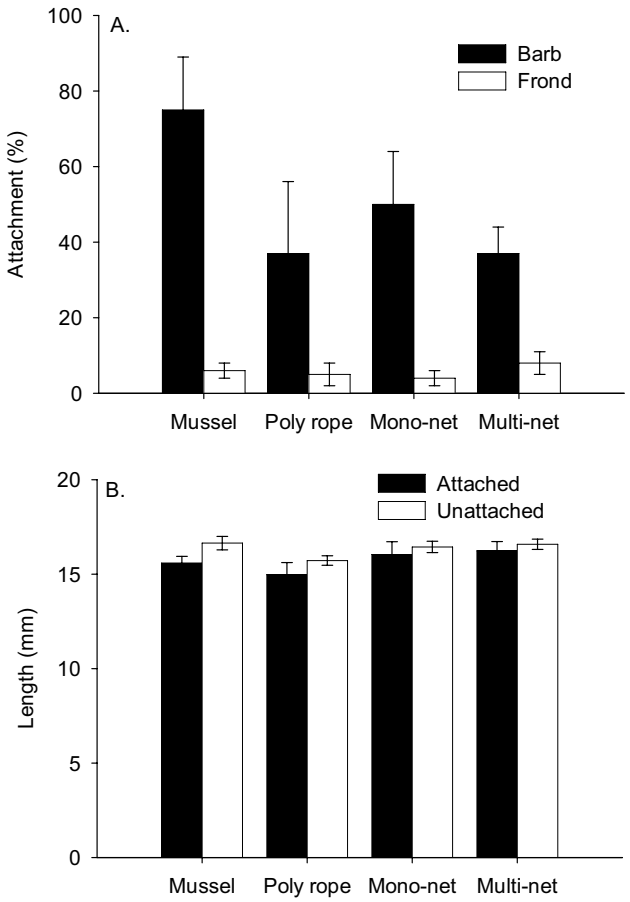

Fig. 5 Attachment of *Asparagopsis armata* fragments on four substrata. Mean (±SE) (A) attachment of barb and frond fragments ($n=10$) and; (B) length of attached (n ranged from 30 – 105) and non-attached (n ranged from 94 – 167) barb fragments on the four substrata

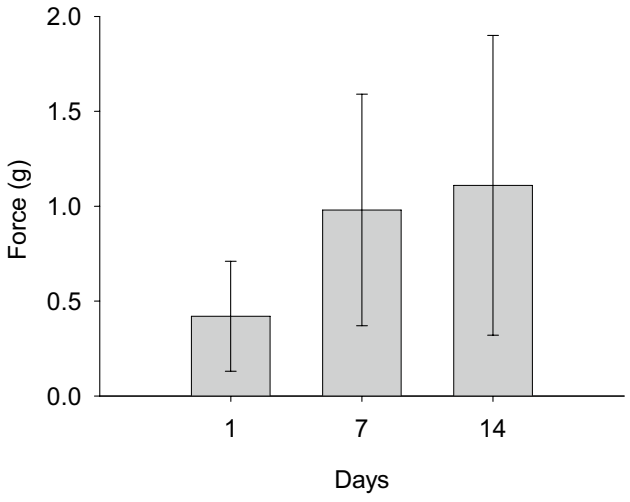

Fig. 6 Mean (±SE) force required to detach *Asparagopsis armata* frond with barb fragments attached to mussel rope tested at 1, 7 and 14 days ($n=10$)

habitat, but also a strategy for sea-based cultivation, where they can be attached to ropes or other substrata and regrow.

The high rates of attachment of barbs and the regrowth in fronds (increases in length and area as well as growth of new barbs) highlights the importance of both traits being present in asexual fragments in *A. armata* for successful regeneration. The lack of regrowth in barbs of *A. armata* has been described in New Zealand (Bonin and Hawkes 1987) but differs to findings for *A. armata* in France where regrowth of barbs occurred (Codomier et al. 1979, 1981; Haslin and Pellegrini 2001). In France barbs regenerated best in natural seawater containing iron sulfate, EDTA and potassium bromide (Haslin and Pellegrini 2001). The lack of regeneration in barbs here compared to France could be due to differences in culture conditions, seasonal effects on regeneration of barbs (our regrowth experiments were done in late winter while those in France were done in autumn/summer Codomier et al. 1979, 1981; Haslin and Pellegrini 2001) or genetically different strains being examined. Current genetic evidence suggests invasive *A. armata* in Europe are similar to lineages collected from Sydney and Melbourne (Chualáin et al. 2004) but these differ from *A. armata* in New Zealand and Tasmania (Preuss et al. 2022), Kennedy et al. Unpublished data). Nonetheless, the lack of regrowth in barbs in our experiments indicate that under natural seawater conditions barbs do not typically regrow. The lack of regrowth from barbs in our experiments also suggests barbs do not strictly fit the definition of specialised vegetatively propagules that when detached go on to regrow and form new individuals (as defined in Cecere et al. 2011). Fragments containing both a barb for attachment and a frond for growth are required for successful asexual propagation in *A. armata* in Tasmania.

A number of other red algae produce structures that allow attachment of asexual propagules (Cecere et al. 2011) including *A. taxiformis* gametophytes from India which have been reported to grow morphologically distinct hooks from apical portions of branches which detach and regrow in culture (Mairh 1977). This has also been observed for *A. taxiformis* in north Queensland (A. Cole personal observations) and highlights a different strategy in the production of asexual propagules between *Asparagopsis* species. In addition, *Bonnemaisonia hamifera* which is also in the family Bonnemaisoniaceae, produces crozier shaped hooks from branches which facilitate asexual propagation by hooking thalli onto neighbouring seaweed or other structures either before or after fragmentation occurs (Breeman et al. 1988). Other red algae produce discs or rhizoidal filaments from asexual fragments which eventually attach fragments to the substratum similar to holdfasts (Perrone and Cecere 1997; Bulboa et al. 2013). In the natural environment, we observed *A. armata* gametophyte barbs that were often firmly attached via a biological matrix to other seaweed at or near the

spines. Attachment of *A. armata* barbs has previously been observed to occur via extension of cortical cells in spines (Bonin and Hawkes 1987) and suggests that once a barb attaches a fragment to a substratum, firm long-term attachment can occur, although we could not replicate this over four weeks in the lab. Although in our experiments some *A. armata* fronds without barbs were recorded as attached to rope or nets, these had mostly settled on top of the rope or mesh and many were easily removed by gentle agitation and thus, are unlikely to remain attached in the field for long under even limited water motion. Moreover, given *A. armata* frond fragments grow barbs, detached frond fragments could remain alive while floating and eventually recruit into natural population or attach to rope in a cultivation via new barbs.

Further evidence of the importance of *A. armata* fragments containing both fronds and barbs is highlighted by the high contamination of barbs in isolation compared to when they had a frond attached to them. The reasons for the lower contamination in barbs when attached to fronds are unclear. *A. armata* gametophytes contain bromoform concentrations up to 1.5% dry weight (Vergés et al. 2008), and a diversity of other haloforms and haloacids which inhibit bacterial colonisation (Paul et al. 2006). Barbs contain gland cells (unpublished data) where the secondary compounds are localised but concentrations of secondary metabolites in barbs are not known. Given barbs did not grow during our experiment, it is likely they stopped producing secondary metabolites and become more susceptible to colonisation by bacteria and epiphytes.

Because *A. armata* fragments with barbs can attach naturally to ropes, there is the potential to utilise that strategy in aquaculture. The fragments of many currently cultivated red algae (*Chondracanthus, Euchema, Kappaphycus, Gracilaria* spp.) do not have natural morphological structures that allow attachment to substrata and hence require labour intensive manual attachment methods for sea-based cultivation. The oldest and simplest methods involve manually attaching small fragments to lines using a tie or inserting them between strands of twine or mesh (Bulboa et al. 2005; Hayashi et al. 2010, 2017) which are then hung in the water column where the fragments regrow and are harvested later. Other methods include where small fragments are placed inside baskets or nets for later harvest (Azanza and Ask 2017). Because some red algal fragments develop secondary attachment structures such as rhizoids or discs post-fragmentation (Pacheco-Ruíz et al. 2005; Bulboa et al. 2013) this life-history strategy may also be used in cultivation. For example, *Chondracanthus* fragments produce secondary discs that attach to mesh in a hatchery which can then be outplanted (Bulboa et al. 2013; Macchiavello et al. 2018). It is likely that an effective natural attachment mechanism in *A. armata* will be advantageous in a hatchery situation as attachment of fragments would be less labour intensive.

The finding that barbs on their own did not regrow has implications for *A. armata* aquaculture. Previous sea-based cultivation of gametophytes in France focused on isolation of barbs only for seeding onto ropes (Seguin et al. 1995; Moigne 1998) but in Tasmania, fragments for seeding need to contain both barb and frond tissue. Given barbs occur every 3–4 cm along branches, fragments for seeding need to be at least ~ 5 cm long to maximise the likelihood of fragments having both barbs and fronds.

Although *A. armata* fragments that contain both a barb and a frond could provide an effective strategy for sea-based cultivation, the type of substratum they are attached to is also important. Attachment rates of fragments to mussel rope was approximately double that of the other potential substrata tested. The 'hairy' structure of the rope presumably provides a structure that barbs readily hook onto. However, maximising the attachment strength of *A. armata* fragments once attached to lines is also likely to be critical. We found attachment strength of single barbs was relatively weak and did not increase over two weeks in the lab. This contrasts to fragments of *Caulerpa filiformis* for which attachment strength to plastic mesh increased over time (Khou et al. 2007). That study also indicated that attachment strength increased with the number of rhizoids per fragment. Given barbs occur every 3–4 cm on thalli, multiple barbs per fragment are likely to provide stronger attachment than a single barb tested here while the longer-term attachment through rhizoidal growth as observed on ropes in cultivation remains to be elucidated.

Conclusion

Fragments containing both a barb for attachment and fronds for growth are required for successful asexual propagation in *A. armata* gametophytes. The abundance of barbs on thalli and the high rates of attachment to potential cultivation lines highlights this strategy for cultivation is highly achievable and could be automated to be successful at an industrial scale. Nonetheless, cultivating gametophytes from tetraspores released in a hatchery could also be industrialised, as it has for other red algae (Oliveira et al. 2000; Wang et al. 2020). The demand for *Asparagopsis* is significant (Kelly 2020) and large-scale cultivation of *A. armata* is required to meet this demand. The farming of *Asparagopsis* is in its infancy however, the asexual propagation of gametophytes is a readily applied strategy to meet the goal of utilising *Asparagopsis* as a feed supplement to reduce methane emissions from livestock.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10811-022-02763-6.

Acknowledgements Funding for this project was provided by Sea Forest Ltd.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions

Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

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