Farming sponges for chemicals with pharmaceutical potential

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Biologically active metabolites present in sponges to deter predation, prevent surface overgrowth, or to fight microbial infection and disease, may be used in the near future to fight and cure human diseases. Clinical trials have shown that some sponge bioactive metabolites have anticancer, antiviral and anti-inflammatory properties. Examples include the anticancer agents discodermolide from the Caribbean sponge *Discodermia dissoluta* (Gunasekera et al. 1990) and isohomohalichondrin B isolated from the New Zealand sponge *Lissodendoryx* n. sp. (Munro et al. 1999). The market value of a marine bioactive metabolite is potentially huge if it is successful in all trials and is approved as a drug. For example, the anticancer metabolite bryostatin-1 from the bryozoan *Bugula neritina* is estimated to be worth US$1 billion per year (Pain 1998).

Up to one kilogram of a bioactive metabolite is required for drug development alone. Unfortunately, many sponges contain only trace amounts of bioactive metabolites and the need for large quantities for trials has at times resulted in the near extinction of a population or a species. The supply issue becomes a much more serious problem if the natural product itself is used in commercial drug manufacture. It is therefore essential to develop methods to guarantee the supply of metabolites for the pharmaceutical industry both for drug development and long-term commercial production. Of the supply methods currently being developed, aquaculture, where sponges are farmed in the sea or on land in seawater tanks, is considered to be the most cost-effective or perhaps only method to guarantee sufficient supplies of some sponge metabolites (Munro et al. 1999). Even if the target metabolite is produced by a symbiont such as a bacterium, aquaculture of the sponge plus symbiont may still be the best method of supplying that metabolite.

History

Although farming sponges for drug production is a recent concept, *in situ* aquaculture of bath sponges for their fibrous skeletons has a long but checkered history. Early attempts in the mid 1800s were largely unsuccessful mainly because no suitable structure was found for growing sponges. Later, in the early 20th century, research showed that sponges grow well when attached to concrete discs or when threaded with wire so that they hung in mid water (Crawshay 1939). Recently, this "hanging" method has been used with modern materials (plastics) with some success (e.g. Verdenal and Vacelet 1990).

Sponge aquaculture for production of chemicals with pharmaceutical potential has a significantly shorter history, with the first trials occurring in the last decade. Unlike bath sponge aquaculture, sponge shape does not determine their market value, so consequently there is considerable flexibility in developing new farming methods for production of biomedically interesting chemicals via aquaculture. This was explored in the first studies by growing sponge explants in mesh structures, either grouped together in scallop lanterns (Battershill and Page 1996) or separately in mesh bags (Duckworth et al. 1997). Although both studies reported good sponge growth, there is still a need to develop structures designed specifically for farming sponges for bioactive metabolite production.

In the last decade there have also been several attempts at farming sponges in seawater tanks on land (Osinga et al. 1999). In land-based aquaculture the sponge farmer has greater control over environmental conditions (such as temperature and salinity) and food supply, thus sponge growth, survival and metabolite production should be greater compared with sea-based aquaculture. Unfortunately, little is known about optimal environmental conditions and feeding regimes and this limitation is hampering full commercial development.

Another possible method to supply sponge metabolites for drug manufacture is cell culture, where the sponge cell responsible for production of the metabolite is grown in flasks (Pomponi and Willoughby 1994). Although this is an exciting supply option, it has been difficult so far to produce a cell line where cells continue to divide. Cell culture may also disrupt the sponge-endosymbiont relation, which may be critical in metabolite production. On the other hand, it may be possible to co-culture the sponge with its symbiont, if the symbiotic relationship is essential for production of the bioactive metabolites

Sponge farming in the sea

Sponge aquaculture capitalizes on the amazing ability of sponges to survive damage and regenerate lost tissue (a survival mechanism to withstand partial predation, disease, and storms). Thus sponges collected from the sea can be cut into many smaller pieces or explants. To promote good survival, at least one side of each explant should be uncut and covered with pinacoderm (the outer cell layer of a sponge). To minimize harvesting impact one third of the collected sponge is left still attached to rock; these cut sponges will heal quickly and over time will regrow lost biomass.

Two factors restricting the commercial development of sponge aquaculture are a poor understanding of how the environment affects the growth, survival and metabolite biosynthesis of sponges, and

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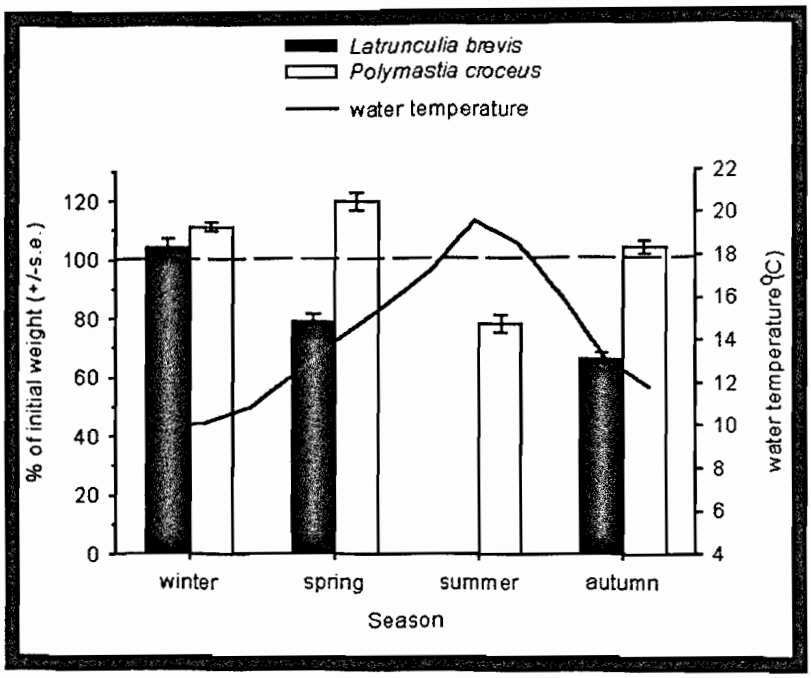

Fig. 1. Mean percent growth of L. brevis *and* P. croceus *explants farmed for two months in each season. Horizontal dashed line represents the initial explant weight.*

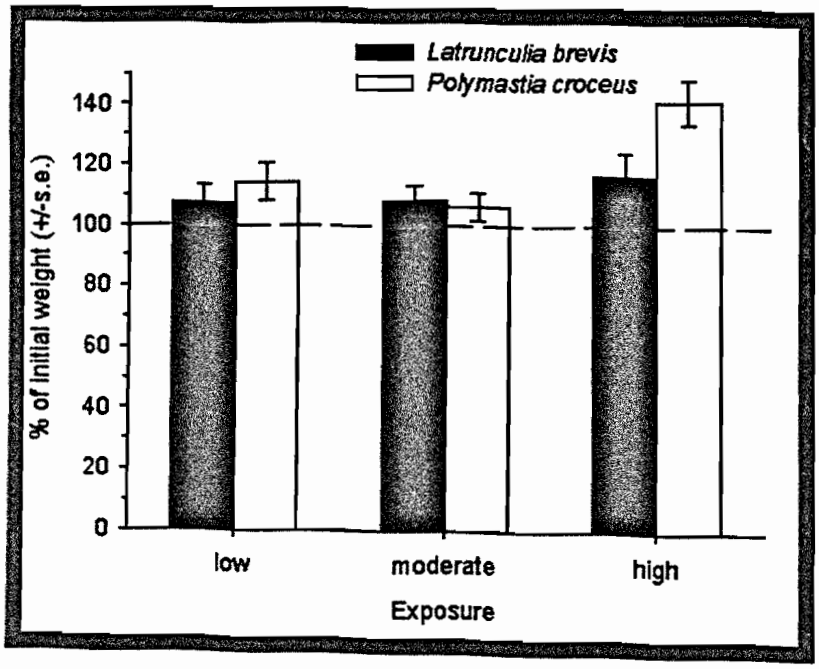

Fig. 2. Mean percent growth of L. brevis *and* P. croceus *explants farmed for two months at three exposures. Horizontal dashed line represents the initial explant weight.*

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the lack of a farming structure that can supply sufficient quantities of bioactive metabolites.

How the environment affects farming

The two major environmental conditions that are likely to influence the growth, survival and metabolite production of farmed sponges and thus directly affect the success of a farming operation are season and exposure. The farming season affects the abundance of ultraplankton (<5µm), the primary food of sponges, and generally increasing in abundance as water temperature rises. Exposure or the degree of water movement affects the availability and supply of ultraplankton to the farmed sponges. To examine their effect on sponge farming explants of two New Zealand sponges, *Latrunculia brevis* and *Polymastia croceus*, both containing metabolites of biomedical importance, were transplanted to three exposures differing in the degree of water movement (high, moderate and low exposure). Each season, about 200 explants of each species were farmed in scallop lanterns for two months, thus ensuring that farming would not run into the next season.

Both survival and growth of *L. brevis* were greatest in winter when the water temperature was lowest (Fig. 1). Low water temperature reduces stress during transplanting and promotes healing of cut surfaces, thus allowing healthy explants to divert more energy into growth. For *P. croceus*, explant survival was similar in winter, spring and autumn. Growth however was greatest in spring (Fig. 1) probably because of greater food abundance during this season. During the summer transplant the toxic alga *Gymnodinium brevisulcatum* bloomed, killing most farmed explants. Toxic blooms and disease outbreaks are a serious problem for aquaculture, and highlight the danger of adverse unpredictable events that cannot be planned for or controlled. This experiment also shows that the effect of the farming season on growth and survival can vary between sponge species.

Exposure or the degree of water movement also influences the growth of farmed sponges. Growth of *L. brevis* and *P. croceus* explants generally increased as exposure increased (Fig. 2). This indicates that to maximize production sponge farms for these species should be located in areas of high water movement. After two months, explants of *P. croceus* farmed at the high exposure had increased in weight by 40% on average, while some explants doubled in size. This shows that high sponge growth is possible. For both species, explant survival was similar between the three exposures.

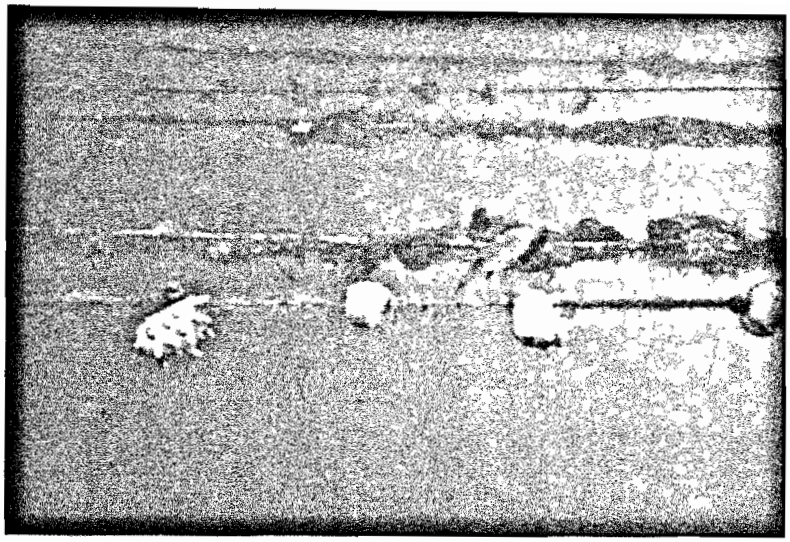

Fig. 3. Explants of P. croceus *and* L. brevis *farmed in rope and mesh arrays. The* P. croceus *explant bottom right is 7x7x6cm in size, 1200 percent its initial size. (photo by A. Duckworth)*

At the end of the farming experiment, production of bioactive metabolites in *L. brevis* and *P. croceus* explants was examined by measuring activity against the P388 murine leukaemia bioassay. This bioassay is a useful indicator for examining general levels of anticancer bioactivity in extracts from organisms as a preliminary screen. For both species, bioactivity of farmed explants was similar among farming seasons and exposures. However, compared with the bioactivity of wild (uncut) sponges, farmed explants were more active indicating they were biosynthesizing more biologically active metabolites, possibly in response to the initial tissue damage. Elevated levels of bioactive metabolites in farmed explants are a very promising result for the development and future success of commercial sponge aquaculture.

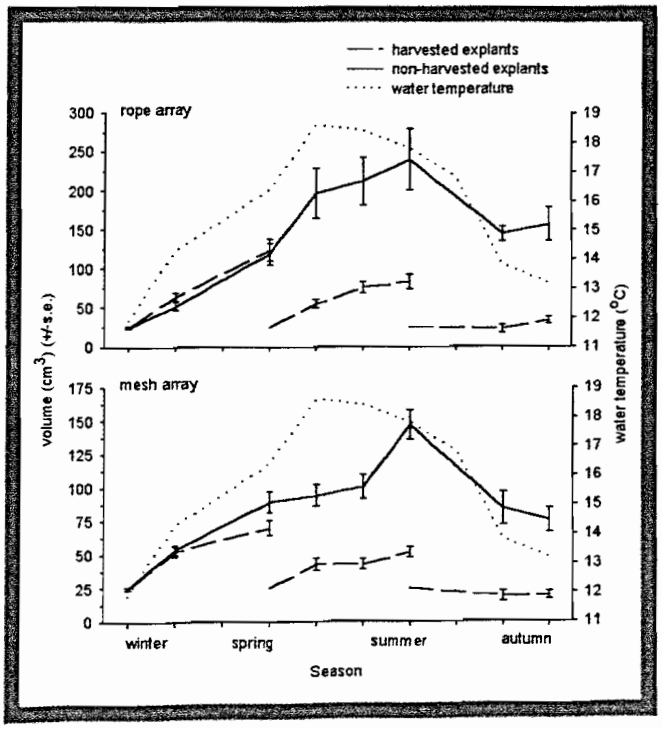

Fig. 4. Mean percent growth of harvested and non-harvested L. brevis *explants farmed on rope and mesh arrays over nine months (from mid-winter to mid-autumn).*

Sponge farming structures

To be suitable for large-scale commercial use a farming structure must be inexpensive, have a low surface area to reduce drag and bio-fouling, and allow cost-effective and efficient harvesting. It must also promote good growth and survival while maintaining high metabolite production. After a series of experiments examining a wide range of possible methods and materials (both natural and artificial), two farming structures were developed: rope and mesh arrays (Fig. 3).

A rope array consists of a length of thin polyvinyl alcohol rope threaded with explants. It is similar to structures used previously to farm bath sponges. A mesh array consists of a mesh tube divided into two by a central rope. Explants rest at the bottom of mesh pockets, made by tying the two mesh sides together in a zig-zag pattern down the mesh tube. This method has never been used before to farm sponges or other marine organisms.

Explants of *L. brevis* and *P. croceus* were farmed for 9 months, from mid winter to mid autumn, in rope and mesh arrays. In addition, some of the explants were harvested in spring and summer (3 month interval). Harvesting involved the cutting and removal of new tissue, leaving behind the original explant "core" to heal and regrow. This examines whether it is possible to capitalize on their regenerative ability and harvest an explant many times, thereby increasing production of sponge tissue and bioactive metabolites.

For both *L. brevis* and *P. croceus*, growth after harvesting was similar between harvested and non-harvested explants in the two farming structures (Fig. 4). This is a promising result for sponge

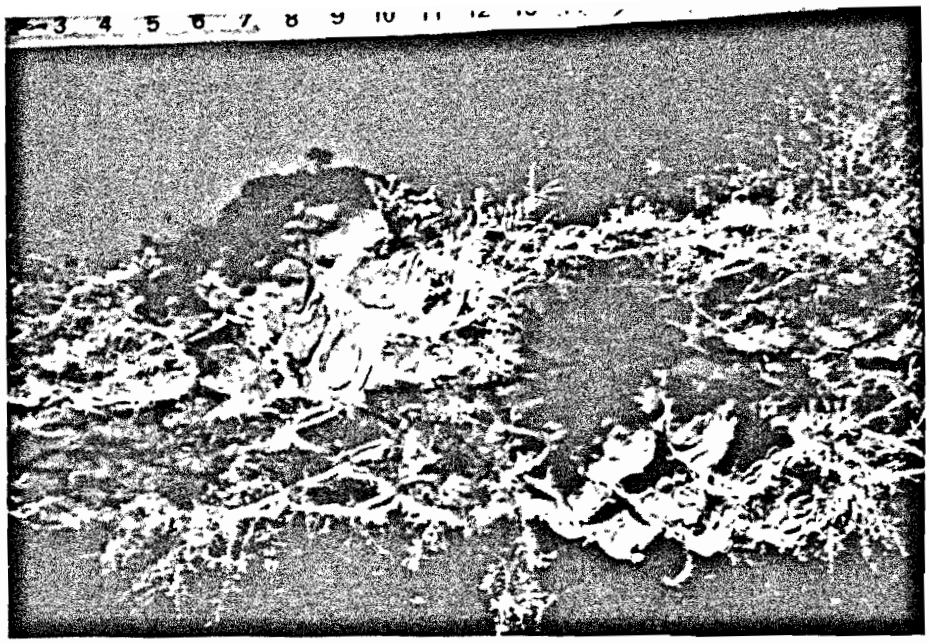

Fig. 5. Explants of L. brevis *growing through the mesh strands of a mesh array. (photo by A. Duckworth)*

aquaculture because it suggests that healing of cut tissue after harvesting does not divert energy away from overall somatic growth. Another promising finding is the very high growth rates recorded in this study: after 6 months, non-harvested *L. brevis* and *P. croceus* sponges had grown by 960% and 740% respectively. Similar to the findings of the previous experiment, sponge growth varied over seasons. Farmed explants of *L. brevis* and *P. croceus* generally grew when water temperature increased but shrunk in size when temperature fell.

Water temperature can also affect explant survival. After the summer, but not the spring harvest, survival of harvested *L. brevis* explants was lower than non-harvested explants. In contrast, final survival was similar between harvested and non-harvested *P. croceus* explants. These results indicate that water temperature is an important consideration when harvesting tissue from some sponges.

Sponge growth and survival also differed between rope and mesh arrays. Overall, growth was greatest in rope arrays (Fig. 4), because the explants are directly exposed to the environment. However, many explants of the soft, fleshy sponge *L. brevis* farmed in the mesh arrays grew partially through the mesh, incorporating the strands into their tissue (Fig 5). This allowed direct exposure of the explant to the outside environment, promoting better growth. Although rope arrays promote growth, the process of threading thin rope through explants can reduce their survival. In contrast, explants in mesh arrays have higher survival, because they are simply placed in mesh and experience minimal damage. For *P. croceus* for example, final survival of explants farmed in rope and mesh arrays was 59% and 96% respectively.

Therefore, both rope and mesh arrays were found to be good sponge farming structures, but differing patterns of growth and survival indicated that the two arrays are most suited for a particular type of sponge depending on its tissue structure. Rope arrays should be used to farm firm sponges such as *P. croceus* that can survive the threading process, while mesh arrays are best for farming soft, fleshy sponges like *L. brevis* that can grow quickly through the mesh strands.

Conclusion

Through a sound knowledge of environmental effects and use of a good farming structure, sponge aquaculture for metabolite production should be commercially viable. To maximize production of sponge tissue and bioactive metabolites, farmed sponges should be transplanted in winter to promote survival and to areas of high water movement to promote growth. To further increase production, farmed sponges can be repeatedly harvested, however the season of harvesting is an important consideration for some species. High growth rates and elevated levels of bioactive metabolites from farmed explants suggests that sponge aquaculture can guarantee sufficient and sustainable supplies of bioactive metabolites for pharmaceutical use.

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