Cah. Biol. Mar. (2011) 52 : 435-439

Fast sporophyte replacement after removal suggests banks of latent microscopic stages of *Laminaria ochroleuca* (Phaeophyceae) in tide pools in northern Portugal

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Abstract: This study investigated the effects of a physical disturbance consisting of the removal of adult kelps (*Laminaria ochroleuca* Bachelot de la Pylaie) and their corresponding understorey turf assemblage in tide pools in northern Portugal. Vertical plots of 0.25 m^2 were scraped in April 2007 in independent tide pools and were monitored one (May) and four (August) months after removal. A rapid sporophyte development was observed in the disturbed plots, attaining mean densities of 40 and 82 young sporophytes.m^{-2} one and four months after disturbance, respectively. Mean blade lengths in August reached 14.97 ± 7.10 cm. The observed recruitment seemed to be the result of a (resumed) development of microscopic forms present on the substrate in a state of suspended growth. These findings suggest the existence of a bank of microscopic forms capable of replacing populations subject to severe physical disturbances, such as dislodgement by winter storms. Whether such a microscopic bank is mainly composed by spores, gametophytes or early sporophyte stages remains unknown.

Résumé : *Le rapide remplacement de sporophytes après éradication suggère l'existence d'une banque de formes microscopiques chez* Laminaria ochroleuca *(Phaeophyceae) dans les cuvettes du Nord Portugal.* Cette étude analyse les effets de la suppression de laminaires adultes (*Laminaria ochroleuca* Bachelot de la Pylaie) et de leur communauté de macroalgues associée dans des cuvettes du Nord du Portugal sur le développement de nouveaux sporophytes. En avril 2007, la roche a été grattée au sein de deux cadrats verticaux (0,25 m^2), chacun dans une cuvette distincte, qui ont été revisités un mois (en mai) et quatre mois après (en août). Un rapide recrutement de sporophytes a été observé dans les parcelles perturbées, avec un recrutement de laminaires atteignant 40 et 82 recrues.m^{-2} respectivement un et quatre mois après la destruction. La longueur moyenne des lames en août a atteint 14,97 ± 7,10 cm. Le recrutement observé semble résulter d'une reprise du développement de formes microscopiques présentes sur le substrat dans un état de dormance. Ces résultats suggèrent l'existence d'une banque de formes microscopiques capables de remplacer les populations de sporophytes soumises à de graves perturbations physiques, comme l'arrachage des thalles adultes résultant des tempêtes hivernales, bien que la forme exacte de cette banque microscopique reste inconnue.

Keywords: Recruitment ● Bank of microscopic forms ● *Laminaria ochroleuca* ● Kelp canopy ● Disturbance ● Life history

Reçu le 16 novembre 2010 ; accepté après révision le 1 avril 2011.
Received 16 November 2010; accepted in revised form 1 April 2011.

Introduction

Many macroalgal species rely on reserves of microscopic stages to compensate for harsh periods that may be responsible for temporal loss of the adult population (Kain, 1964; Dayton, 1985; Hoffmann & Santelices, 1991; Blanchette, 1996; Kinlan et al., 2003; Carney & Edwards, 2006). Kelp beds (Orders Laminariales and Tilopteridales) are known for being extremely dynamic, as evidence from both descriptive and experimental studies indicates high temporal and spatial variability (Dayton et al., 1992 & 1999; Reed et al., 2006; Bartsch et al., 2008) and occur in environments that may be subject to extreme variability in essential resources (light, nutrients and rock space) and other important limiting factors (e.g. temperature, herbivory). Unpredictable changes result in frequent and drastic reductions in population density whose extent of recovery depends on the reproductive strategy of the species. Kelps demonstrate a heteromorphic life cycle in which microscopic life stages (either haploid or diploid) seem to be more tolerant to stressful conditions than the macroscopic diploid adult stage (Hoffmann & Santelices, 1991). By delaying the development of microscopic stages to adult populations until favorable conditions occur, they enhance the likelihood of a successful sporophyte recruitment. This delayed recruitment has been inferred as the consequence of the time lag between the production of zoospores and the macroscopic recruitment observed for several macroalgal species (Dayton, 1985; Blanchette, 1996; Edwards, 2000). The microscopic stages are thought to remain in the substrate in a dormant stage and are likely to form banks of microscopic forms, responsible for the recovery of sporophyte's populations after disturbances once their development is resumed from specific environmental cues. The exact abiotic factors that regulate the survival and development of these microscopic stages are unclear as well as which microscopic stage (zoospore, gametophyte, or embryonic sporophyte) is responsible for recruitment delay. Nonetheless, it is widely recognized that the existence of these forms can act as a reserve that constitutes an invaluable potential for recovery after disturbances (Kennelly, 1987; Blanchette, 1996; Kinlan et al., 2003). These banks of microscopic forms have aroused considerable attention as they might determine macroscopic recruitment and population demography and have been compared to terrestrial seed banks (Hoffmann & Santelices, 1991; Edwards, 2000). However, the information regarding these stages has been limited by the difficulties of studying them in the field, mostly due to their microscopic size (Schiel & Foster, 2006). The kelp communities on the Portuguese coast have been poorly documented (Ardré, 1970; Pérez-Ruzafa et al., 2003) until recent global mapping efforts (Assis et al., 2009), which documented a decline of kelp communities in Portugal over recent decades. This awareness strengthens the need for understanding the role of microscopic life stages that determine macroscopic recruitment.

We studied the recruitment response of a population of *Laminaria ochroleuca* Bachelot de la Pylaie 1824 in tide-pools in northern Portugal (Esposende) to a severe physical disturbance over a period of four months.

Materials and Methods

The experiment and observations were conducted in Esposende (S. Bartolomeu do Mar, 41º34'25.97"N-8º48'0.81"W, northern Portugal), which is located in a natural park (Parque Natural do Litoral Norte). The sites were located on an intertidal zone comprising variable-sized tide pools where the predominant kelp is *L. ochroleuca*, and where the kelp *Saccorhiza polyschides* dominates the subtidal zone below. The adult sporophytes in this zone occur in a somewhat scattered pattern inside the tide pools and their density is therefore variable.

Two independent vertical plots of 0.25 m^2 (Q1 and Q2) inside different rocky tide pools (density of sporophytes in each sampled plot was of 8 and 4 individuals.m^{-2}, respectively) were totally scraped during low tide with a chisel in April 2007 and monitored for kelp recruitment in the following months of May and August of the same year. The tide pools exhibited an irregular shape, with a maximum depth of 1.0 and 1.5 m, and a width varying from ~ 0.5 to 1.0 m. The material removed inside the plots comprised sporophytes of *L. ochroleuca* and the dense turf-forming assemblage of macroalgae beneath their canopy. The sporophytes that were removed in each plot were the only existing sporophytes in each tide pool.

All the observed recruits ($\geq$ 0.5 cm) inside the scraped plots were counted and measured in length of the blade in May and August, except for one of the plots in the month of May, where it was only possible to obtain the total count of recruits (due to the tide conditions in this period).

Size distribution frequencies were tested for normality using the Kolmogorov-Smirnov test ($\alpha = 0.05$).

The scraped material was sorted in the laboratory and no recruits were found among the macroalgal assemblage. It is important to mention that the blades of the removed sporophytes did not have visible reproductive structures (sori). A large section of each tidepool was used to study population demography of *L. ochroleuca* at the same time and this information was used to compare natural sporophyte recruitment outside the cleared experimental plots.

Results

One month after the induced disturbance, macroscopic recruits (small blade stages with a minimum length of 0.5 cm) were visible inside the disturbed plots, in a mean density of 40 recruits.m^{-2} (Table 1). The mean density of recruits doubled from May to August, but this was mostly due to a very strong density increase in plot 1 (Table 1). Over the same period, the mean length of recruits exhibited about a seven-fold increase (Table 1). Size-frequency distributions of *L. ochroleuca* recruits were normally distributed (Kolmogorov-Smirnov normality test, $p > 0.200$) and showed conspicuous differences in population structure between the months of May and August, indicating not only growth of the blades but also further recruitment during the sampling interval (Fig. 1).

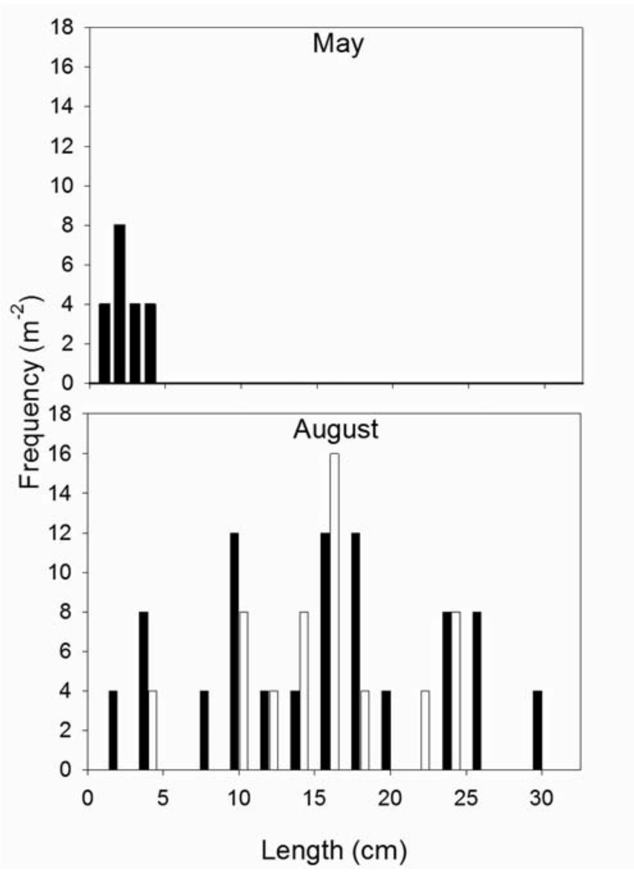

Figure 1. *Laminaria ochroleuca.* Size-frequency distribution of recruits in two plots Q1 (black bars) and Q2 (white bars). Measurements for Q2 in May were not performed due to the tide level. N = number of recruits for the size interval.

Figure 1. *Laminaria ochroleuca.* Distribution des fréquences de tailles des recrues dans les deux quadrats Q1 (barres noires) et Q2 (barres blanches). Les mesures pour Q2 en mai n'ont pas été effectuées en raison du niveau des marées. N = nombre de recrues pour l'intervalle de taille concerné.

Table 1. *Laminaria ochroleuca.* Total densities (m^{-2}) and mean length (cm) of recruits inside the scraped plots. Q1 and Q2 represent 0.25 m^2 vertical plots inside two different tide pools.

Tableau 1. *Laminaria ochroleuca.* Densités totales (m^{-2}) et longueur moyenne (cm) des recrues à l'intérieur des parcelles grattées. Q1 et Q2 représentent les parcelles (cadrats) verticales de 0.25 m^2 à l'intérieur de deux cuvettes rocheuses intertidales.

	Density		Length	
	Q 1	**Q 2**	**Q 1**	**Q 2**
May	20	60	2.02 ± 1.29	-
August	92	72	14.91 ± 7.95	15.04 ± 5.86

During the entire study period, no recruitment of *L. ochroleuca* sporophytes was observed outside the disturbed plots, where the turf-forming assemblage of macroalgae and adult individuals of *L. ochroleuca* were left intact. Some Chlorophyta (*Ulva* sp.) were also observed to recruit into the plots but considerably less than *L. ochroleuca* individuals.

Discussion

A rapid replacement of the removed kelp population occurred through a successful recruitment of *L. ochroleuca* individuals. As no reproductive individuals were present during the removal, it seems likely that the microscopic stages that gave rise to the rapid visible sporophyte recruitment were already present under the extirpated adults. Although it would theoretically be possible that recruitment could originate from outside the studied tide-pools, we consider this unlikely because a) no comparable recruitment occurred in natural areas followed for demography, b) in other tide pool kelps recruits arise mainly from the canopy directly above them (Santelices et al., 1995), and most of all because c) removal took place before the onset of the natural recruitment season. The sporophytes from the tide pools did not exhibit any reproductive structures, and the reproductive season of *L. ochroleuca* in this region lasts from April-May to November-December (when sori become visible, T. Pereira, personal communication). Even if any neighbouring subtidal sporophytes were already reproductive at the time of the study, it seems highly unlikely that these could have contributed to the observed recruitment in May, because they would need to be releasing spores whose germination would originate the microscopic forms (i.e., completed the cycle between spores to gametophytes, gametes, zygotes and early sporophytes) in the time period between the removal and the occurrence of the first visible recruits. This suggests that the microscopic forms must have been established during the previous reproductive season. The time lag between the end of the reproductive season and the visible recruitment points towards a period of quiescence, during which microscopic forms remain in a state of suspended growth until their development is triggered.

Survival of microscopic stages in the field has been reported to be short for several kelp species (Hsiao & Druehl, 1973; Devinny & Volse, 1978; Deysher & Dean, 1984 & 1986; Hoffmann & Santelices, 1991; Edwards, 2000), but the relatively long-lasting reproductive season of *L. ochroleuca* might allow a prolonged and relatively continuous replacement of the microscopic stages that make up the bank.

The observed development of the kelp recruits was rather fast, compared to other studies in which observable kelp juveniles started appearing two months after canopy removal (Santelices & Ojeda, 1984; Blanchette, 1996). The observed rapid development of sporophyte recruits can be attributable to microscopic gametophytes and/or sporophytes. Although both gametophytes and microscopic sporophytes could constitute the bank of miscroscopic forms, some authors (Hoffmann & Santelices, 1991; Carney & Edwards, 2006) emphasized the role of the gametophytes. In contrast, Kinlan et al. (2003) argued that it is mainly the embryonic sporophytes that make up the microscopic bank. Furthermore, other studies found no evidence of the importance of banks of microscopic forms (Reed et al., 1988). In our case, the exact life-history stage that composes the bank of microscopic forms responsible for the population recovery remains undetermined. The precise environmental cue triggering the recruit development is also obscure. The most obvious cue seems to be the change in light quantity and quality, as light intensity, composition and photoperiod have been found to play determinant roles in the development of several life-history stages of different species of *Laminaria* (Hsiao & Druehl, 1973; Lüning & Neushul, 1978; Lüning 1980 & 1981). The removal of adult sporophytes and their understorey assemblage of macroalgae may, in fact, represent an increased availability of light to the bare substrate and set off spore germination, gametogenesis or development of young sporophytes. Although microscopic sporophytes could have been present at the moment of canopy removal and formed the source of new sporophyte recruitment, the gametophyte is more frequently reported as the probable microscopic stage arresting development and building up a bank of microscopic forms (Hsiao & Druehl, 1973; Dayton, 1985; Blanchette, 1996; Ladah et al., 1999; Edwards, 2000). Temperature and nutrients are also important factors contributing to the development of microscopic stages (Hoffmann & Santelices, 1982), although these are difficult to correlate with canopy removal.

The relationship between macroalgal longevity and the persistence of banks of microscopic forms is not established, as the evidence thus far for the existence of these banks comes both from annual and perennial macroalgal species (Santelices et al., 1995 & 2002). To date, little is known about demography of *L. ochroleuca* and many of its life history traits such as age to fertility, life expectancy, growth patterns and reproduction are assumed to be similar to *Laminaria hyperborea* (Birkett et al., 1998). As a perennial species, the investment made into a reserve of latent microscopic forms can be related to persistence in a variable environment. In the studied Atlantic coast, the severity of physical disturbances is high due to the frequency and intensity of storms that dislodge kelps by water motion, resulting in significant canopy losses. The existence of a bank of latent microscopic forms of potential recruits allows to compensate for such canopy losses when environmental conditions improve. Persistence of such banks for perennial species like *L. ochroleuca* might perhaps be adjusted to the periods of increased storm probability. The association between the effects of seasonal disturbances such as winter storms and heteromorphic life histories has been proposed (Clayton, 1988) and is thought to be the result of adaptation to a variable environment.

The observations of this study suggest the existence of a microscopic bank of latent recruitment stages in *L. ochroleuca,* in populations situated in intertidal pools. Recruitment from such a bank can play a crucial role in the dynamics of kelp populations, preventing local extinctions caused by severe disturbances and deserve further investigation regarding quantification, origin, composition and survival in the field. These findings would greatly improve the knowledge and understanding of kelp ecosystems and allow for better management practices.

Acknowledgements

This study was funded by the Ecokelp project (ANR 06 BDIV 012, France) and FCT (Portuguese Science Foundation) and FEDER. AHE was supported by FCT scholarship SFRH/BPD/63703/2009.

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