



# Effects of depth and water flow on growth, survival and bioactivity of two temperate sponges cultured in different seasons

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Received 7 January 2004; received in revised form 9 March 2004; accepted 8 August 2004

## Abstract

Successful in-sea aquaculture of sponges for metabolite production requires a sound understanding of the effects of environmental factors on explant growth, survival and metabolite biosynthesis. Using short-term experiments, we examined the effects of season, water flow and depth on the culture response of the New Zealand Demospongiae *Latrunculia wellingtonensis* (Alvarez, Bergquist and Battershill) and *Polymastia croceus* (Kelly–Borges and Bergquist). Sponge pieces were cultured seasonally for 2 months at depths of 5 and 10 m at three sites close to parent populations but characterised by differing water flow: high, medium and low. Survival of *L. wellingtonensis* and *P. croceus* was lowest in summer, possibly because of the relatively higher water temperature increasing stress during translocation. Growth of farmed sponges varied greatly among seasons, being greatest for *L. wellingtonensis* in winter and *P. croceus* in spring, and lowest for both species in autumn. Seasonal variation in growth may result from seasonal variation in food abundance and/or periodic investment of metabolic resources in reproduction. Within each season, growth of both species was greatest overall at the high-flow site, with increases up to 12.5% in weight per month. Culture depth influenced the final size of transplanted sponges by interacting with season and water flow. Bioactivity, a

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measure of metabolite biosynthesis, was high for both species regardless of the environmental condition.

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*Keywords:* Sponge; Growth; Survival; Metabolite biosynthesis; Season; Water flow

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## 1. Introduction

In-sea aquaculture is one possible method of supplying sufficient and sustainable quantities of sponge metabolites that have pharmaceutical potential as drugs or as biomedical tools (Osinga et al., 1999). As with other cultured marine organisms where biomass production is the goal, successful sponge aquaculture demands a thorough understanding of the effect of environmental factors, such as seasonal influences of water temperature, on growth and survival. In addition, sponge aquaculture for metabolite production also requires an understanding of the environmental effect on metabolite biosynthesis.

Although the first culture trials of sponges failed largely because of a poor understanding of environmental factors (Moore, 1908; Crawshay, 1939), few studies since have investigated the importance of the environment on sponge growth, survival and metabolite biosynthesis. Verdenal and Vacelet (1990) discovered that growth and survival of *Spongia* species were reduced in heavily polluted water, possibly because of the high load of sediment smothering the explants. Wilkinson and Vacelet (1979) and Duckworth and Battershill (2003a) found that growth of some sponges increased as water flow increased, while other species grew better in sheltered, low-flow areas (Duckworth et al., 1997). Growth of transplanted sponges may also vary between depths (Wilkinson and Vacelet, 1979; Contini, 1995) or among seasons, the latter possibly related to temporal variation in water temperature and food abundance (Duckworth and Battershill, 2003a). Seasonal variation in ambient water temperature may also influence sponge survival (Moore, 1908; Battershill and Page, 1996) and metabolite biosynthesis (Turon et al., 1996). Lastly, culture depth through its influence on light intensity can affect metabolite production in some sponge species (Thompson et al., 1987; Kreuter et al., 1992). These studies suggest that the main environmental factors that could influence sponge farming success are season, water flow and depth. It is unclear, however, which environmental factors are most important when farming sponges, or how they interact to affect the culture response.

To determine the effect and possible interactions of the major environmental factors on the farming success of sponges, we transplanted explants of two New Zealand Demospongiae, *Latrunculia wellingtonensis* (Alvarez, Bergquist and Battershill) and *Polymastia croceus* (Kelly–Borges and Bergquist) in each of the four seasons to depths of 5 and 10 m at three sites differing in their degree of water flow: high, medium and low. The culture period for each season was 2 months to ensure that growth would not run into the next season. The seasonal growth, survival and bioactivity of *L. wellingtonensis* and *P. croceus* in natural populations are described in Duckworth and Battershill (2001).

Neither species contains photosynthetic symbionts that may aid growth. *L. wellingtonensis* contains bioactive metabolites called discorhabdins which have strong antitumor and antimicrobial properties (Perry et al., 1988; Lill et al., 1995), while *P. croceus* contains a bioactive metabolite that has strong cytotoxic properties (National Cancer Institute, personal communication). Wellington Harbour, located at the southern end of the North Island, New Zealand, was chosen as the farming location for this study due to the range of environmental conditions occurring there (Northcote, 1998). Situated in a temperate latitude, Wellington Harbour is a well-mixed, semienclosed body of water, 85 km<sup>2</sup> in area (Maxwell, 1955; Booth, 1975; Heath, 1977). Except during storms, water flow in the harbour results from tidal currents (Carter, 1977).

## 2. Methods

### 2.1. Collecting sponges and cutting explants

Near the onset of each season (July, winter; October, spring; January, summer; and May, autumn), approximately 40 *L. wellingtonensis* and 40 *P. croceus* were collected from the south coast of Wellington. To minimise harvesting impact, up to one third of each sponge was left attached to a rock. Remaining cut sponges of these two species heal and regrow quickly (Duckworth, 2003).

All collected sponges were kept in tanks with running seawater at ambient temperature sourced from Wellington Harbour and transplanted to experimental field sites as soon as possible, normally within 2 days. *L. wellingtonensis* and *P. croceus* were separated into different 50-l aquaria to prevent any antagonistic interactions between the two species. Sponges were cut with scalpels under running seawater into cube-shaped explants, approximately 16 cm<sup>3</sup> (2.5×2.5×2.5 cm) in size. A preliminary experiment determined that 16-cm<sup>3</sup>-sized explants exhibited high survival while allowing for high replicate explant production from the collected sponges. All explants possessed at least one uncut side covered with pinacoderm. It was necessary to bring sponges to shore and cut them up in aquaria to allow us to fully randomise explants to treatments, thus blocking across differences in initial condition and genotype.

### 2.2. Experimental design

In each season, explants of *L. wellingtonensis* and *P. croceus* were transplanted to three sites, differing in the degree of water movement or flow: a high-flow site at the entrance to Wellington Harbour, and medium- and low-flow sites within the harbour. At each site, sponges were cultured at depths of 5 and 10 m. Because Wellington Harbour is relatively shallow, with an average depth of 14 m (Heath, 1977), it was not possible to culture sponges at a greater depth at all three sites.

Three replicate culture arrays, approximately 15 m apart, were established at each site. An array consisted of one scallop lantern at each depth linked by rope pulled taut by a subsurface buoy and anchored with heavy weights to the substrate (Fig. 1). A scallop lantern is a 1.5×0.5 m cylindrical net divided into 10 compartments and covered with

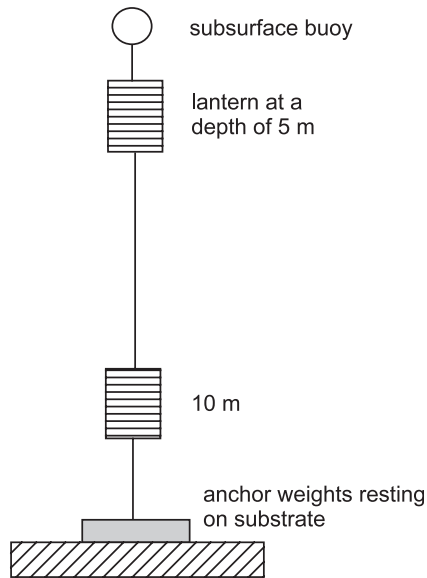


Fig. 1. Schematic diagram of the culture array, showing scallop lanterns at 5 and 10 m depths.

large nylon mesh, with a mesh cover of 10% (i.e., 90% of the lantern is open to the flow of water). Each scallop lantern was situated at least 2 m above the substrate to reduce the possibility of resuspended bottom sediments smothering the explants. Scallop lanterns have been used successfully before to grow sponges (Battershill and Page, 1996; Duckworth and Battershill, 2003b). For each species, eight explants were placed all together inside a randomly chosen and separate compartment in each lantern. In each season, 144 explants (i.e., 8 explants  $\times$  2 depths  $\times$  3 arrays  $\times$  3 sites) of each species were farmed for approximately 2 months and thus ensuring that growth would not run into the next season. After each seasonal experiment, all culture arrays were removed and cleaned thoroughly. This study ran for 1 year from July 1998 to June 1999.

### 2.3. Monitoring farming response

Growth was determined by wet-weighing the explants (to 0.1 g) at the start and end of each experiment. In a previous study (Duckworth and Battershill, 2003b), we found that both species when disturbed by handling will self-contract and expel most excess water over a 30-min period. Thus, to obtain reliable weight data, explants were disturbed by handling 30 min before weighing. Because “self-contracting” is a natural process (Duckworth and Battershill, 2001), this method would have no long-term affect on the culture response of either species. At the start of each season, 50 representative explants, each of *L. wellingtonensis* and *P. croceus*, were wet-weighed to determine their average initial weight (Table 1). Analysis of variance (ANOVA) for each species determined that initial explant weights were significantly different between seasons for both *L. wellingtonensis* ( $F_{df(3,196)}=7.80$ ,  $P<0.001$ ) and *P. croceus* ( $F_{df(3,196)}=14.39$ ,  $P<0.001$ ).

Table 1  
Mean initial explant weight (g) of *L. wellingtonensis* and *P. croceus* ( $n=50$ ) explants in each season

Season	<i>L. wellingtonensis</i>	<i>P. croceus</i>
Winter	14.5 (0.5)	13.7 (0.4)
Spring	16.9 (0.5)	16.6 (0.5)
Summer	17.7 (0.5)	17.1 (0.5)
Autumn	15.4 (0.5)	13.9 (0.5)

Standard errors in parentheses.

This probably resulted from experimental error in cutting the explants to slightly different sizes but could reflect seasonal differences in cellular density of sponges. Relative sponge size does not influence the growth or survival for *L. wellingtonensis* or *P. croceus* (Duckworth and Battershill, 2001), thus, small interseason differences are considered unlikely to affect final results. However, these seasonal differences prevented the use of final explant weight to compare environmental factors. Instead, percentage weight of each explant was examined:

$$\% \text{ weight} = \text{final weight} / \text{mean initial weight} \times 100$$

Explants were monitored haphazardly during each seasonal transplant, their survival recorded, and any fouling organisms such as algae and bushy bryozoans that could have reduced the water flow to explants were removed from the lantern mesh.

To assess overall metabolite biosynthesis between farming conditions, explant extracts of *L. wellingtonensis* and *P. croceus* were analysed using a P388 murine leukaemia bioassay, which is a useful indicator for examining general levels of bioactivity in extracts from marine organisms (Blunt et al., 1990). The chemical analysis procedure is described in Duckworth and Battershill (2001). Because of the expense of doing the bioassays, only two extract replicates, each consisting of five explants, were analysed per treatment. Bioactivity is expressed as an  $IC_{50}$ , with an  $IC_{50} < 1500$  ng/ml considered very active (Lill et al., 1995).

#### 2.4. Data analysis

Three-way analysis of variance (ANOVA) was used to examine the relative effect of season, water flow and depth on the growth and survival of *L. wellingtonensis* and *P. croceus*. To meet ANOVA assumptions for each species, the growth data were log-transformed, and the survival data were arcsine-transformed. Statistical analysis was not done for explant bioactivity because of low replication number ( $n=2$ ).

In late summer of 1998, the toxic dinoflagellate *Karenia brevisulcata* bloomed in Wellington Harbour, killing many marine organisms (Wear and Gardner, 2001) including all *L. wellingtonensis* and most *P. croceus* explants. This highlights the susceptibility of aquaculture to adverse stochastic events that cannot be adequately planned for or controlled. For explant survival, statistical analysis used the data recorded shortly before the bloom. This meant that the summer culture period was 6 weeks, instead of the planned 8 weeks. For explant growth, only data from winter, spring and autumn were analysed.

### 2.5. Physical conditions

Water temperature was recorded daily at the medium-flow site at a depth of 5 m by the NIWA Mahanga Bay Hatchery. Using temperature data loggers, water temperature was also recorded for 1 month (May 1998) at the three sites and two depths.

Relative water movement was measured between sites and depths by comparing the erosion of “clod cards”. Made of plaster of Paris, clod cards erode at a consistent rate relative to water movement and therefore give a qualitative measure of water movement (Jokieli and Morrissey, 1993). At each site, one clod card was tied to the top of each lantern on two arrays and left in the water for 2 days (12 clod cards used in total). The need for suitable diving weather restricted this experiment to a relatively fine spell of settled weather, otherwise clod cards could not be retrieved. They were left in the water for 50 h and results represent a conservative estimate of water movement among sites and depths.

## 3. Results

### 3.1. Physical conditions

Water temperature was lowest during winter (August) and highest during summer (February; Fig. 2). Mean water temperatures per seasonal transplant period were winter, 10.6 °C; spring, 14.5 °C; summer, 18.5 °C; and autumn, 14.0 °C. Water temperature varied little between sites and depths with mean differences <0.2 °C, consistent with other studies reporting that water is well mixed in Wellington Harbour (Maxwell, 1955; Booth, 1975; Heath, 1977).

Erosion of the clod-cards differed significantly among sites (two-way ANOVA:  $F_{df(2,6)}=81.5, P<0.001$ ) and depths (two-way ANOVA:  $F_{df(1,6)}=68.7, P<0.001$ ). Results of a Tukey–Kramer Multiple Comparison test determined that all three sites were significantly different from one another: high>medium>low-flow. Erosion decreased from the high-flow to the low-flow site and also decreased with depth within each site

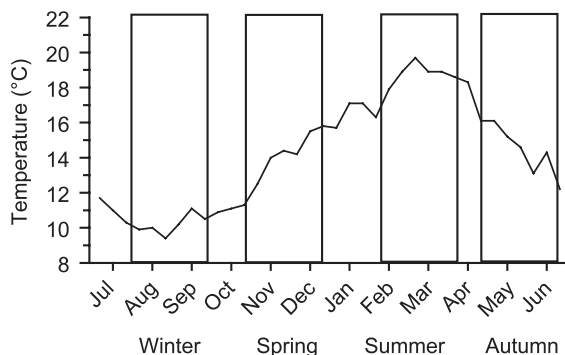


Fig. 2. Water temperature in Wellington Harbour across seasons. The boxes represent the growing period used in this study for each season.

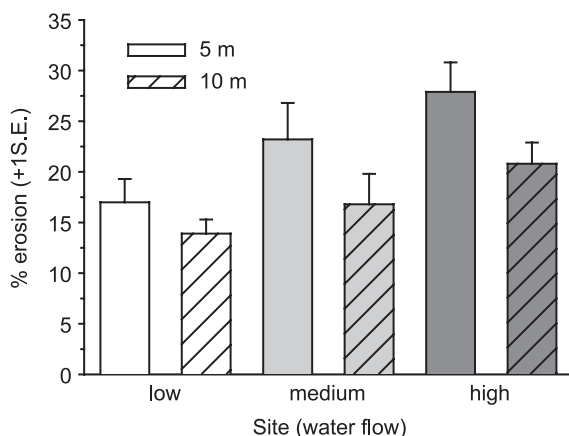


Fig. 3. Water movement, measured by percent erosion of clod-cards among sites and depths. As erosion increases, water movement increases.

(Fig. 3), indicating decreasing water movement from the high- to the low-flow site and decreasing water movement with depth within each site. Recorded water flow rates were  $0.19\text{--}46\text{ ms}^{-1}$  at the exposed site (Carter, 1977),  $0.13\text{ ms}^{-1}$  at the moderately exposed site and  $0.015\text{ ms}^{-1}$  near the sheltered site (Truebridge et al., 1978).

### 3.2. Growth of farmed sponges

Growth of *L. wellingtonensis* was significantly affected by the interaction of water flow and depth (Table 2), being highest at 10 m at the high-flow site but at 5 m at the medium- and low-flow sites (Fig. 4a). Water flow also influenced the final size of *L. wellingtonensis*, with increasing water flow generally promoting better explant growth (Fig. 4a). Overall final weight was 103% of initial explant weight at the high-flow site, 93% at the medium-flow site, and 90% at the low-flow site. Growth also varied between seasons, being greatest during winter when most explants surpassed their initial weight (average=110%) and least during autumn (64%; Fig. 4a).

Table 2

Summary of analysis of variance results for growth of *L. wellingtonensis* (Lw) and *P. croceus* (Pc) between seasons, water flows and depths

Factor	Lw		Pc	
	Degrees of Freedom	F ratio	Degrees of Freedom	F ratio
Season	2	37.74***	2	12.06***
Water flow	2	4.63*	2	25.29***
Season*water flow	4	0.93	4	4.22**
Depth	1	0.42	1	0.74
Season*depth	2	0.12	2	7.35***
Water flow*depth	2	6.17**	2	7.77***
Season*water flow*depth	4	0.7	4	7.71***
Error	197		387	

Probability: \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ .

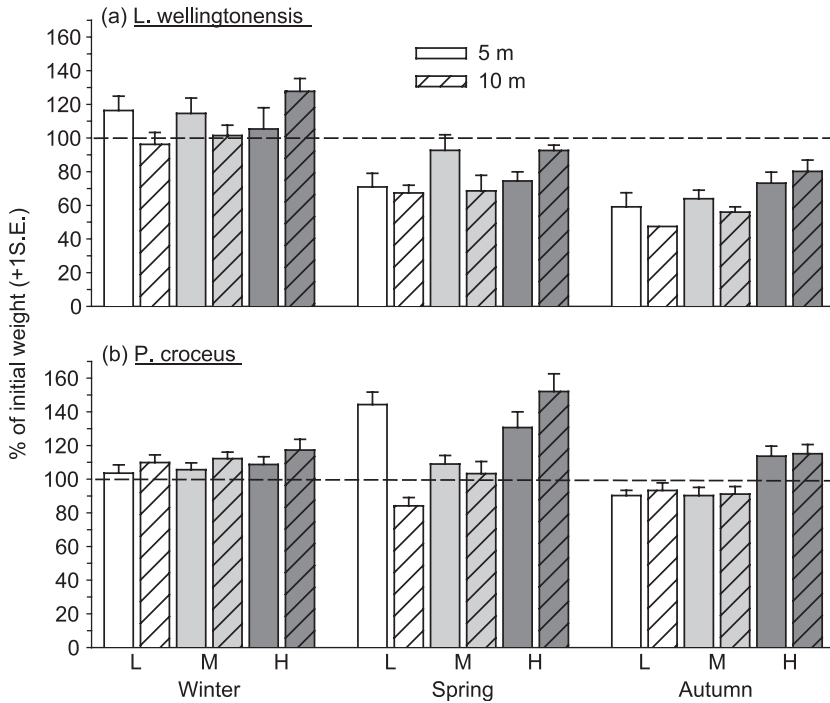


Fig. 4. Growth (% of initial weight) after 2 months of (a) *L. wellingtonensis* and (b) *P. croceus* farmed in different seasons, sites and depths: L—low-flow site, M—medium-flow site and H—high-flow site. Dashed lines represent initial weight (100%). Error bars represent variation between explants.

Statistical analysis of *P. croceus* growth showed a significant season\*water flow\*depth interaction (Table 2). Greatest growth occurred for explants cultured during spring at a depth of 5 m at the low-flow site and at 10 m at the high-flow site (Fig. 4b). These explants grew on an average by 8 g or 50% of their initial weight in 2 months. Comparing seasons, overall growth was greatest during spring (average=120%) and least during autumn (98%; Fig. 4b). *P. croceus* transplanted to the high-flow site grew the most during each season (Fig. 4b). Overall final weight was 125% of initial explant weight at the high-flow site, 102% at the medium-flow site and 104% at the low-flow site.

### 3.3. Survival of farmed sponges

The survival of *L. wellingtonensis* differed greatly between the four seasons (Table 3). Final survival was highest in winter and lowest in summer, being 78% and 24%, respectively (Fig. 5a; note that, for each species, the summer transplant used survival data recorded shortly before the toxic algal bloom). Water flow also had an effect on the survival of *L. wellingtonensis*. During autumn, more explants survived at the medium-flow site than at the high- and low-flow sites (Fig. 5a). This resulted in a significant season\*water flow interaction term (Table 3). Depth had no effect on the survival of transplanted *L. wellingtonensis* (Table 3).

Table 3

Summary of analysis of variance results for survival of *L. wellingtonensis* (Lw) and *P. croceus* (Pc) between seasons, water flows and depths

Factor	Lw		Pc	
	Degrees of Freedom	F ratio	Degrees of Freedom	F ratio
Season	3	23.34***	3	9.99***
Water flow	2	3.09	2	0.85
Season*water flow	6	3.38**	6	1.73
Depth	1	0.00	1	0.44
Season*depth	3	0.76	3	0.04
Water flow*depth	2	1.12	2	1.21
Season*water flow*depth	6	0.43	6	0.82
Error	48		48	

Probability:  $p < 0.01$  and  $p < 0.001$ .

The final survival of transplanted *P. croceus* differed significantly between seasons (Table 3), being highest in spring (98%) and lowest in summer (79%; Fig. 5b). Neither water flow nor depth had any significant effect on *P. croceus* survival (Table 3). Compared

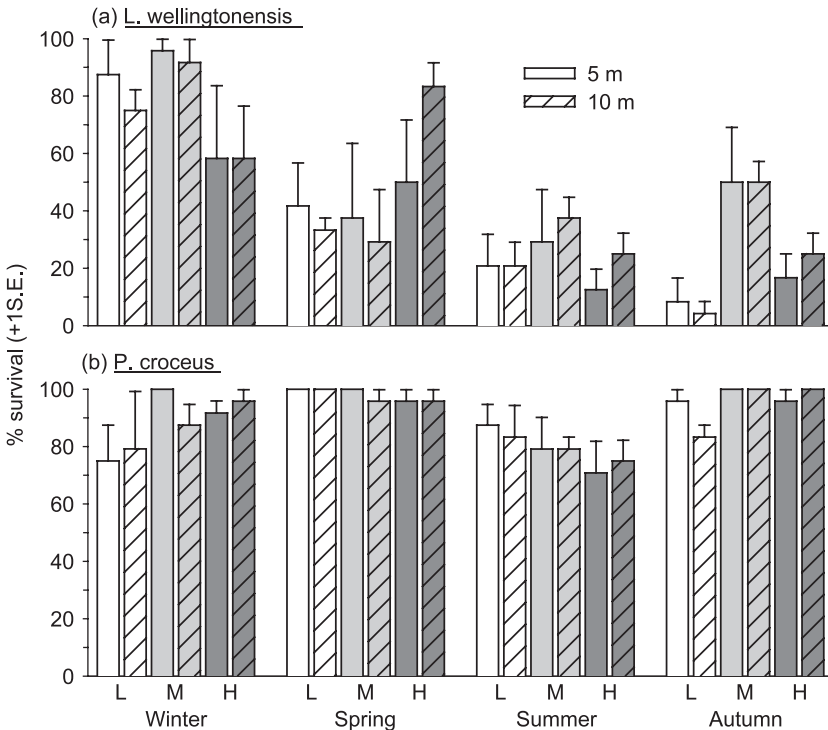


Fig. 5. Percent survival after 2 months of (a) *L. wellingtonensis* and (b) *P. croceus* farmed in different seasons, sites and depths: L—low-flow site, M—medium-flow site and H—high-flow site. Error bars represent variation between lanterns.

with *L. wellingtonensis*, *P. croceus* was a hardier species with >90% of the transplanted explants surviving. Percentage survival of *P. croceus* in a lantern did not correlate with survival of *L. wellingtonensis* (canonical correlation:  $r=0.012$ ,  $P=0.92$ ). Dead explants of both *L. wellingtonensis* and *P. croceus* were often covered with an unidentified white fungal film. The sponge tissue beneath this film was black and pungent.

### 3.4. Bioactivity of farmed sponges

For each species, the bioactivity of farmed sponges was similar between seasons, water flows and depths (Fig. 6). The large variation in some treatments probably resulted from the low number ( $n=2$ ) of extracts that were bioassayed. Using the Lill et al. (1995) definition of high activity ( $IC_{50} < 1500 \text{ ng ml}^{-1}$ ), farmed sponges of both *L. wellingtonensis* and *P. croceus* were very active, regardless of treatment. No explants of either species became fouled by macroorganisms such as algae or ascidians at any time, possibly because of the presence of biologically active metabolites (Duckworth and Battershill, 2001).

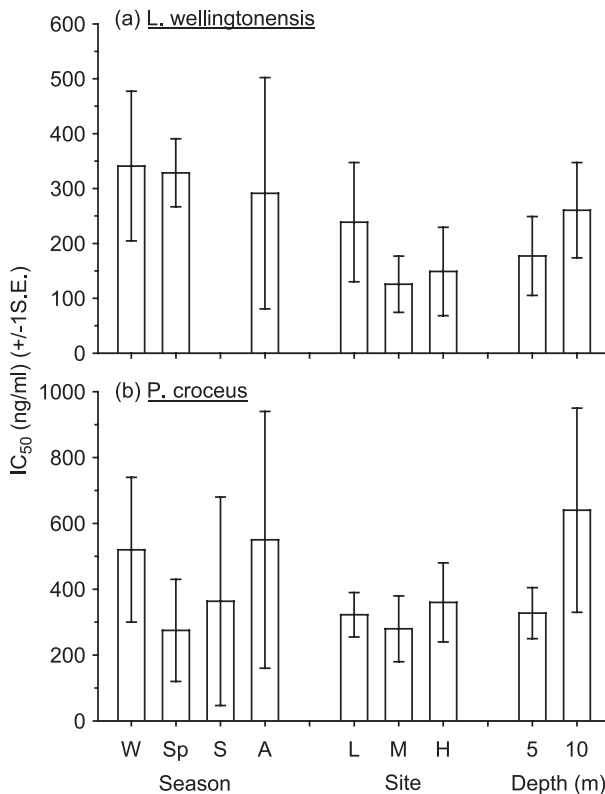


Fig. 6. Final bioactivity ( $IC_{50}$ ) of (a) *L. wellingtonensis* and (b) *P. croceus* farmed in different seasons, sites and depths: L—low-flow site, M—medium-flow site and H—high-flow site. As  $IC_{50}$  decreases, bioactivity increases. Error bars represent variation between extracts. There was no data for *L. wellingtonensis* in summer.

#### 4. Discussion

This study demonstrates that environmental factors have strong effects on the growth and survival of cultured sponges. Survival of transplanted *L. wellingtonensis* and *P. croceus* varied greatly between seasons, with survival lowest in summer when water temperature was highest. In comparison, survival of *L. wellingtonensis* and *P. croceus* in populations adjacent to the exposed site was similar over all seasons (Duckworth and Battershill, 2001). These results suggest that the survival of transplanted sponges is greatly affected by water temperature. Cooler water promotes survival in several ways. Respiration is lower in cooler water (Burlando et al., 1992; Cheshire et al., 1995), and this reduces stress during transplanting. Cooler water also promotes quicker pinacoderm healing (Duckworth et al., 1997) and reduces microbial growth (Hummel et al., 1988; Vacelet et al., 1994), both of which may reduce the chance of infection. To promote high sponge survival, and thus culture success, this study suggests that transplanting and farming should be started during winter.

In each season, explant survival was greater for *P. croceus* than for *L. wellingtonensis*, being most notable in summer when final survival (after 6 weeks) was 79% and 24%, respectively. A difference in explant survival is common between species (Verdenal and Vacelet, 1990; Duckworth et al., 1997) and probably stems from physiological differences in the response to reorganising cut tissues into fully functional explants. One possible consequence of *P. croceus* being a hardier species is that its explants were comparatively healthier and able to divert more energy into growth. This may explain the greater overall growth of *P. croceus* compared to *L. wellingtonensis* in this study.

Growth of both species varied greatly between seasons, being greatest for *L. wellingtonensis* in winter and for *P. croceus* in spring. Both species had a mean weight loss in autumn. These results largely agree with an ecological study done on these two species (Duckworth and Battershill, 2001). Sponge growth has been correlated with water temperature for several other temperate species (Johnson, 1979; Barthel, 1986), and it may result from seasonal variation in food abundance and/or reproductive investment. Sponges feed primarily on plankton  $\leq 10 \mu\text{m}$  (Reiswig, 1971), which in temperate coastal waters, including New Zealand, generally increase as water temperature rises and decrease as water temperature falls (Tamigneaux et al., 1995; Vant and Safi, 1996). The overall negative growth of *L. wellingtonensis* in spring may have resulted from its poor survival during the spring transplant. Because gametogenesis in sponges involves the transformation of choanocytes (feeding cells) into sperm sex cells (Simpson, 1984), variation in growth over time may also result from seasonal variation in reproductive investment (Simpson, 1984). *P. croceus* is probably reproductively active in summer and early autumn (Ayling, 1980; Duckworth and Battershill, 2001), correlating with periods of relatively poor growth. In contrast, *L. wellingtonensis* appears reproductively active throughout the year (Duckworth and Battershill, 2001).

Being active suspension feeders, sponges are capable of generating water currents to supply food and thus energy for growth. However, this study clearly shows the importance of ambient water flow to sponge growth and farming success. Within each season, growth of transplanted *L. wellingtonensis* and *P. croceus* was greatest overall at the high-flow site. This result supports the findings of other studies that have found that high water flow

promotes the growth of active suspension feeders such as sponges (Wilkinson and Vacelet, 1979; Lenihan et al., 1996). High water flow can increase growth and final size of active suspension feeders directly through increased availability of food (Lenihan et al., 1996), indirectly by breaking down the food-depleted boundary layer around the organism (Leichter and Witman, 1997), or by increasing the internal flow through sponges (Vogel, 1974, 1977). An additional benefit of high water flow may be a greater transfer of oxygen into sponge tissue (Gatti et al., 2002).

Although increasing water flow can promote the growth of sponges, the relationship is not linear, and final size can be reduced in areas of abnormally high water flow. *L. wellingtonensis* explants cultured at the high-flow site, adjacent to where they naturally occur, grew poorest at the shallow depth of 5 m where water flow is greatest. Explant growth at the medium- and low-flow sites, however, was greatest at 5 m. Reduced growth at very high water flows has also been recorded for other sponge species (Duckworth et al., 1997; Leichter and Witman, 1997) and may result from reduced feeding efficiency as found for other suspension feeders (Eckman and Duggins, 1993) or a need for the organism to invest more resources in skeletal structure and repair to shallow water surge damage (Palumbi, 1984).

Although the farming environment greatly affected both growth and survival of *L. wellingtonensis* and *P. croceus*, it had little influence on overall metabolite biosynthesis, as bioactivity for each species was similar among seasons, sites and depths. Farmed sponges of both species were very active, however, and generally more bioactive than wild conspecifics (Duckworth and Battershill, 2001). High bioactivity levels may result from increased biosynthesis in response to the injury and tissue damage suffered when sponges were cut to make explants. In addition, farming all explants of a species in a single compartment may have caused “chemical warfare” between the explants, possibly further promoting bioactivity. Overall, the variation in growth and survival of *L. wellingtonensis* and *P. croceus* in this study clearly indicates that successful sponge culture requires a comprehensive understanding of the interactions among physical and biological variables.

## Acknowledgements

Many thanks to our colleagues at NIWA, particularly Christopher Woods and Pete Notman, for their help with this study. Thanks also to Prof. Dame Patricia Bergquist for helpful comments and three anonymous referees for critically reviewing this manuscript. This study was done under the University of Canterbury/NIWA Centre of Excellence in Aquaculture and Marine Ecology programme and was funded by the New Zealand Foundation for Research, Science and Technology.

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