TITLE:

Offshore cultivation methods affects blade features of the edible seaweed *Saccharina latissima* in a bay of Galicia, Northwest Spain

THE RUNNING TITLE:

Variation of blade features in *Saccharina latissima* cultured

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Abstract.— Changes in blade morphology of *Saccharina latissima* may be of importance for its commercial cultivation. Blade features were compared between the cultivation in horizontal rope and in hanging rope during its reproduction period (early autumn and early spring of next year). Cultivation experiments were conducted from February on a sheltered coastal area of a bay of Galicia (N.W. Spain). According to the results, the morphological differences were significant in area of blade which affected to blade biomass, although only significantly during the reproduction period of early spring. Moreover, the cultivation method significantly affects always significantly to "substantiality value", an index that express the blade quality for human consumption. The variation in morphological features of *S. latissima* blade seems that were caused by different hydrodynamics of both cultivation methods.

Key words: hanging culture, horizontal culture, hydrodynamics, kelp, morphology.

The brown seaweed *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl & Saunders, previously known as *Laminaria saccharina* (Linnaeus) Lamouroux, has attracted commercial interest as food for human consumption in Europe in recent years. This kelp is one of native European seaweeds that have more potential for be commercial culture in these waters. Thereby several offshore cultivation trials with this specie have been carried out with success in the Irish Sea, United Kingdom [10]; White Sea, Russian [4]; North Sea, Germany [3]; and in Atlantic Sea, Spain [5, 19, 20]. In a middle term perspective, *S. latissima*, commonly known as "sugar kombu" or "kombu royale", is considered as a probable aquaculture species in the Atlantic coasts of Spain.

Macroscopic stage of *Saccharina latissima* consists of undivided blade without midrib connected to a branched holdfast by a stipe. However, its morphology can vary considerably with environmental conditions, such as wave exposure. So, in *Laminaria* sensu lato (*Laminaria* and *Saccharina*) individuals from wave exposed habitats have short, narrow and thick blades, whereas those from sheltered habitats have long, wide and thin blades [7, 8, 11, 17]. Morphological blade features have very important implications in assessing the quality of *Saccharina japonica* (Areschoug) Lane, Mayes,

Druehl & Saunders for human consumption. Thus, "substantiality value" is a simple and quick method based in morphological blade characters commonly applied to Asian cultivated *S. japonica* ("kombu"), in order to assess its quality [12, 13, 15]. Other key blade features with commercial interest are those related to productivity, such as biomass [6, 15]. So far, the effect of the offshore cultivation methods on the blade features of *Saccharina latissima* is unknown.

In this study, we have performed offshore cultivation experiments with culture ropes in a hanging and horizontal position to examine the variation of blade features in *Saccharina latissima* during the reproduction period between these two culture methods used in Asia for *Laminaria* sensu lato. Cultivation experiments were conducted in a coastal bay of Galicia (Northwest Spain) opened into the northwest Atlantic Ocean. Considering the current commercial interest for *S. latissima*, this paper provides valuable information for its future aquaculture in bays coastal.

MATERIAL AND METHODS

Cultivation experiments with *Saccharina latissima*

String with attached seedlings of *Saccharina latissima* was produced from gametophytes under controlled environmental conditions using the European method [18]. It was produced in the Spanish Institute of Oceanography (IEO), as described in previous studies [20, 21]. All seedlings were originated from the same germplasm collection of IEO (cultivation gametophytes storage), so that any heritable differences in morphology would be minimal.

Cultivation experiments with *Saccharina latissima* were carried out on the Ría de Ares y Betanzos, in a small bay opened into the northwest Atlantic Ocean in the Galician coast (Northwest Spain). The culture site was located at the inner zone of the Ria, a sheltered coastal area devoted mainly to commercial mussel farming (see Fig. 1). Culture raft with horizontal ropes and hanging rope (garland-type) were deployed as

illustrated in Fig. 2 in the culture site. Horizontal rope culture consisted of ropes suspended horizontally at 2-2.5-metres depth attached to buoys that were fixed to the bottom by anchors. While, hanging rope culture (garland-type), included ropes attached to buoys that were midway weighted by a small stone, therefore, ropes remain hanging in an oblique position between 2 to 4 meters of detph. This hanging rope culture is commonly used in Asia as a modification of the traditional vertical hanging method, with the advantage of decreasing depth gradually and minimizing the shadow effect of the seaweeds.

Strings with seedlings of *Saccharina latissima* were outplanted in February 2002. Seedlings were inserted on the culture rope by rolled and reinforced with soft tape. Depth was increased about 4 meters during summer months in both culture methods in order to allow *S. latissima* survive more easily under higher summer temperatures. Blades of *S. latissima* were collected in October 2002 and in March 2003 when begin to develop reproductive tissues (sorus), life stage in which have developed all adult morphological traits. It represents a total of 237 and 374 days (8 and 12 months approximately) of culture in the sea.

Measurement of morphological features

Four samples of culture rope were randomly collected during the reproduction period in the same depth (about 2-3 metres) to avoid the influence of depth on development of *Saccharina latissima* in both cultivation methods. Twenty individuals were selected from the five longest individuals collected in each sample; these are the most interesting from the commercial point of view as occurred in kelp farming. Several morphological characters of blade were recorded for each individual: wet weight, length and maximum width. Moreover, the surface areas were estimated from length and maximum width according to Abe *et al*. (1998) [1]. All measurements were done from fresh individuals. In addition, "substantiality value" (SV) of blade was calculated according to the formula give by Kawashima (1984, 1993) [12, 13]:

$$SV\ (mg\ cm^{-2}) = \text{wet weight (mg)} / \text{length (cm)} \times \text{maximum width (cm)}$$

Comparisons of blade morphological features between hanging and horizontal rope culture were performed using unpaired two-tailed Student's t-test assuming unequal variances (Table 1). To minimize the risk of a type I error, differences were considered significant if the probability value (*P* value) is less than 0.01. Statistical analysis was tested using SPSS® statistical package software for Microsoft Windows.

RESULTS

Blade features of *Saccharina latissima* during the two reproduction period (early autumn and early spring of next year) are presented in Table 1. Results obtained for wet weight, length, width and area of the blades from horizontal rope culture were greater than that those from hanging rope culture, whereas thickness from hanging rope culture was greater than from horizontal rope culture. Except for length, all differences were statistically significant at 0.01 level, altought for wet weight only during second reproduction period (early spring). Moreover "substantiality value" of blade was significantly greater in the hanging rope culture than in horizontal rope culture. Other morphological characteristic worth to point out is that blade from hanging rope culture was covered with deep bullations (i.e. depressions), in contrast to blade from horizontal rope with hardly bullations.

DISCUSSION

Results of this study show that cultivation method affects blade features of *Saccharina latissima*. Obtained values for wet weight, length, width and area of blade differ between horizontal and hanging rope culture, however, differences for length are not significant statistically at 0.01 level, and for wet weight only during early spring of next year. The blade area was greater between 37% and 24% in horizontal rope culture than in hanging rope culture during early autumn and early spring of next year respectively. At this point, it is worth to signalise that despite the significant differences in blade area, biomass blade during first reproduction period (early autumn) is not

significantly affected by such morphological variation. Similarly, Gerard (1987) using laboratory simulations with different longitudinal tension (equivalent to different water flow rates), had also found that biomass production rates of *S. latissima* after 2 weeks were not affected by differences in blade area, even at low light-levels [8]. However, biomass would be expected to be proportional to photosynthetic area as has been found by Lüning *et al*. (1973) in *S. latissima* with blades amputated at various lengths [14]. In our study, there were no significant differences in biomass during early autumn would be interpreted as a result of the proximity to summer season where the plant for survival makes use of the reserves accumulated during the winter and spring [9]. In anyway, the culture method could affect to biomass between 20% and 34% during early autumn and early spring of next year respectively. However, it must be interpreted only to individual level. No data were available for assessment of biomass per farm unit.

Regarding to quality, we have to look at "substantiality value" results (measurement of the quality for human consumption). This value was greater between 23% and 16% in hanging rope culture than in horizontal rope culture during early autumn and early spring of next year respectively. These results especially in hanging rope culture, are similar to the ones reported by Mairh *et al*. (1991) for *Saccharina japonica* in Japan [15], therefore, in relation to quality, *S. latissima* can be considered an excellent local analogous substitute for the Asian "kombu".

Taking into account the factor responsible of changes in morphological features of *Saccharina latissima* blade, our results showed that individuals cultured in horizontal rope culture formed a long, broad and thin blade without almost any bullations. In contrast, hanging rope culture formed a short, narrow, thick blade with deep bullations. Similar variation in blade morphology has been observed for some species of *Laminaria* sensu lato (e.g. *Saccharina japonica* and *Saccharina longicruris* (Bachelot de la Pylaie) Lane, Mayes, Druehl & Saunders) in response to wave exposure [7, 11]. In laboratory simulations, Gerard (1987) has demonstrated that *S. latissima* subjected to constant longitudinal tension (as would be expected in higher water flow rates) had significantly narrower blades compared to those that had not experienced the same stress [8]. In this

respect, Neushul *et al*. (1992) measured in situ water movement over kelps farms and showed that the tension on the main horizontal line of the farm produced a greater water motion velocity than untensioned (i.e. slack) under moderate and calm conditions of wave exposure [16]. In our experiment, the hanging culture rope was tensioned by a small weight, this tension is easily transmitted throughout the culture rope, while, the tension in the horizontal rope culture is the one given for the structure of culture raft, and it is technically complex to tense enough the structure to transmit tension over the entire culture rope, so it is usually slack. Summing up, morphological variations observed in our study seem that are caused by different hydrodynamics of cultivation methods.

Finally, concerning to the most favourable sea cultivation method, our experiments indicate that hanging rope culture has a better water motion, however, it has the disadvantage of being low resistant to wave action than horizontal rope culture. Moreover, hanging rope culture is technically easier to assemble since the tension of culture rope with weights does not require having a much tensioned culture raft structure. In general, hanging rope culture is best suitable for coastal areas where there are low wave action (i.e. sheltered sites) as would be the inner zones of semi-closed bay (e.g. Rias), though horizontal rope culture is better adapted to coastal areas where there is moderately but not excessively strong wave exposure as might be at the mouth of the bay or inlet. Recently, Buck and Buchholz (2004) has described a suitable method, in open ocean, known as the offshore-ring, which supports fairly exposed sites with rough conditions [2].

CONCLUSIONS

In this study, we evidenced that the cultivation method affected blade features of *Saccharina latissima* in addition difference significant to area, biomass and "substantiality value" were found. The blade area was greater in horizontal rope culture than in hanging rope culture and in addition the biomass of blade was also greater, but it must be interpreted only to individual level. The "substantiality value" (measurement of the quality for human consumption) was greater in hanging rope culture than in horizontal rope culture. These values especially in hanging rope culture were similar to

the reported for *Saccharina japonica* cultivation, making it an excellent local analogous substitute for the Asian "kombu". The variation in morphological features of *S. latissima* blade seems that were caused by different hydrodynamics of both cultivation methods.

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TABLES

Table 1. Blade features (means ± SD) of *Saccharina latissima* in the cultivation with horizontal rope and with hanging rope during the reproduction periods, and significant differences in these characters between both culture methods analyzed by Student's *t*-test (n = 20 individuals in each period)

Blade features	Culture duration		Culture method		Student's *t*
	Measured*	days	Horizontal rope	Hanging rope	*P*-value
Blade wet weight	October 02	237	87.0 ± 33.4	69.5 ± 22.3	0.060
(g)	March 03	374	152.6 ± 52.0	99.7 ± 27.9	**0.000**
Blade length	October 02	237	80.6 ± 19.1	69.7 ± 13.4	0.044
(cm)	March 03	374	112.3 ± 22.3	103.7 ± 20.6	0.221
Blade width	October 02	237	19.2 ± 2.0	14.2 ± 1.9	**0.000**
(cm)	March 03	374	21.5 ± 3.3	13.0 ± 1.6	**0,000**
Blade area	October 02	237	0.16 ± 0.05	0.10 ± 0.02	**0,000**
(m^2)	March 03	374	0.25 ± 0.08	0.19 ± 0.03	**0,000**
"Substantiality value"	October 02	237	54.2 ± 8.7	70.9 ± 21.6	**0,004**
(mg cm^{-2})	March 03	374	62.5 ± 10.5	74.6 ± 14.6	**0.005**

*Blades of *S. latissima* were collected when it begin to develop reproductive tissues (sorus). Significant differences to a confidence level of *P*-value < 0.01 are in bold type

FIGURE CAPTIONS

Fig. 1. Location of the culture site (closed square) in the Ría de Ares y Betanzos, western coast of Galicia (N.W. Spain)

Fig. 2. Schematic representation of the horizontal rope culture (a) and hanging rope culture in garland-type (b)

FIGURE 1

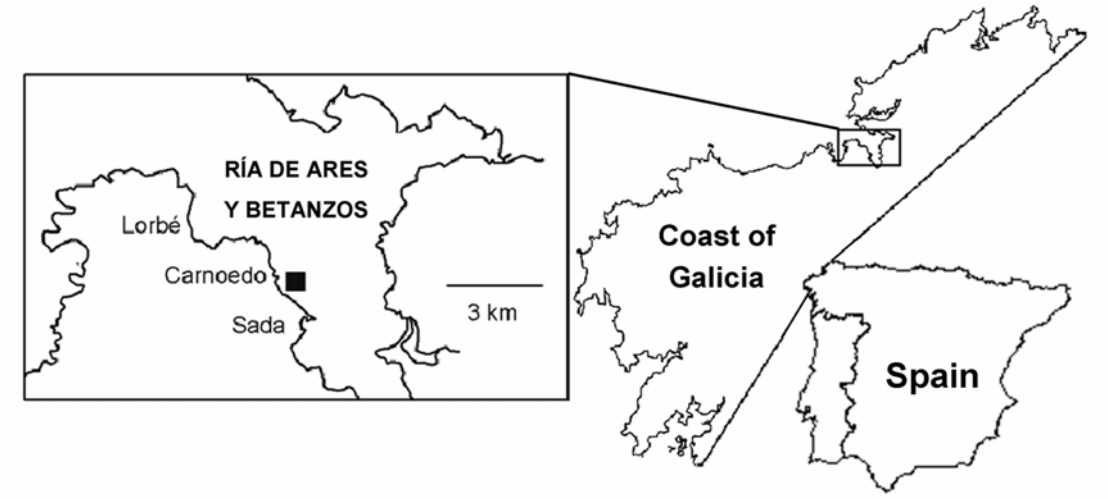
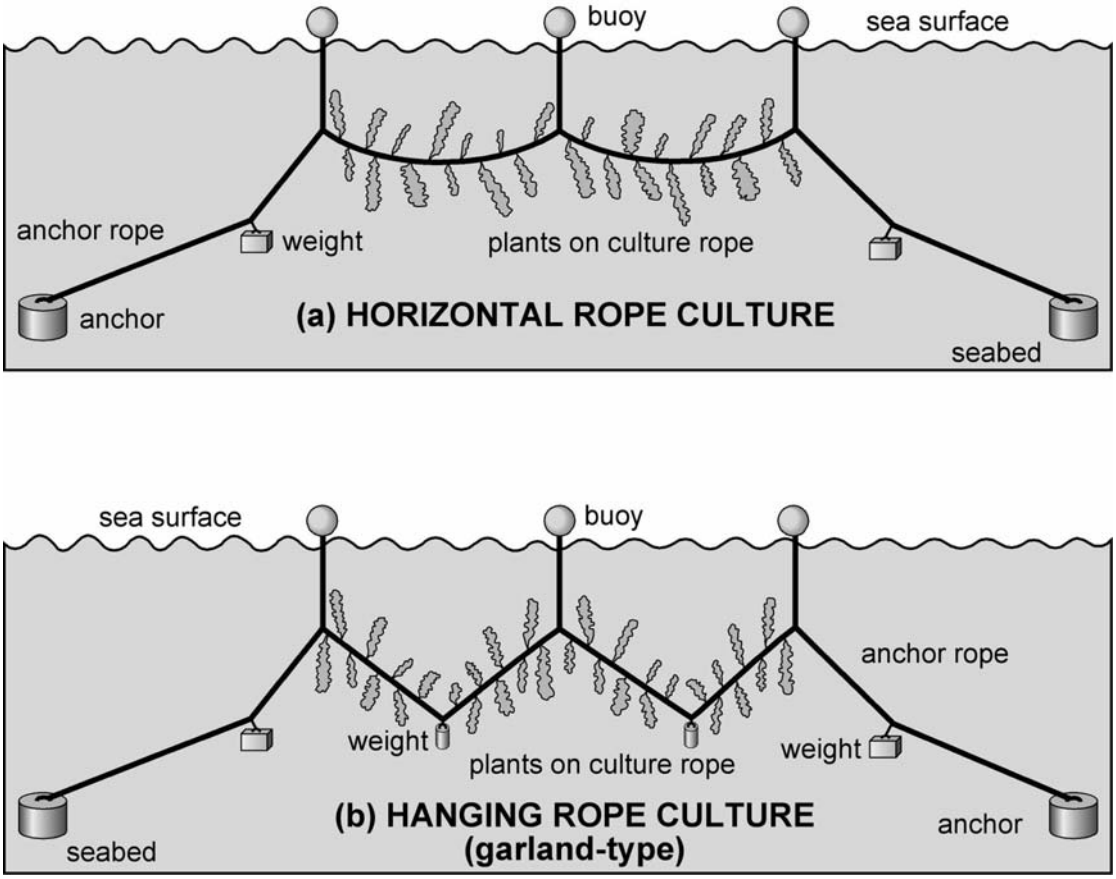

RÍA DE ARES
Y BETANZOS
Lorbé
Carnoedo
Sada
3 km
Coast of
Galicia
Spain

FIGURE 2


buoy
sea surface
anchor rope
weight
plants on culture rope
anchor
(a) HORIZONTAL ROPE CULTURE
seabed
sea surface
buoy
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plants on culture rope
weight
seabed
(b) HANGING ROPE CULTURE
(garland-type)
anchor