

Trophic and biotic interactions in *Laminaria digitata* beds: which factors could influence the persistence of marine kelp forests in northern Brittany?

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Abstract: In this paper, we first reviewed the trophic ecology studies carried out on Laminaria digitata beds, at two rocky areas of Northern Brittany (France), Batz Island and Ar Pourven, displaying contrasted ecological conditions. The general trophic structure did not vary between the two sites, with a wide diversity of filter-feeders and predators, and only 14% of grazers. The results of stable isotope analyses allow drawing a simplified model for the food web associated to L. digitata beds. Kelp-derived organic matter is channeled into two distinct trophic pathways, the particle feeding-based and the grazing-based, which are coupled with higher trophic levels. The L. digitata detritic pathway contributes most to the diet of filter-feeders inhabiting sheltered sites. Although detritus feeding seems to be the main pathway transferring matter from kelps to the upper trophic levels, we showed that the direct grazing of kelps by herbivores can be locally important. Indeed, the gastropod *Helcion pellucidum* based exclusively his diet on the consumption of L. digitata and therefore its grazing pressure was studied on L. digitata sampled in two sites of Bay of Morlaix. In both sites, the herbivory pressure was significantly higher on reproductive adult plants than juvenile algae and the distribution of grazing damages showed strong variations along L. digitata thalli. To study the specific algal responses against herbivory, a laboratory-controlled system was established using L. digitata / H. pellucidum model. Defense-related gene transcripts were monitored in grazed tissues of L. digitata, showing the induction of molecular responses upon grazing. Altogether, the functioning of kelp-associated food web and the contribution of L. digitata are likely to be affected by two important factors, hydrodynamism and nutritive values of the food source. However, our results suggest that specific induced defense responses upon grazing and chemical interactions in natural environment could also interfere with trophic links in kelp beds.

Résumé : Interactions trophiques et biotiques des peuplements à Laminaria digitata : quels facteurs peuvent réguler la persistance des forêts de laminaires en Bretagne nord ? Dans cette article, nous avons tout d'abord établi une synthèse des analyses du réseau trophique associé à ces grandes algues brunes dans deux sites rocheux situés sur la côte Nord de la

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Bretagne, l'Ile de Batz et Ar Pourven, qui présentent des caractéristiques écologiques contrastées. La structure générale de ce réseau ne varie pas entre les deux sites étudiés, avec une grande diversité de filtreurs et prédateurs, et seulement 14% d'herbivores. Les résultats d'analyses isotopiques ont permis d'établir un schéma simplifié du réseau trophique associé aux laminaires. Celui-ci se divise en deux voies alimentaires distinctes: l'une particulaire dérive de la décomposition des algues alors que l'autre est fondée sur leur broutage. Toutes deux sont couplées aux niveaux trophiques supérieurs. La contribution de la voie détritique dérivant de L. digitata est plus importante pour l'alimentation des filtreurs dans le site protégé. Bien que la consommation de ces particules détritiques soit la principale voie de transfert des laminaires vers les niveaux trophiques supérieurs, nous avons montré que la consommation directe des laminaires par des herbivores peut localement être importante. Ainsi, le gastropode Helcion pellucidum se nourrit exclusivement de L. digitata et nous avons donc étudié la pression de broutage de cet herbivore sur des laminaires récoltées dans deux sites de la Baie de Morlaix. Sur l'ensemble des deux sites, les algues adultes, présentant des sores, sont plus broutées que les juvéniles, et ceci de façon significative. Les herbivores se répartissent également de façon hétérogène le long du thalle. Afin d'étudier la physiologie de l'algue en présence de brouteurs, nous avons établi un système de broutage de L. digitata par H. pellucidum dans des conditions contrôlées de laboratoire. L'expression de gènes marqueurs des réactions de défense a été mesurée dans des tissus broutés ou non et a montré l'induction spécifique de réponses moléculaires suite au broutage. Globalement, le fonctionnement et la structuration du réseau trophique associé aux laminaires semblent être affectés par deux facteurs importants : les conditions hydrodynamiques des sites étudiés et les variations de la valeur nutritive des algues. Cependant nos résultats suggèrent que des réponses spécifiques de défense contre les herbivores ainsi que des interactions chimiques entre espèces pourraient aussi moduler les liens trophiques dans les champs de laminaires.

Keywords: Laminaria digitata • Kelp • Food web • Trophic interactions • Chemical ecology

Introduction

Kelp species of the genus Laminaria (Phaeophyceae) are one of the dominant brown algae of both Atlantic and Pacific rocky shores of temperate regions. They form ecologically important, extensive seaweed beds, also called kelp forests, which occur from the upper sublittoral fringe downwards (for recent review, see Bartsch et al., 2008). Worldwide, kelp associated communities have long been considered to be regulated by the strong top-down control exerted by sea-urchins, sometimes leading to total eradication of kelps and their associated fauna in high urchin density areas (Dayton, 1985). Such overgrazing of kelps has been reported in different places of the world coastal ocean, including European waters (Bartsch et al., 2008). It sounds however that L. digitata (Lamouroux) beds depart from this general paradigm. In Brittany, the most common urchins Echinus esculentus (Linnaeus, 1758), Sphaerechinus granularis (Lamarck, 1816) colonize habitats at a depth greater than the lowest limit of distribution of L. digitata (Arzel, 1998), thereby making unlikely any significant effect of grazing by these consumers on kelp populations. Surprisingly, in spite of their ecological importance along the coasts of North-Western Europe, until recently, no study addressed the issue

of trophic functioning of L. digitata-associated communities. For instance, although animal communities associated to L. digitata beds have been characterized in different European areas (e.g. Le Hir & Hily, 2005), the identity of species likely to consume L. digitata still remained unclear. Recently, studies aiming at understanding the trophic structure and functioning of these ecosystems have been carried out in Northern Brittany (Schaal et al., 2009, 2010a & 2011). One of the main findings of these studies was that the food web was based on a wide array of food sources, L. digitata clearly not being the dominant source sustaining the community (Schaal et al., 2010a). In fact, only one grazer species, the gastropod Helcion pellucidum (Linnaeus, 1758), consistently displayed a high trophic specialization of this kelp (Schaal et al., 2010a). Although other species (e.g. the topshell Gibbula cineraria (Linnaeus, 1758)) seemed to be able to occasionally rely on L. digitata, the main sources consumed by grazers in high exposure sites were red algae (e.g. Palmaria palmata (Linnaeus), Mastocarpus stellatus (Stackhouse), and microbial biofilms. Interestingly, an important spatial variability was observed in the functioning of the food web, L. digitata being consumed by a higher diversity of grazers in sheltered conditions (Schaal et al., 2009). This variability could be related to a higher

nutritive value of kelps in sheltered conditions (higher protein content, lower C/N ratio). Kelps detritus, however, represented an important source for suspension-feeders and deposit-feeders (until more than 90% of the diet). The predominance of the detritus pathway over the fresh kelps grazing pathway has been related to the effects of microbial degradation of kelps, which results in increased nitrogen content, as well as in decreased herbivore-deterrent secondary metabolites, in detritus (Tenore et al., 1984; Norderhaug et al., 2003). Hence, although the study of factors affecting the availability of kelps to their associated consumers still remain relatively poorly understood and deserve further attention, these preliminary results suggest a paramount role of biochemical aspects. In particular, the low diversity of grazers able to efficiently feed on kelps raises some issues about the suitability of kelps as a food source for grazers. Brown algae are often considered to be a poor food source for grazers because of the presence of herbivore-deterrent phenolic compounds in their tissues (Hay & Fenical, 1988). However, among northern Atlantic rocky shore-associated brown algae, L. digitata has one of the lowest content in phenolic compounds (Connan et al., 2004), suggesting that this alga relies on other defense mechanisms to prevent extensive grazing.

All plants, from benthic algae to terrestrial seed plants, are subject to attack by grazers. Pathogen-induced defenses in macroalgae are now well documented and reveal common features with innate immunity of plants and metazoans (Weinberger, 2007). Land plants have also evolved sophisticated responses to cope with herbivores, such as transcriptional reprogramming and emission of specific chemical volatile active compounds (Wu & Baldwin, 2010). In terrestrial ecosystems, these defensive plant traits are likely to structure the complex community associated with the plant (Poelman et al., 2008). In contrast, while predation has been considered as major factor in driving the dynamics of natural communities and populations of marine algae, little is known about molecular and chemical algal responses induced by grazers. Most of studies on herbivore-induced responses in algae are based on feeding preference assays looking at differences in palatability between grazer-exposed and control plants. In 2007, Toth & Pavia reported a meta-analysis based on several previous published data, suggesting that seaweeds, as land plants, can also sense and respond to woundingrelated cues and further develop resistance against herbivores. For instance waterborne compounds produced by the grazed brown alga Ascophyllum nodosum (Le Jolis) affect the population dynamics of herbivores and also predators (Toth et al., 2007; Coleman et al., 2007). It has also recently been suggested that resistance to herbivores may be induced in advance by waterborne cues and spread effectively throughout a Fucus vesiculosus (Linnaeus, 1753) belt (Haavisto et al., 2010). In filamentous red algae, brominated secondary metabolites seem to play an important role in feeding deterrence (Paul et al., 2006) and the susceptibility to herbivore varies between sex and lifehistory stages in *Asparagopsis armata* (Harvey, 1855) (Vergès et al., 2008). Therefore all these data strongly suggest that herbivore-seaweed interactions should play a direct role into the structuring of alga-based food web. Up to now, the induction of specific anti-herbivore defense in species of Laminariales has been only reported for *Saccharina japonica* (Areschoug) (as *L. japonica*, Molis et al., 2008), whereas kelps are known to protect themselves against oxidative stress generated by both abiotic and biotic factors (for review, Bartsch et al., 2008).

In this context, this paper reviews recent data and presents complementary results on both the animal communities inhabiting *L. digitata* forests and food web organization in these particular coastal ecosystems. Based on these results, a laboratory-controlled system was established and targeted gene transcripts were monitored in *L. digitata*, showing molecular defense responses induced by grazing. These data are discussed in terms of putative factors affecting food web functioning and of research perspectives for studying both the nature and the ecological role of kelp-herbivore interactions in their natural environment.

Materials and methods

Algal material for grazing laboratory procedures

Both *Laminaria digitata* sporophytes and *Helcion pellucidum* were collected from the surrounding shores of Roscoff (Brittany, France) and maintained at 14°C in running filtered seawater (FSW), in white light of 25 µmol photons m⁻² s⁻¹ and with a photoperiod of 10 h light and 14 h darkness. Squared pieces of tissues (5 x 5 cm) of *L. digitata* thalli were cut off, rinsed in FSW for 24 hours and independantly put in contact with 5 individuals of *Helcion pellucidum* for up to 5 days. For gene expression studies, five herbivores were put on young *L. digitata* (4-6 cm in length), and the algae were frozen in liquid nitrogen after 6, 12 and 24 hours, stored at -80°C until RNA extraction. Control sporophytes (without grazer) were submitted to the same treatment and each experiment was repeated 3-10 times.

Gene expression analyses

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR) analyses were conducted as described in Cosse et al. (2009). qRT-PCR expression analyses of genes were normalized to transcript levels of the actin reference gene in the same samples. This relative expression was then used to calculate fold changes upon grazing compared with controlled conditions. The 8 selected defense-related genes encode vanadium-dependent bromoperoxidase 1 and 3 (vBPO1, vBPO3), vanadiumdependent iodoperoxidase 1 and 3 (vIPO1, vIPO3), glucose-6-phosphate dehydrogenase (g6pd), 6-phosphogluconate dehydrogenase 2 (6pgd2), thioredoxin (trx), and glutaredoxin (grx).

Food web

An extensive list of macroinvertebrates and demersal fish species inhabiting Laminaria digitata beds was built based on repeated observations carried out during field work on three sites surveyed in the framework of the ECOKELP project: Ar Pourvern site (3°57.55'W-48°42.71°N), Duons (3°55.59' W-48°43.66'N) and Toull ar Zarpant (4°2.62'W-48°76.26'N). Those sites were representative of the diversity of abiotic conditions encountered in northern Brittany L. digitata beds (in term of wave exposure), and characterized by typical associated macroalgal communities (e.g. Fucus serratus (Linnaeus, 1753), Himanthalia elongata (Gray, 1821), and a wide array of understory Rhodophyta). All samplings were carried out between low water marks of neap and spring tides. Based on literature data concerning the trophic ecology of these species and recent food web studies based on stable isotope data (Schaal et al., 2009, 2010a & b & 2011), a general model of trophic functioning within L. digitata beds was built, emphasizing the major trophic links between taxa of different trophic levels, and the main factors that have been observed to affect these links.

H. pellucidum pressure in natural populations

The effect of *H. pellucidum* pressure was studied in two of the sites (Ar Pourven and Duons) sampled in area determined for the food-web analysis. At each location, kelp canopy was removed from haphazardly placed plots of 0.25 m² in spring (March) and autumn (September) of 2007 in order to follow species succession (see Engelen et al., 2011). A total of 18 plots were followed in Ar Pourven (7 eradicated in spring, 6 in autumn and 5 untreated controls) and 13 in Duons (5 eradicated in spring, 5 in autumn and 3 untreated controls). At the end of the experiment, in autumn 2009, all canopy was removed from all 21 plots. For each plot, the reproductive status (i.e., either mature or juvenile) and the presence/absence of H. pellucidum were recorded for each L. digitata individual. In addition, the location of the grazer in the different parts of the thallus (i.e., stipe, meristematic zone and/or blade) was noted in order to examine the extent of variation in grazing defense or grazing palatability within individual.

In order to test if algal defense varied during the kelp life

history, we assessed the difference of grazing pressure in reproductive adults and in juveniles. For each plot, the frequency of grazed adults was computed as the number of adults bearing *H. pellucidum* over the total number of adults. In addition, the frequency of grazed juveniles was calculated for each plot in the same way. Frequency of grazing was compared between age classes (juvenile and adult), taking into account the effect of canopy removal (Treatment) and variation between sites using a three way factorial model ANOVA:

Yijkn = μ +Ai + Bj + Ck + (AB)ij + (AC)ik + (BC)jk + eijkn (1) where Yijkn is the frequency of grazing of the nth individual sampled in the ith Site, exposed to the jth treatment type and belonging the k-th Age class. μ is the mean frequency of grazing, Ai is the fixed effect of the i-th Site, Bj is the fixed effect of the j-th Treatment, Ck is the fixed effect of the k-th age class. (AB)ij, (AC)ik and (BC)jk are respectively the interaction effect of 'site x treatment type', 'site x age class' and 'treatment type x age class' and eijkn is the error term.

The ANOVA was conducted using the general linear model procedure of MINITAB (version 13.2 MiniTab Inc. 1994, State College USA). Data were not transformed since they meet the homoscedasticity requirement of ANOVAs. Multiple comparisons of means were performed using the Tukey test.

Results

Animal diversity associated to Laminaria digitata and trophic functioning of the habitat

150 animal species were found within *L. digitata* beds (Table 1). In terms of diversity, the assemblage was dominated by filter-feeders (65 species) and carnivores (predators and scavengers, 48 species). The grazer trophic guild was represented by 21 species, while only 15 deposit-feeding species were found. Crustaceans (33 species, mainly amphipods and decapods) and molluscans (34 species, mainly gastropods) were the most diversified taxa.

The global trophic functioning of kelp-associated communities was characterized by the co-occurrence of two main pathways (Fig. 1). The first one is based on fresh primary production (macroalgae and biofilm), which is consumed by grazers (gastropods mainly), which form the dominant part of crustacean predators (decapods). The only species consistently relying on *L. digitata* is the gastropod *Helcion pellucidum*. Although other species (e.g. *Gibbula cineraria, Acanthochitona fascicularis* (Linnaeus, 1767)) appeared to be able to efficiently feed on kelps, this trophic link was clearly as temporally and spatially consistent as for *H. pellucidum*. In fact, most grazers primarily relied on



Figure 1. *Laminaria digitata*. General simplified scheme of food web structure in associated communities. The connections between the four trophic levels are based on published (Schaal et al., 2009 & 2010a) and unpublished data: the main trophic links (involving the highest number of species) are represented by thick arrows, while secondary links are represented by dotted arrows. Putative positive or negative factors affecting the trophic interactions are indicated by grey arrows and discussed in the text. POM = particulate organic matter (both sedimented and suspended).

Figure 1. *Laminaria digitata.* Schéma général simplifié de la structure du réseau alimentaire dans les communautés associées. Les connections entre les quatre niveaux trophiques sont basées sur des données publiées (Schaal et al., 2009 & 2010a) et non publiées: les principaux liens trophiques (incluant le nombre important des espèces) sont représentées par des flèches épaisses, tandis que les liens secondaires sont représentés par des flèches en pointillés. Les facteurs présumés positifs ou négatifs qui affectent les interactions trophiques sont indiqués par des flèches grises et discutés dans le texte. POM = matière particulaire organique (sédimentée ou en suspension).

red algae (*Palmaria palmata, Mastocarpus stellatus*) and biofilms (epilithic and epiphytic) for food. In term of diversity, this pathway only involves a minor part of the community associated to *L. digitata* (see Table 1). The pathway including most of the consumers is based on particulate organic matter (POM), this is mostly composed of macroalgae detritus. POM is consumed by suspensionfeeders and deposit-feeders, which represent most of the part of gastropods predators. Both pathways contribute to the diet of demersal fish (Norderhaug et al., 2005). The relative dominance of these two pathways appears to be largely controlled by hydrodynamism (Schaal et al., 2009), nutritive value (Schaal et al., 2009 & 2010a), and chemical factors (Bartsch et al., 2008).

In situ *surveys of* Helcion pellucidum *grazing impacts on L. digitata*

During the field surveys of the two sites of Duons and Ar Pourvern in the Bay of Morlaix, we noticed that *H. pellucidum* was the only gastropod that can be found in very high densities on kelp's fronds (as illustrated in Fig. 2A). These *in situ* observations were confirmed by isotopic

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Table 1. List of common animal species found associated to the kelp Laminaria digitata in Ar Pourven, Duons and Toull ar Zarpant.Tableau 1. Liste des espèces animales communes récoltées en association à l'algue Laminaria digitata à Ar Pourven, Duons et Toull ar Zarpant.

Trophic group	Taxa	Species	Trophic group	Taxa	Species
Suspension-feeders	Porifera	Clathirna coriacea			Ascidia mentula
		Dysisdea fragilis			Botryllus schlosseri
		Grantia compressa			Dendrodoa grossularia
		Halichondria panicea			Didemnum candidum
		Hymeniacidon sanguinea			Didemnum maculosum
		Leuconia gossei			Distomus variolosus
		Pachimatisma johnstonia			Fragarium elegans
		Phorbas fictitius			Lissoclinum argylense
		Phorbas plumosum			Lissoclinum perforatum
		Suberites domuncula			Molgula sp.
		Sycon ciliatum	Deposit-feeders	Annelida	Amphitrite edwardsii
		Tethya aurantium			Amphitrite gracilis
	Cnidaria	Aglaophenia pluma			Amphitrite sp.
		Aglaophenia sp.			Eupolymnia nebulosa
		Amphisbetia operculata			Nicolea venustula
		Campanularia hincksii			Platynereis dumerilii
		Plumularia setacea		Crustacea	Ampithoe ramondi
		Sertularella gaudichaudi			Anapagurus hyndmanni
	Annelida	Amphiglenia mediterranea			Athanas nitescens
		Pomatoceros triqueter			Dynamene bidentata
		Sabella pavonina			Elasmopus rapax
		Sabellidae sp.			Galathea squamifera
		Serpulidae sp.			Gammarus locusta
	~	Spirorbis spirorbis			Gammaridae sp.
	Crustacea	Balanus perforatus			Melita sp.
		Chthamalus montagui	Grazers	Crustacea	Idotea granulosa
		Elminius modestus			Idotea neglecta
		Jassa falcata		N C 11	Idotea pelagica
		Pisidia longicornis		Mollusca	Acanthochitona crinitus
		Porcellana platycheles			Acanthochitona fascicularis
	Mallagaa	Semibalanus balanolaes			Barleela unijasciata
	Monusca	Anomia ephippium			Callichtion septemvalvis
		Crassostrea gigas			Gibbula cineraria
		Hiatalla aratica			Gibbula umbilicalis
		Mytilus adulis			Haliotis tubarculata
	Bryozoa	Alexonidium hirsutum			Halcion pollucidum
	Dry020d	Alevonidium sn			hinhing striatus
		Ricellaria ciliata			Leptochiton asellus
		Celleporella hvalina			Leptochiton cancellatus
		Crisia denticulata			Leptochiton sp
		Crisia eburnea			Littoring obtusata
		Electra pilosa			Patella ullyssiponensis
		Escarella immersa			Patella vulgata
		Escaroides coccinea	Carnivores	Cnidaria	Anemonia viridis
		Flustrellidra hispida			Bunodactis verrucosa
		Membranipora membranacea			Urticina felina
		Microporella ciliata		Annelida	Aphroditidae sp.
		Schizoporella sp.			Harmothoe imbricata
		Turbicellepora avicularis			Harmothoe impar
	Echinoderma	ta Cucumaria sp.			Harmothoe sp.
		Ophiothrix fragilis			Lepidonotus clava
	Chordata	Applidium pallidum			Marphysa sanguinea
		Applidium punctatum			Perinereis cultrifera
			1		-

	Syllis hyalina Svllidae sp.		Nassarius incrassatus Nucella lapillus
Crustacea	Cancer pagurus		Ocenebra erinacea
	Liocarcinus arcuatus		Ocinebrina aciculata
	Liocarcinus holsatus		Onchidorodidae sp.
	Liocarcinus sp.		Trivia arctica
	Necora puber		Tricia monacha
	Nymphon brevirostre	Echinodermata	a Asterina gibbosa
	Pagurus bernhardus		Henricia sanguinolenta
	Palaemon serratus		Luidia ciliaris
	Paragnathia formica	Chordata	Centrolabrus exoletus
	Pilumnus hirtellus		Ciliata mustela
	Pirimela denticulata		Conger conger
	Synisoma lancifer		Crenilabrus melops
	Xantho incisus		Gobius paganellus
	Xantho pilipes		Labrus bergylta
Mollusca	Calliostoma zizyphinum		Lepadogaster lepadogaster
	Diodora graeca		Taurulus bubalis
	Lamellaria perspicua		

analyses showing that this small grazer effectively fed on L. digitata (Schaal et al., 2010a). The results of the ANOVA on the frequency of grazed algae are given in Table 2. The frequency of grazed algae was significantly different between sites - two times greater in Ar Pourvern (0.637 \pm 0.065) than in Duons (0.300 ± 0.066) - and among age classes - more than 3 times higher in Adults (0.777 ± 0.064) than in juveniles (0.212 ± 0.050) - but did not vary according to the type of treatment (canopy removal in spring or in autumn or untreated control). Therefore the data for the juveniles or adults were pooled per site, leading to the observation of grazing impact on 151 juveniles and 83 adults (234 in total) in Ar Pourven and 393 juveniles and 48 adults (441 in total) in Duons (Fig. 2B). Even if none of the interaction were significant, the interaction Site x Age Class was nearly significant (Table 2). The Tukey multiple comparison of means showed that frequencies of grazed juveniles were not significantly different between sites $(0.333 \pm 0.077 \text{ at Ar Pourven and } 0.133 \pm 0.039 \text{ at Duons}).$ In contrast, the adult plants were significantly more grazed than juvenile algae in both sites, but at Ar Pourven most of the reproductive algae (0.940 ± 0.025) featured Helcion damages on their thallus, whereas significantly lesser adults (0.467 ± 0.110) were grazed at Duons (Fig. 2B).

The distribution of *H. pellucidum* and/or grazing damages was also recorded along *L. digitata* thallus, which was divided in three main parts, the stipe, the meristem and the blade. At Ar Pourven the grazer distribution between juveniles and adult plants was similar, whereas it looked quite different between the two age groups at Duons (Fig. 2C). Globally, *H. pellucidum* was found on only on blades (29 and 38% in Ar Pourven and Duons, respectively) and on stipes (8 and 28%), or on both blades and stipes of

grazed algae (40 and 22%). The meristematic zone was rarely grazed, excepted when all the three parts featured grazing impacts. In fact thalli totally covered by H. *pellucidum* could represent up to 25% of the infested adult algae, as found in the highly impacted zone of Ar Pourven.

Preliminary results on L. digitata/H. pellucidum interaction studies

Even if the grazing of L. digitata by H. pellucidum was sometimes highly intensive, it probably rarely caused the death of adult plant since it was preferentially the distal reproductive part of the blade that was heavily grazed and not the vital parts of the plant (i.e; stipe and meristem). This apparently "non-lethal" and specific grazing interaction with H. pellucidum was therefore a model of interest to study the changes in L. digitata physiology upon grazing. We first established the best conditions for reproducing grazing effects on blades of young or adult L. digitata in laboratory. Following the first contact with H. pellucidum, clearest areas appeared where the small grazers fed on the blade. These grazed zones were clearly visible after 12 hours and progressively increased during the 5-days experiment (see inset, Fig. 3A). These grazed zones seem to correspond to epidermal and outer cortex removals, since no deeper injury appeared such as blade holes. To monitor the molecular algal responses upon grazing, we have followed the expression of 8 oligoguluronate-inducible genes, by qRT-PCR, in L. digitata plantlets grazed during 24-hours by H. pellucidum in laboratory (Fig. 3B). Six of the 8 tested genes (vBPO1, vBPO3, vIPO1, g6pd, 6pgd2, Trx) have shown a relative increase of their transcript number in grazed algae compared to control ones, and the strongest response (3- to 10-fold

Α В Frequency of grazed algae per age class С 1 □ Juveniles Adults 0.8 83 0.6 b 0.4 a b 48 151 0.2 а 393 0 Duons Ar Pourven С Meristen Stipe 90 Blade only Total number of grazed algae 80 Meristem only Stipe only 70 Stipe +Blade 60 Meristem +Blade 50 Stipe +Meristem 40 All algal parts 30 20 10 0 Juveniles Adults Juveniles Adults Ar Pourven Duons

changes) occurs between 12 and 24 hours after the first contact between herbivores and algae (Fig. 3B). In order to compare these results with environmental samples, we measured the expression level of these 8 genes in different parts of grazed *L. digitata*, collected during a low tide. The general trend is clearly different from the laboratory experiments and only 3 genes showed a stronger expression level in grazed areas compared to healthy tissues. This pattern was observed in the meristematic zone for vBPO1 and vBPO3 genes and in the stipe and the blade regions for 6pgd2 expression (Fig. 3C).

Figure 2. Laminaria digitata. In situ observations of Helcion pellucidum grazing effects. A. Example of L. digitata thallus highly infested by H. pellucidum at the Duons site. B. Occurrence of H. pellucidum on L. digitata sampled in two observation sites (Ar Pourven and Duons). The frequency of grazed algae (with grazers and/or grazing mark) was reported to the total number of sampled individuals (indicated on each bar diagram) according to age class. Adults are L. digitata sporophytes with sores and juveniles represent all the non fertile algae. Data sharing a letter were not significantly different according to multiple comparisons of means performed using the Tukey test. C. Distribution of H. pellucidum over L. digitata thalli sampled in two observation sites (Ar Pourven and Duons). For each age class, the total number of grazed algae was classified according to the localization of either grazers or grazing marks along the thallus.

Figure 2. Laminaria digitata. Observations in situ sur les effets de broutage d'Helcion pellucidum. A. Exemple d'un thalle de L. digitata fortement infesté par H. pellucidum sur le site de Duons. B. Présence d'H. pellucidum sur L. digitata échantillonné dans deux sites d'observation (Ar Pourven et Duons). La fréquence des algues broutées (avec brouteurs et/ou une marque de broutage) a été rapportée au nombre total d'individus échantillonnés (indiqué sur chaque diagramme) en fonction de la classe d'âge. Les adultes sont les sporophytes de L. digitata avec des sores et les juvéniles représentent toutes les algues non fertiles. Les données présentant la même lettre ne sont pas significativement différentes d'après le test statistique de Tukey de comparaisons multiples des moyennes. C. Distribution d'H. pellucidum le long des thalles de L. digitata échantillonnés dans deux sites d'observation (Ar Pourven et Duons). Pour chaque classe d'âge, le nombre total d'algues broutées a été classé selon la localisation des brouteurs ou des margues de broutage le long du thalle.

 Table 2. Results of ANOVA on the effects of site, treatment

 and age classes on the frequency of grazed L. digitata.

Tableau 2. Résultats d'ANOVA sur les effets du site, du traitement et des classes d'âge sur la fréquence de broutage de *L. digitata.*

Source of variation	Degree of Freedom	Mean Squares	F	P-values
Site	1	1.42376	21.58	< 0.001
Treatment	2	0.08776	1.33	0.273
Age class	1	3.52651	53.45	< 0.001
Site x Treatment	2	0.12353	1.87	0.164
Site x Age class	1	0.26124	3.96	0.052
Treatment x Age class	2	0.10232	1.55	0.222
Error	52	0.06598		
Total	61			



Figure 3. *Laminaria digitata.* Set up of grazing laboratory-controlled experiments with *Helcion pellucidum* following defense gene expression. **A.** Increase of grazed surface on *L. digitata* co-incubated with *H. pellucidum*. The grazed surface were quantified by measuring paler grazed area reported to darker un-grazed tissues using imaging analysis (relative unit), during two independent experiments. Data are means of $n = 10 \pm SD$ and an example of grazed *L. digitata* tissues after 5 days is presented as inset. **B.** Relative changes in expression levels of 8 defense-related genes (see the full names in the Material and methods section) in young plantlets of *L. digitata* grazed by *H. pellucidum* in laboratory-controlled conditions. Data are means of $n = 3 \pm SD$. **C.** Relative changes in expression levels of 8 defense-related genes to un-grazed areas of *L. digitata* directly sampled and frozen on the shore. Data are means of $n = 10 \pm SD$.

Figure 3. *Laminaria digitata.* Mise au point d'une expérience de broutage contrôlée en laboratoire par *H. pellucidum* par suivi de l'expression de gènes de défense. **A.** Accroissement des surfaces broutées chez *L. digitata* co-incubé avec *H. pellucidum*. Les surfaces broutées ont été quantifiées par le rapport entre les zones broutées plus claires et le reste du thalle plus foncé en utilisant l'analyse d'images (unité relative), au cours de deux expériences indépendantes. Les données sont la moyenne de 10 mesures \pm SD et l'encart présente un exemple de tissu de *L. digitata* brouté pendant 5 jours. **B.** Changements d'expression relative de 8 gènes de défense (voir les noms complets dans la section Matériel et Méthodes) chez de jeunes plantes de *L. digitata* broutées par *H. pellucidum* en conditions contrôlées de laboratoire. Les données sont la moyenne de 3 expériences \pm SD. **C.** Changements d'expression relative de 8 gènes de défense de défense dans des tissus broutés de *L. digitata* en comparaison à des tissus non broutés, échantillonnés et directement congélés sur la grève. Les données sont la moyenne de 10 mesures \pm SD.

Discussion

As deduced from isotopic analyses, kelps can be transferred in food web through two principal pathways, direct grazing or detritus feeding. Overall, the different results of this study, i.e. low diversity of grazers compared to other trophic guilds, only one species consistently feeding on kelps and active defenses of kelps against grazing, suggest that L. digitata associated communities are not controlled by top-down regulation. The fact that most of kelp production enters food webs through the detritus pathway also supports the absence of top-down control. Although detritus feeding sounds to be the main pathway transferring matter from kelps to the upper trophic levels, it seems that the direct consumption of kelps by herbivores could be locally and punctually important. The functioning of this particular food web and the contribution of L. digitata are likely to be affected by two important factors, hydrodynamism (Schaal et al., 2009) and nutritive value of sources (Schaal et al., 2010a), but preliminary results on L. digitata/H. pellucidum interactions suggest that others factors such as induced defense and chemical signaling could also interfere with trophic interactions in kelp beds (Fig. 1).

A well-documented example of activated defense in kelps is the oxidative burst in response to oligoalginates in L. digitata (for reviews Cosse et al., 2007; Weinberger et al, 2007). Alginates are the main polysaccharide components of brown algal cell walls. In fact, the capacity to recognize specifically oligoguluronates fragments and to react by the massive external production of reactive oxygen species was confined to alginate-rich taxa with complex thallus morphology, such as Laminariales (Küpper et al., 2001 & 2002). This chemical specific perception is likely to mimic an external damage that could be due to biotic attack by a pathogen or a grazer and the timescale of defense responses spread from a few seconds (perception of the chemical elicitor) to several weeks for the establishment of an effective resistance (Table 3). The black-box of molecular defense regulations was starting to fill in with the identification of fifty up regulated genes in elicited L. digitata over a 24-hour time-course. These genes were related to oxidative stress responses, production of antimicrobial secondary compounds or cell wall strengthening (Cosse et al., 2009). This set of defenserelated genes provided the first molecular markers to explore the responses of L. digitata upon grazing by H. pellucidum.

Kelps as food source: a poor nutritional quality for direct consumers versus active herbivore deterrence

By comparing the sources consumed by grazers and their respective nutritive value (as assessed by their total organic

matter content, C/N ratios, lipids content and proteins content) it appears that grazers inhabiting kelp forests consume preferentially sources characterized by high protein content and low C/N ratios, and that lipid content plays a minor role in determining the palatability of a food source to grazers (Schaal et al., 2010a). Accordingly, L. digitata sounds to represent a source of low nutritive value for grazers inhabiting these ecosystems. The higher importance of L. digitata for grazers at Ar Pourven is in fact related to a higher nutritive value of this kelp on this sheltered site (higher protein content, lower C/N ratios) (Schaal et al., 2009), thus supporting the hypothesis that the nutritive value of the source seems to be a dominant factor determining herbivore food choice in L. digitata beds. Similarly, previous studies on other kelp species concluded that fresh kelps were not a suitable food source for most consumers owing to their low nitrogen content and the presence of herbivore deterrent secondary metabolites in their tissues (Duggins & Eckman, 1997; Norderhaug et al., 2003).

However, in situ surveys also strongly support a preference for adult L. digitata by grazers even in plots where removal treatments have increased the densities of juveniles (see Engelen et al., 2011). This clearly supports age- and tissue-specific consumption patterns of L. digitata by H. pellucidum (Fig. 2). This was already observed for tissues in several laboratory studies showing that older and reproductive blade parts were preferred to meristematic tissues, probably due to the relatively high polyphenol content of the latter (Bartsch et al., 2008). A recent report has also shown that the blade was the preferred part, without elemental composition differences among kelp parts (Molis et al., 2010). Our hypothesis is that the differences in H. pellucidum grazing pressures could be related to strong tissue- and/or age-specificity in induceddefense responses, as observed when various tissues of adult L. digitata sporophytes were compared in their response with oligoguluronates. The young blade tissues were the most reactive parts to elicitation (Küpper et al., 2001). Indeed, gene expression studies clearly showed that young plantlets of L. digitata were able to perceive and respond to grazers through activation of gene expression (Fig. 3), with a time lag when compared to oligoguluronates-elicitation (Cosse et al., 2009). Another physiological trait likely to explain the lowest infection of juveniles by H. pellucidum (Fig. 2) could be the level of iodine content, which can reach highest concentrations in young plants (Küpper et al., 1998) and which is likely to be involved into active defense processes in L. digitata (Cosse et al., 2009). In fact, kelps with sori were significantly lower in iodine concentrations than non-fertile plants (Ar Gall et al., 2004). Interestingly, the meristem of grazed algae featured the lowest infection pattern (Fig. 2C) and the

Table 3. Active defense responses following oligoguluronate elicitation in *Laminaria spp.*, from early perception to effective resistance.

Tableau 3. Réactions	de défense active	après l'élicitation	par des ol	igoguluronates	chez Lan	<i>ninaria</i> spp., d	e la percept	ion précoce à
la résistance établie.								

Defense step	Physiological responses	Time-scale	References
Attack perception	Chemical elicitor perception (Oligoguluronates)	a few seconds	Küpper et al., 2001 & 2002
Signal transduction	Pharmacological approaches : Kinases, phosphatases Phospholipases Ions Fluxes (Ca ²⁺ , K ⁺ , Cl ⁻)	1-15 min	Küpper et al., 2001 Cosse et al., 2009
Early responses	Reactive Oxygen Species I ₂ , Volatile Halo-Carbons Aldehydes	up to 1 h	Küpper et al., 2001 & 2002 Küpper et al., 2008 Goulitquer et al., 2009
Gene regulation	GST C5 epimerases ~ 50 upregulated genes (oxidative stress, antimicrobial compounds, cell wall strengthening)	~1-48 h	Hervé et al., 2008 Tonon et al., 2008 Cosse et al., 2009
Resistance	Decrease of the infection with the endophyte Laminariocolax tomentosoides	~1-2 weeks	Küpper et al., 2002

strongest expression of halogen-related genes (Fig. 3C) in natural conditions. In this context, a tradeoff between resource allocation to defense related to survival and growth during the first stages of development versus reproduction may also be hypothesized, as seen for different life-cycle stages in red algae (Vergès et al., 2008). Moreover, the grazing of the reproductive blades probably facilitates frond detachment and drifting and therefore might increase long distance dispersal.

Multiple and complex interactions with environment

The preliminary results on gene expression of grazed algae in laboratory-controlled conditions compared with *in situ* samples (Fig. 3) suggest that either external stimuli or abiotic stressors (low tide for instance) have stronger impact than grazing on the expression of selected genes. These data point out the need for specific molecular or chemical markers, to be used in natural populations for further functional studies on specific seaweed-herbivore interactions. The establishment of a laboratory-controlled grazing system with *L. digitata/H. pellucidum*, provides cues to better understand in one hand the constraints of this food selection for the mollusk and in the other hand the possible defense mechanisms which might be activated by grazing, in comparison with those activated by other inducers of stress responses.

In recent laboratory experiments, the "non-lethal" grazing impacts of a gastropod stimulated consumption by

isopods of L. digitata thallus (Molis et al., 2010). According to the authors, this pattern is better explained by indirect effect, such as a better accessibility, than by previous grazing-induced changes. Another study revealed that the induction of chemical anti-herbivore responses depends not only on the type of the grazers but also on the type of algae on which the grazer feeds (Molis et al., 2008). It seems obvious that the efficient induced responses upon grazing are combined and integrated into the global physiological response of the alga to all external stimuli in natural environment. Measurements in rock tide pools containing L. digitata detected the presence of a cocktail of volatile aldehydes (Goulitquer et al., 2009), alkenes (Broadgate et al., 2004) and halogenated compounds (Jones et al., 2009). The coastal environment provides kelps with a wealth of potential chemical signals. For instance, aldehydes produced by stressed L. digitata are potential signals to warn-off kelps and activate potential toxic compounds for herbivores, as shown in terrestrial environment (Dicke & Baldwin, 2010). It raises the question of the effects of these chemical compounds on anti-herbivore defense induction at the community level and for the kelp-associated food web functioning.

Towards functional ecology of trophic interactions in kelp beds

Together with poor nutritional quality for direct consumers (Schaal et al., 2008, 2010a & b) and with highly evolved

capacities to adapt their physiology to environmental stressors, natural populations of L. digitata seem to display an efficient response for resisting to grazers and other attackers. These properties may contribute to the persistence of L. digitata in the absence of external strong disturbances. Further work still remains to be carried out to both identify and test the main factors affecting the stability of this complex ecosystem with a spatio-temporal perspective. Bottom-up processes are linked to the abundance of the dominant food source supporting the food web, that is, kelp detritus. The study of interactions between kelps, associated-microorganisms and their potential competitors for space in this habitat would therefore provide promising insight for a thorough understanding of ecosystem functioning within European kelp forests.

Moreover, to understand the consequences of active algal responses in a community context, it is important to expand research from individual interactions to multispecies interactions. As already suggested by Toth & Pavia (2007), future investigations concerning induced defenses in marine algae would benefit from more complex hypotheses including the genetic and biochemical mechanisms, cost and constraints of defensive seaweed responses, as well as the effects of these responses on herbivores and whole food web. Using kelp-grazing experiments in laboratory-controlled conditions, largescale chemical and transcriptomic approaches could be developed to monitor both molecular and chemical responses of algae in a context of herbivory, and identify specific markers of grazing defenses. These new tools will be integrated into mesocosm experiments, in situ food web and ecological surveys to test the hypothesis of interactions between food web structuring and defense responses of kelps. Our data showed that the effect of canopy removal had no effect on the frequency of grazers when evaluated 24 months after the disturbance. However, the effect of disturbance such as harvesting probably affects differently the food web and kelp defense during kelp beds recovery. For a complete assessment of grazing effects on kelp population dynamics, grazing impacts on all life history stages, including meiospores and gametophytes should be included, together with both a spatial and temporal perspective in the studies of trophic and biotic relationships within kelp forests.

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