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Mosaic genetic structure and sustainable establishment of the invasive kelp *Undaria pinnatifida* within a bay (Bay of St-Malo, Brittany)

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Abstract: Macroalgae can represent a significant component of biological introductions in coastal areas. One such example is the Asian kelp *Undaria pinnatifida*, which has been both intentionally and unintentionally introduced throughout the world. The alga was first introduced in the Bay of St-Malo (English Channel, France) for cultivation in the early 1980s, where it subsequently escaped and established a number of spontaneous satellite populations in the wild. We here document the present-day distribution of *U. pinnatifida* in this bay based on a grid sampling design. The alga was observed in most of the surveyed sites (84%). To test if this broad distribution of *U. pinnatifida* could be at all associated with the annual supply of propagules released from the local farm, we evaluated population genetic diversity and connectivity throughout the bay using nine microsatellite loci. We detected limited gene flow that could not be attributed to any isolation-by-distance mechanism, or to habitat type. In further support of a lack of direct connection between farms and satellite populations, spontaneous populations in the open bay were strongly differentiated from farmed populations. Future studies should investigate environmental disturbance, including commercial and recreational nautical activities, which may promote further spread of this pioneer alga.

Résumé : Les macroalgues forment une composante importante des espèces introduites en zones côtières, comme en témoigne l'algue asiatique *Undaria pinnatifida* introduite intentionnellement et accidentellement à une échelle mondiale. Cette algue a été introduite pour la première fois dans la Baie de St-Malo au début des années 1980 (Atlantique, France) à des fins de culture d'où elle s'est échappée pour former des populations spontanées dans la baie. Utilisant une grille d'échantillonnage pour documenter sa distribution actuelle à l'échelle de la baie, l'algue a été observée dans la plupart des sites prospectés (84%). Cette large couverture spatiale n'est certainement pas due uniquement à des échappements annuels de propagules depuis la ferme présente dans cette baie. Pour tester cette hypothèse, la diversité génétique et la connectivité des populations établies dans la baie a été analysée avec 9 microsatellites. D'importantes limites aux flux de gènes ont été détectées sans relation avec la distance entre populations ou les types d'habitats occupés. En outre, les populations non cultivées sont génétiquement différentes des populations cultivées. Dans ce contexte, les facteurs à l'origine de perturbations environnementales, dont les loisirs nautiques et transports maritimes, devraient être l'objet d'une plus grande attention car pouvant faciliter l'expansion d'*U. pinnatifida*, une espèce pionnière.

Keywords: Biological invasions ● Diving survey ● Genetic diversity ● *Undaria pinnatifida* ● Anthropogenic pressure

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Introduction

Biological introductions strongly influence the long-term stability and natural evolution of ecosystems (see Molnar et al. (2008) for a review in marine ecosystems). Seaweeds represent a significant part of these introductions in marine systems. Williams & Smith (2007) reported 277 introduced seaweed species in the world. The number of records for introduced seaweeds varies among regions, which is partially explained by lack of species inventories and the loss of expertise in algal taxonomy (Williams & Smith, 2007; Lyons & Scheibling, 2009). In the North-East Atlantic, 79 seaweed introductions have been recorded. Among them is an emblematic marine invader, the Asian kelp *Undaria pinnatifida* (Harvey) Suringar.

Together with the well-known invasive alga, *Caulerpa taxifolia* (Vahl) Agardh, 1817, *U. pinnatifida* is the only seaweed on the Global Invasive Species Program's list of the "100 of the World's Worst Invasive Alien Species". This status can be partly explained by the extent and rapidity of its worldwide introduction: this kelp has established populations over a wide range of habitats, coastal areas and biogeographic provinces through various introduction vectors, from shipping to farming (Voisin et al., 2005). *U. pinnatifida* has also been named as one of the five most hazardous seaweeds in Europe by Nyberg & Wallentinus (2005).

The first record of *U. pinnatifida* outside its native range dates from 1971: it was accidentally introduced in Europe, possibly concomitantly to the deliberate introduction of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) in the Thau Lagoon (Mediterranean Sea). Following this accidental introduction, this edible kelp was deliberately introduced in Brittany (France) in the 1980s for cultivation. Soon after farms had been established, individuals escaped into the wild and formed satellite populations (i.e. non-farmed populations, here after called "spontaneous populations") in natural (rocky or mixed habitats at the low shore fringe) and artificial (e.g. marinas) habitats. This process has been well documented around Ushant Island and in the Bay of St-Malo (Castric-Fey et al., 1993; Floc'h et al., 1996).

The Bay of St-Malo is one of the first places where an *U. pinnatifida* farm was established in France (Perez et al., 1984; Castric-Fey et al., 1993; Floc'h et al., 1996). The farm was established in the early 1980s upstream of the dam located in the Rance estuary (Fig. 1). Castric-Fey et al. (1993) noted that the presence of *U. pinnatifida* in 1992 was concomitant to a '*population explosion in the Rance estuary'*. In addition, its presence in several locations in the open bay (downstream of the dam, Fig. 1) was documented in the early 1990s, roughly 10 years after its primary introduction (Girard-Descatoire et al.,1997; Fig. 1). The farm established in the Rance estuary may have been a source of propagules for the establishment of the spontaneous populations because *U. pinnatifida* individuals are harvested from the cultivation ropes after they have reached sexual maturity (and thus after massive spore release; Fletcher & Farrell, 1999). In addition, 'reeling in' the ropes may promote the spread of drifting thalli (unintentionally detached from the ropes). Field observations and molecular studies (Castric-Fey et al., 1993; Floc'h et al., 1996; Voisin et al., 2005) all point out that farming activities are most likely to be the primary vector of establishment of spontaneous populations in the wild.

In addition to records of primary settlement of spontaneous populations, other features make the Bay of St-Malo particularly interesting to study regarding the establishment of *U. pinnatifida*. First, a variety of suitable habitats coexist in and around the bay (natural or artificial habitats; marine to brackish waters). Field experiments and observations suggest that this alga tolerates a large range of environmental conditions although it has a preference for artificial substrates (like pontoons in marinas or buoys in natural habitats), disturbed habitats and substrates that are not densely covered by other algal species in shallow waters (Floc'h et al., 1996; Valentine & Johnson, 2003). *U. pinnatifida* is considered as an opportunistic and pioneering species able to colonise marginal habitats (Sliwa et al., 2006). Second, the Bay of St-Malo is a large megatidal bay with a mean tidal range of 12 m, the highest in Europe, which generates important currents that may facilitate the natural spread of *Undaria*. This alga has limited natural dispersal ability with spores and gametes that have a lifespan of only a few hours. However, high velocity currents may facilitate its spread including through the dispersal of drifting mature thalli (Forrest et al., 2000). Finally, the original *U. pinnatifida* farm is still running, whereas since the early 2000s *Undaria* is no longer being farmed anywhere else in Northern Brittany (except one farm located in the Bay of Morlaix, several hundreds of kilometres west of St-Malo). The Rance estuary farm may still be a source of spores and drifting thalli for the settlement of new individuals in previously colonised or new sites. However, given the semi-annual to annual characteristics of this alga in Brittany (Wallentinus, 2007; pers. obs.) and the low dispersal ability of this species, we did not expect the farmed populations to still be the main source of founding individuals or migrants for spontaneous populations except for those located close by. This hypothesis can be tested using a population genetics approach based on microsatellites already developed for *U. pinnatifida* (Daguin et al., 2005). Such analyses can also provide useful insights on the relative strength of genetic drift and migration, two important evolutionary forces.

In this context, this study sought (1) to map the present-day distribution of *U. pinnatifida* in the Bay of St-Malo

using a grid sampling design, thereby setting a baseline for future comparisons, (2) to assess genetic diversity and connectivity among patches in relation to original farm sources, and (3) to look for correlations between habitat types, near- and offshore locations and salinity gradients. These data and future surveys may provide useful information for coastal management options in controlling future establishment of *U. pinnatifida* populations.

Materials and Methods

Survey and population sampling

To examine the present-day distribution of *U. pinnatifida* in the Bay of St-Malo, we selected 37 sites (Fig. 1) representative of various natural and anthropogenic habitats. These sites were chosen based on a grid approach, using a mesh size of roughly 1-2 square nautical miles. Sites in natural environments were mostly shallow subtidal or intertidal rocky habitats with depths ranging from +1.5 to -10 m (Castric-Fey et al., 1993; Girard-Descatoire et al., 1997). Surveyed sites were selected according to these broad criteria which covered a large range of environments (with various levels of wave energy, currents, salinity, turbidity). Sites no. 18 and 19 are the two marinas of the city of St-Malo. Note that site # 20 is an old abandoned fish pool.

The dam built in 1963-1966 in the Rance estuary drastically modified the environment in the upstream portion of the estuary, in particular the tides (Bonnot-Courtois et al., 2002). This may influence the connectivity between populations located upstream and downstream of the dam. In addition, as the only farm established in the Bay of St-Malo is located upstream of the dam, populations located downstream of the dam might be differentially seeded by farmed populations. We thus examined and sampled populations in both areas. Upstream sites (sites # 32, 33 and 35-38, covering roughly 13 km²; Fig. 1) are in areas characterized by different salinity levels (30-34 for sites # 32-36, 20-30 for site # 37 and below 20 for site # 38, see Bonnot-Courtois et al., 2002). The 31 downstream sites were selected over a large area covering roughly 65 km². All sites were visited in May 2009 except sites # 18-20 and 23, which were visited in mid-March 2009. Except for these four sites, marinas and the farm, all observations and sampling were done by scuba-diving, each site being visited by two divers for 10-15 min. For each site, the geographical coordinates were recorded and entered into an ArcGIS layer (ESRI, ver. 9).

At every site, except when only few individuals were found, a small piece of blade tissue (5 cm²) was collected from at least 30 individuals. Tissue was dried in silica gel for future DNA analysis. In addition, we included a farmed population sample (site # 34 in Table 1 & Fig. 1) coming from the only farm (C-Weed company) where *Undaria* is cultivated in the Bay of St-Malo. For this sample, the sporophytes were sampled on ropes by the farmer. We also included 32 individuals from the "Biocéan" farm located in the Bay of Morlaix (North Brittany), which was, at the time of the sampling, the only other *Undaria* farm still in operation in Northern Brittany. The seedlings used in this farm in 2009 came from the farm located in the Rance estuary (P. Podeur, pers. com.). However, this second farmed population sample came from a different batch from the one sampled in St-Malo, thus being an additional sample of the population cultivated in the St-Malo farm.

DNA extraction and microsatellite genotyping

For each sample, 29-32 individuals were analysed. For each individual, total genomic DNA was extracted from 5-10 mg of dried tissue using the Nucleospin Multi96 Plant extraction kit (Macherey & Nagel) according to the manufacturer's instructions (excluding the recommended incubation step at 65°C) and diluted to 1:100 prior to PCR. Amplifications of 10 microsatellite loci were carried out according to the protocols described in Daguin et al. (2005) except that forward primers were labelled with ABI dyes and a multiplex procedure (*i.e.* the use of several pairs of primers within the same PCR) were used. Three multiplex PCRs were performed with the following combination of loci and dyes: (1) 4G2 (NED), 1C1 (PET), 1B5 (FAM), 4C12 (VIC); (2) 1B2 (NED), 1H5 (FAM), 1G2 (VIC), 4E9 (PET); and (3) 2C1 (FAM), 2E8 (VIC). Multiplex PCR products were diluted before being processed on an ABI 3130xl DNA sequencer: (1) 1:16; (2) 1:25 and (3) 1:100. In addition, taking advantage of different allele sizes, multiplexes 2 and 3 were pooled in equal proportion before being processed on the sequencer. Two microlitres of each multiplex (1) and (2+3) mixture was added to 5 µl of loading buffer containing 0.07 µl of size standard (GeneScan-500 LIZ) and 4.93 µl of Hi-Di formamide (Applied Biosystems). The loading mix was denatured at 92°C for 3 min and run in an ABI prism 3130XL DNA sequencer (Applied Biosystem) with POP7 polymer and 50 cm capillaries.

Genotypes obtained were scored using GENEMAPPER ver. 4 software (Applied Biosystems). Preliminary analyses showed important and consistent heterozygote deficiencies at the 4E9 locus when compared to all the other loci, suggesting null alleles: this locus was thus excluded from subsequent genetic analyses.

Statistical analyses of the microsatellite dataset

Genetic diversity and departure from random mating.Statistical independence across the nine study loci was verified using Fisher's exact test computed for all locus

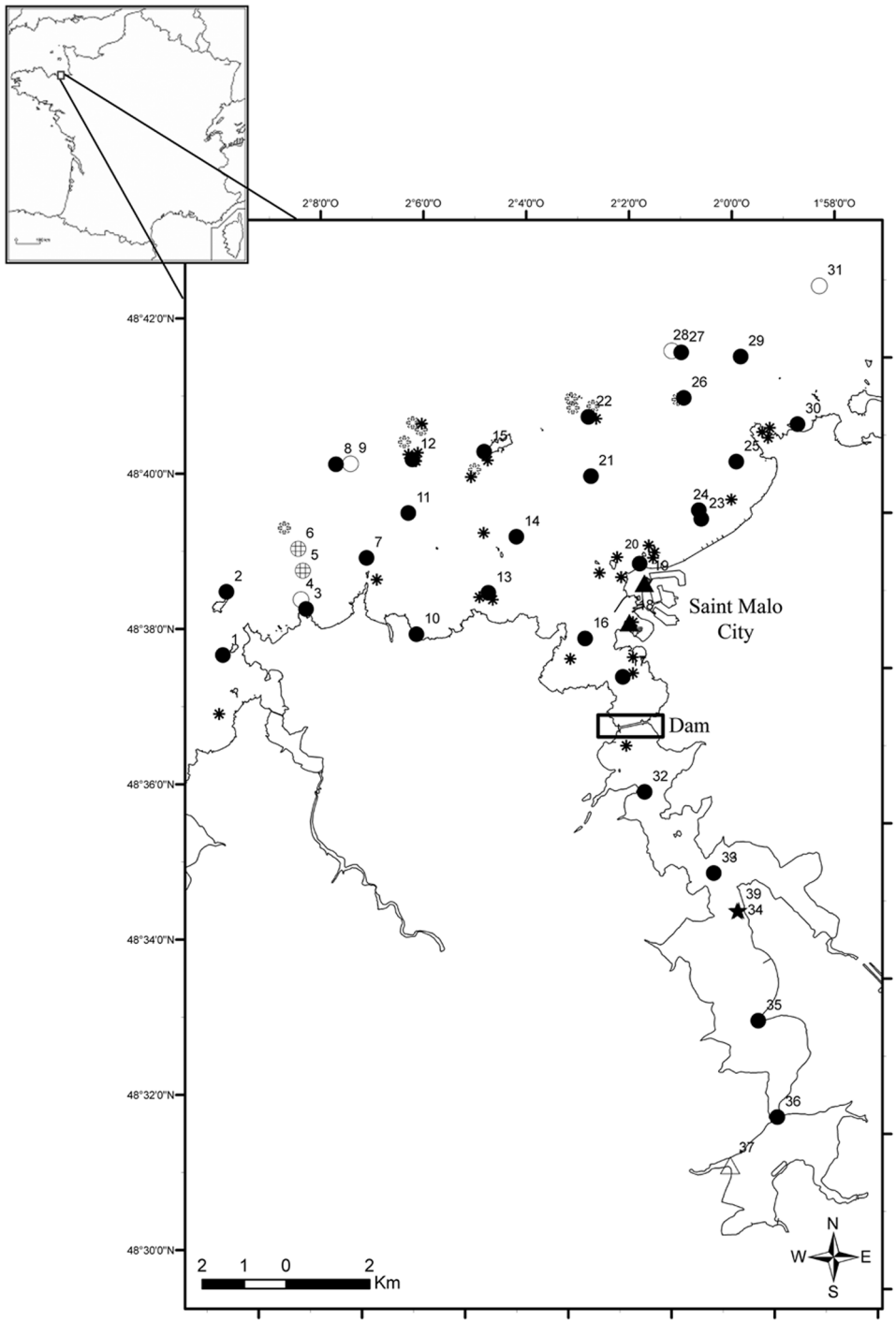

Figure 1. *Undaria pinnatifida.* Location of the 38 sites surveyed in the St. Malo area (spring 2009). Triangles, spontaneous populations in marinas; stars, farmed populations; circles, spontaneous populations in natural environments. Black: abundant, hatched: rare, unfilled: absent. Asterisks (black asterisk : presence, white asterisk: absence) refer to previous records reported in Girard-Descatoire et al. (1997).

Figure 1. *Undaria pinnatifida.* Localisation des 38 sites étudiés dans la Baie de St-Malo (printemps 2009). Les ports, la ferme et les sites en milieu naturel sont représentés respectivement par des triangles, une étoile et des cercles. Symbole noir : espèce abondante, hachuré : espèce rare, blanc : espèce absente. Les astérisques font référence aux données d'observations indiquées dans l'article de Girard-Descatoire et al. (1997) : astérisque noir indiquant la présence d'*U. pinnatifida* et blanc son absence.

Table 1. *Undaria pinnatifida.* Sampling and genetic diversity characteristics for 38 localities surveyed in 2009 in St-Malo's bay. For each site, its name (along with previous records, if any, see footnote (1)) is given with its geographic location and the type of habitat (F: farm, SM: spontaneous population in marinas, SW: spontaneous populations in wild environments). The number of individuals genotyped (N_{genot}) is provided with genetic diversity indices: the number of alleles (N_{all}), gene diversity (H_e) and heterozygote deficiency (f) with its associated probability (as determined from exact tests).

Tableau 1. *Undaria pinnatifida.* Echantillonnage et indices de diversité génétique des populations dans 38 sites prospectés en 2009 dans la baie de St-Malo. Pour chaque site, son nom (avec des indications d'observations antérieures, voir la note de bas de tableau (1)) est indiqué avec ses coordonnées géographiques et le type de population (F : populations cultivées, SM : populations spontanées de ports, SW : populations spontanées dans des milieux naturels). La présence et le nombre d'individus génotypés (N_{genot}) est indiqué avec les indices de diversité génétique suivants : le nombre d'allèles (N_{all}), l'hétérozygotie attendue (H_e) et un indice de déficit en hétérozygotes (f) avec la probabilité associée (test exact de déviation aux proportions attendues sous l'équilibre de Hardy-Weinberg).

ID	Sample site(1)	Latitude N	Longitude W	Habitat	N_{genot}(2)	N_{all}	H_e	f	P
1	Les Perronnias	48.62814	2.15783	SW	32	2.44	0.419	0.265	0.003
2	Ile Agot	48.64175	2.15783	SW	32	2.78	0.419	0.056	0.167
3	Plage Longchamp	48.63903	2.13177	SW	32	2.11	0.204	0.148	0.050
4	Pointe de la Garde Guérin	48.64099	2.13357	SW	A	-	-	-	-
5	Platier des Lardières	48.64725	2.13351	SW	R	-	-	-	-
6	Balise Nerput (nearby site: 1989, A)	48.65187	2.13540	SW	R	-	-	-	-
7	Moulin St Lunaire (nearby site: 1994, P)	48.65075	2.11309	SW	32	2.56	0.253	0.232	10^{-6}
8	Balise Buarats Ouest n°2	48.67046	2.12470	SW	32	2.33	0.276	0.095	0.315
9	Balise Buarats	48.67072	2.12004	SW	A	-	-	-	-
10	Pointe Bellefard	48.63498	2.09555	SW	32	2.67	0.387	0.069	0.253
11	Les Cheminées	48.66089	2.10038	SW	32	2.67	0.381	-0.028	0.763
12	Pierre des Portes (1993-1994, P)	48.67244	2.10003	SW	32	2.89	0.430	0.088	0.432
13	Le Rochardien (1992-1994, P)	48.64472	2.07301	SW	30	2.78	0.425	0.105	0.258
14	La Pierre Salée (nearby site: 1994, P)	48.64620	2.06675	SW	32	2.78	0.396	0.011	0.463
15	Le Grand Murier (1993-1994, P)	48.67498	2.07695	SW	32	2.78	0.396	0.027	0.687
16	Tonne (site nearby : 1990 P)	48.63609	2.04082	SW	32	2.89	0.360	0.178	0.029
17	Bizeux (nearby site: 1993-1994, P)	48.62830	2.02791	SW	32	2.78	0.355	0.160	0.091
18	Bas Sablons (1992-1994, P)	48.57413	1.98270	SM	29	3.00	0.414	0.094	0.522
19	Bassin Vauban	48.64862	2.02267	SM	32	2.44	0.306	0.479	10^{-6}
20	Fort National (1991-1993, P)	48.65281	2.02450	SW	32	2.89	0.374	0.179	0.001
21	Les Pierres Aux Normands	48.67101	2.04186	SW	32	2.67	0.372	0.008	0.484
22	La Grande Conchée (1994, P)	48.68371	2.04375	SW	32	2.78	0.372	0.030	0.505
23	Rocher Des Thermes (nearby site: 1994, P)	48.66314	2.00537	SW	32	2.89	0.410	0.117	0.021
24	Rocher de l'Aiguille (nearby site: 1994, P)	48.66498	2.00628	SW	NS	-	-	-	-
25	Le Grand Dauvier	48.67585	1.99503	SW	32	2.89	0.418	0.005	0.409
26	Les Petits Pointus (1990 A)	48.68892	2.01320	SW	32	2.56	0.362	0.027	0.869
27	Bouée Saint Servantine	48.69864	2.01485	SW	32	2.67	0.414	0.171	0.077
28	Saint Servantine	48.69883	2.01798	SW	A	-	-	-	-
29	Les Grands Pointus	48.69846	1.99550	SW	32	2.78	0.326	0.027	0.994
30	Plage du Val (nearby site : 1986-1994, P)	48.68463	1.97585	SW	32	2.78	0.396	0.017	0.932
31	Balise Rochefort	48.71452	1.97127	SW	A	-	-	-	-
32	Pointe de Cancavale (1990 P)	48.60387	2.01879	SW	32	2.78	0.423	0.131	0.663
33	Ile Aux Moines	48.58726	1.99499	SW	32	2.56	0.417	0.123	0.181
34	Cweed (established in 1983)	48.57952	1.98621	F	32	1.67	0.240	-0.158	10^{-6}
35	Pointe Garot	48.55609	1.97794	SW	32	3.00	0.434	0.145	0.085
36	Pont St Jean	48.53563	1.97007	SW	32	3.00	0.412	0.031	0.781
37	Port de Plouer	48.52469	1.98450	SM	A	-	-	-	-
38	Port de Lyvet	48.48944	2.00203	SM	A	-	-	-	-
39(3)	Biocéan-Roscoff	-	-	F	32	1.67	0.234	-0.069	0.921

(1) Previous records in the same site or in a nearby site indicated in Girard-Descatoire et al. (1997) are given in parentheses (year(s) of the observation and presence (P) *vs*. absence (A) of *U. pinnatifida*).

(2) When no (A) or only few (R) individuals were observed, the population was not sampled. At site 24, *U. pinnatifida* was abundant but not sampled (NS).

(3) In 2009, seedlings used in the Biocéan farm, located near Roscoff in the Bay of Morlaix, came from St. Malo (See Materials & Methods)

Table 2. *Undaria pinnatifida.* Output of AMOVA testing for the effects of population location or habitat on the genetic structure. F_{sc} and F_{ct} (and their associated *P* values) indicate the genetic differences among populations within groups and among groups, respectively. Significant values for group effects are underlined. Populations within each group ({populations in group *i*} vs. {populations in group *j*}) are indicated by population number as provided in Table 1 and Figure 1. SM, spontaneous populations sampled in marinas; SW, spontaneous population sampled in wild environments.

Tableau 2. *Undaria pinnatifida.* Résultats d'AMOVA testant les effets de localisation des populations et du type d'habitat sur la structure génétique des populations. F_{sc} et F_{ct} (et les valeurs de probabilités P associées) indiquent respectivement la différence génétique entre populations au sein des groupes ou entre groupes. Les valeurs significatives de ces estimateurs sont soulignées. Les groupes sont décrits de la façon suivante : ({populations du groupe *i*} vs. {populations du groupe *j*}) avec les numéros de populations tels qu'indiqués dans le tableau 1 et la figure 1. SM et SW correspondent respectivement aux populations échantillonnées dans des ports et dans le milieu naturel.

Effects	Definition of groups	F_{sc}	*P*	F_{ct}	*P*
"Location" effect					
Nearshore vs. Offshore	{1, 3, 10, 13, 16, 17, 20, 23, 25, 30} vs. {11, 12, 15, 21, 22, 26, 27, 29}	0.055	$<10^{-4}$	-0.001	0.603
Western vs. eastern locations	{1-3, 7, 8, 10-12} vs. {23, 25-27, 29, 30}	0.079	$<10^{-4}$	-0.006	0.915
Downstream vs. Upstream site - including farmed population(1) }	{1-3, 14, 16, 21, 27, 29-30 vs. {32-36}	0.097	$<10^{-4}$	<u>0.041</u>	<u>0.009</u>
Downstream vs. Upstream site without farmed population(1)	{1-3, 14, 16, 21, 27, 29, 30} vs. {32-33, 35-36}	0.067	$<10^{-4}$	0.001	0.405
SW populations only Downstream vs. upstream(1)	{1-17, 20-31} vs. {32-33, 35-36}	0.060	$<10^{-4}$	0.001	0.394
"Habitat" effect					
SM vs. Farms vs. SW(1)	{18, 19} vs. {34, 39} vs. {all other samples}	0.063	$<10^{-4}$	<u>0.105</u>	<u>0.001</u>
SM vs. SW(1)	{18, 19} vs. {1-17, 20-33, 35-36}	0.061	$<10^{-4}$	0.012	0.166
SM vs. SW-downstream(1)	{18, 19} vs. {1-17, 20-31}	0.067	$<10^{-4}$	0.010	0.206
SM vs. SW-upstream	{18, 19} vs. {32-33, 35-36}	0.026	$<10^{-4}$	0.026	0.070
Farms vs. SW(1)	{34, 39} vs. {1-17, 20-33, 35-36}	0.062	$<10^{-4}$	<u>0.169</u>	<u>0.003</u>
Farms vs. SW-downstream(1)	{34, 39} vs. {1-17, 20-31}	0.069	$<10^{-4}$	<u>0.178</u>	<u>0.003</u>
Farms vs. SW-upstream	{34, 39} vs. {32-33, 35-36}	0.036	$<10^{-4}$	0.137	0.062

(1) Same qualitative results were obtained with other subsets of populations located in the bay

pairs over all populations and for each population separately in Genepop ver. 4.0.7 (Rousset, 2008). Genetic diversity was analysed using the same software by computing the mean number of alleles per locus (N_{all}) and Nei's unbiased expected (H_e) heterozygosity. Deviation from Hardy-Weinberg equilibrium was tested within each population using the exact test as implemented in Genepop. The inbreeding coefficient (*f*) was estimated for each population and group with the same software. To adjust for multiple comparisons in the above-mentioned tests, the false discovery rate (FDR) was controlled: q-values were computed using the QVALUE routine in the R package (Storey, 2002). Parthenosporophytes can be obtained in laboratory. We checked for their presence in the study populations. Parthenogenesis in *U. pinnatifida* is characterized by a chromosome doubling of haploid female gametophytes. We computed the likelihood of multilocus homozygotes over the nine loci by using the software GenClone (Arnaud-Haond & Belkhir, 2007).

<u>Overall genetic structure and isolation by distance model</u>. The spatial distribution of genetic diversity was first analysed by calculating Weir & Cockerham's *F* statistics using the software FSTAT ver. 9.3.2 (Goudet, 1999). Deviation from zero was tested using a G-test (10 000 randomisations) and results were adjusted for multiple comparisons as described above. To picture the genetic relationship between all the study populations, a principal component analysis (PCA) on genetic data was carried out using the software PCA-GEN (http://www2.unil.ch/popgen/softwares/pcagen.html).

A null model in population genetics when dispersal is spatially limited is the isolation-by-distance (IBD) model. IBD predicts a correlation between geographical and the genetic distance for each pair of populations. Using a

Mantel test (10 000 permutations) implemented in the Genepop software, we tested this model by testing for the independence of two pairwise matrices (Rousset, 1997): one with an estimate of the genetic distance ($F_{st}/(1-F_{st})$) and the other with the natural log of geographic distance (the shortest distance along the coastline).

Clustering of the genetic diversity. To test for the significance of particular groupings, e.g. according to spatial location or habitats, an analysis of molecular variance (AMOVA) was conducted using Arlequin ver. 3.5 (Excoffier & Lischer, 2010). AMOVA partitions the total genetic variance into several variance components and two fixation indexes are estimated: F_{sc} and F_{ct} indicating the genetic differentiation among populations within groups and among groups, respectively. Significance levels of F_{sc} and F_{ct} were assessed by non-parametric permutation of genotypes (10 000 permutations). We tested if the overall genetic structure could be explained by the following effects (see Table 2 for a detailed definition of groups): (1) the location of populations upstream vs. downstream of the dam, (2) the location of nearshore and offshore populations, (3) the location of the populations along a east-west gradient, and (4) habitat type (i.e. natural habitat, marina or farm). For all these analyses, when an unbalanced number of populations per group was observed, we first used the whole data set and then repeated the analysis on a random subset of populations for the over-represented groups. The same qualitative results were obtained.

Given that *F*-statistics and AMOVA analyses operate at the population level and thus make strong assumptions about *a priori* grouping (Manel et al., 2005), we also used an individual-based clustering approach implemented in the software STRUCTURE ver. 2.3.3 (Pritchard et al., 2000). The clustering model attempts to find the number of clusters (K) that best explains the genetic data, assuming Hardy-Weinberg and linkage equilibrium within clusters and then estimates admixture proportions for each individual (i.e. the genetic membership of each individual to each cluster). We performed simulations using an admixture model with correlated allele frequencies. Using the CBSU server (available at http://cbsuapps.tc.cornell.edu/index.aspx), five independent runs (each with 5.10^4 iterations following a burn-in of 10^5 iterations) were carried out for each prior value of the number (K) of clusters, set between 1 and 30 (the maximum number of samples in the data set).

Finally, for detecting the proportion of individuals likely to originate from recent migrant individuals, we used assignment tests based on Rannala & Mountain's method implemented in the software Geneclass 2.0 (Cornuet et al., 1999). For each individual, a marginal probability of belonging to each of the putative source populations was computed, based on their allele frequencies. Each individual was then assigned to the source for which it obtained the highest probability. All sampled sites were considered as putative sources.

Results

Presence of U. pinnatifida in the Bay of St-Malo

U. pinnatifida was present at 31 of the 37 sites (84%) surveyed in the Bay of St-Malo (Table 1 & Fig. 1). Where present, *U. pinnatifida* was abundant in every site but sites # 5 and 6 (where only a few individuals were observed) and non-destructive sampling from each of 30-40 individuals could be performed within 10 minutes. For the six sites where not a single individual of *U. pinnatifida* could be found, one was likely to be out of the species' depth range (# 28) and two (# 37 & 38) where in the most estuarine (i.e. low salinity level) part of the area surveyed.

Genetic diversity at the population level

We obtained multilocus genotypes for 955 *U. pinnatifida* (see N_{genot} in Table 1) corresponding to 30 sites: (1) 28 sites where *U. pinnatifida* was abundant and (2) two farms. Of the 36 tests for linkage disequilibrium performed for each pair of loci over all populations, 10 locus pairs were significant after FDR correction. At the population level (1080 tests with 240 not done due to lack of polymorphism), 26 were significant after FDR correction (15 populations with one to five locus pairs showing disequilibrium). However, no pairs of loci were found to be in linkage disequilibrium in every population.

Over the total dataset, the number of alleles varied across loci (from two for locus 4C12 to six for loci 1B2 and 2C1), with an average of 3.78 ± 1.39 alleles per locus. The mean number of alleles across loci per population was 2.65 ± 0.33 across populations. The mean number of alleles per population was variable with the lowest value (1.67) in the two farms and the highest (3.00) in two populations near the Rance estuary farm (# 35 & 36) and one of the two study marinas (# 18). The expected heterozygosity (*i.e.* gene diversity) was similar across populations (0.35 to 0.44, Table 1).

Only seven out of 30 populations showed a significant departure from Hardy-Weinberg equilibrium (HWE) suggesting that reproduction is generally panmictic. For six of the seven populations with HWE deviations, the observed heterozygote deficiencies only occurred for one to three loci out of the nine analysed. A Walhund effect (i.e. coexistence of genetically differentiated sub-groups in a sampled site) was thus the most likely explanation. Conversely, in one population (the "Bassin Vauban" marina, site # 19), a very

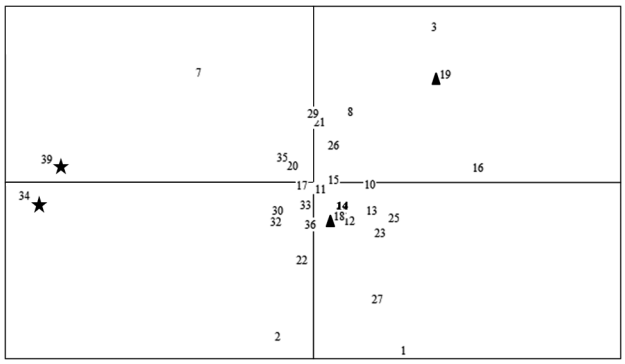

Figure 2. *Undaria pinnatifida.* Output of a PCA analysis on genetic data illustrating the genetic distances between populations in the St-Malo area. Axis 1 represents 42.85% of the genetic variance ($P < 10^{-4}$) and axis 2 represents 13.03% of genetic variance ($P = 0.992$). The populations are numbered as indicated in Table 1 and Figure 1. The two marinas (# 18 & 19) and the two farm samples (# 34 & 39) analysed are indicated by triangles and stars, respectively.

Figure 2. *Undaria pinnatifida.* Résultat de l'ACP sur données génétiques illustrant les distances génétiques entre populations dans la Baie de St-Malo. L'axe 1 représente 42,85% de la variance génétique ($P < 10^{-4}$) et l'axe 2, 13,03% ($P = 0,992$). Les populations sont numérotées comme présenté dans le Tableau 1 et la Figure 1. Les deux ports (n°18 & 19) et les deux échantillons cultivés (n°34 & 39) sont indiqués respectivement par des triangles et des étoiles.

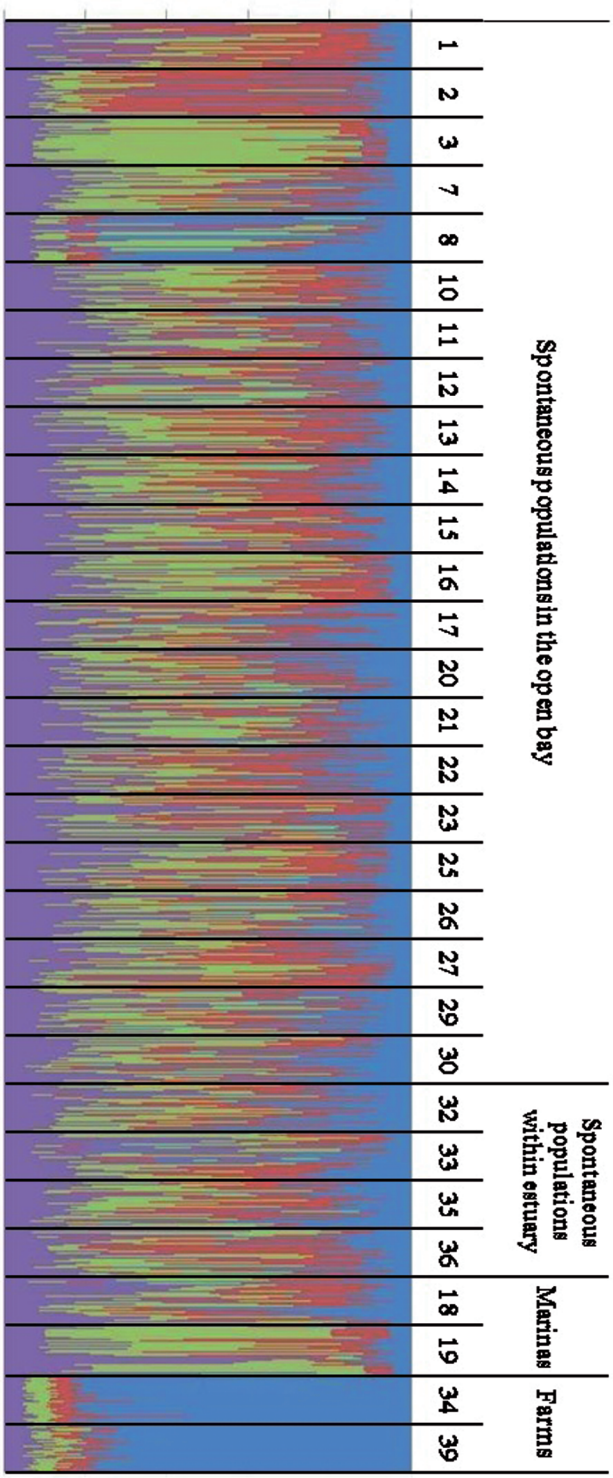

Figure 3. *Undaria pinnatifida.* Results of the clustering analysis performed with the software Structure. Four clusters were identified. Each cluster is represented by a different colour. Individuals are ranked according to the location where they were sampled (population number and name). Each population is separated by a thick black line. Each individual is represented by a thin vertical line divided into *k* (1 to 4) different coloured segments which give the individual membership to each of the four identified clusters.

Figure 3. *Undaria pinnatifida.* Résultat de l'analyse par regroupement réalisée avec le logiciel STRUCTURE. Quatre groupes ont été identifiés. Chaque groupe est représenté par une couleur différente. Les individus sont ordonnés par localités (le numéro et le nom de la population est indiqué en haut). Chaque population est séparée par une ligne noire. Chaque individu est représenté par une fine ligne verticale composée de *k* (1 à 4) segments de couleur différente représentant l'appartenance de l'individu à chacun des quatre groupes identifiés.

high heterozygote deficiency was observed ($F_{is} = 0.479$, $P < 10^{-6}$) and supported by seven of the nine loci suggesting inbreeding. Regarding the likelihood of parthenogenesis at the population level, only three multilocus genotypes were found to be homozygous at every locus. Only one, found in "Bassin Vauban", was repeated (three times) and unlikely to be produced by sexual reproduction only. Partheno-sporophytes may thus occur in this population.

Genetic structure in the Bay of St-Malo according to a priori population definition

Significant genetic structure was observed among the 30 study sites ($F_{st} = 0.099$, $P < 10^{-6}$). Among 435 pairwise comparisons (between populations), only 33 did not show significant genetic differentiation. In Figure 2, the two-dimensional PCA shows the relative genetic distance among the study populations and highlights the genetic isolation of the two farm samples (# 34 & 39). Interestingly, the two marinas were genetically differentiated. One marina population (# 18) was found to be genetically similar to many spontaneous populations sampled in wild habitats whereas the other marina population (# 19) was isolated from almost all other samples.

When analysing a north-south transect along the estuary (sites 14, 15, 16, 17, 32, 33, 35, 36), no isolation-by-distance pattern was observed (Mantel test, $P = 0.500$). The same holds true when considering the 20 sites located downstream of the dam (i.e. the open bay, Mantel test, $P = 0.112$). The genetic distance between populations was therefore not related to their geographic distance.

Results of the AMOVA are provided in Table 2. Neither distance to the shore nor location in the western vs. eastern part of the bay could explain the overall genetic structure. Conversely, we detected a slight "farm effect". For instance, significant structuring was observed when comparing downstream to upstream populations, including the farms (# 34 & 39, # 39 coming from St-Malo as detailed in the Material and Methods section). However, this structuring was no longer observed when the farmed populations were excluded from the analysis (*cf.* rows 3 and 4 in Table 2). The same pattern was observed when testing directly for a "habitat effect". Significant structuring was initially observed among groups ($F_{ct} = 0.105$, $P = 0.001$), but this "habitat" effect was no longer significant when comparing only marinas and populations established in natural habitats ($P = 0.166$) either over the whole study or when considering only the upstream ($P = 0.206$) or the downstream ($P = 0.070$) areas. Conversely, the farmed populations and spontaneous populations established in natural habitats were genetically different. When splitting these spontaneous populations into two subsets according to their upstream or downstream location, the farmed populations were not significantly different from spontaneous populations established upstream in the Rance estuary ($F_{ct} = 0.137$, $P = 0.062$), whereas farmed populations were found to be different from spontaneous populations established in the open bay. This result was supported by pairwise comparisons: the mean F_{st} value was 0.280 when comparing the farm in the Rance estuary with any of the spontaneous populations sampled in natural habitats from the open bay, whereas it dropped to 0.187 when comparing the farmed populations and the spontaneous populations established in the upstream part of the Rance estuary.

Individual clustering and assignment methods

The Bayesian clustering analysis indicated that the most likely number of "true populations" (i.e. panmictic units) over the whole study sites was four. Most individuals were admixed with variable proportions of ancestry to each of the four clusters (Fig. 3). The two farmed populations showed a very different pattern from all other samples with a high probability of every individual belonging to one cluster (blue colour in Fig. 3). Population no. 7 also showed a particular pattern compared to the other non-farmed populations: 59.4% of individuals showed a very high proportion of their genome typical of the cluster characteristic of the farm samples (individuals with a large proportion of blue colour). Populations # 2 and 3 showed a specific pattern: # 2 displayed a high proportion of genetic membership to one given cluster (in red) and # 3 with another cluster (in green).

Assignment tests showed that the proportion of individuals assigned to their sampling locality (*i.e.* self-assignment) was variable, ranging from 3.13% for populations # 23 and 30 to 81.25% in population # 3 (Fig. 4). In most of the populations, a large range of putative sources was found and this pattern was particularly strong for the populations located in the eastern part of the bay (# 23-30) and upstream of the dam (# 32-36, excluding the farm # 34). The farm, # 34, was distinctive as all individuals were self-assigned (i.e. the highest membership probability was observed in the farm). Interestingly, the individuals not assigned to the site where they were sampled were not preferentially assigned to a neighbouring population, showing that there was little effect of the spatial distance on the genetic similarity between populations.

Discussion

The primary introduction of *U. pinnatifida* in Europe and, in particular, along the Atlantic and Channel coasts is due to seaweed farming activities, with its deliberate introduction in Brittany (Castric-Fey et al., 1993; Fletcher

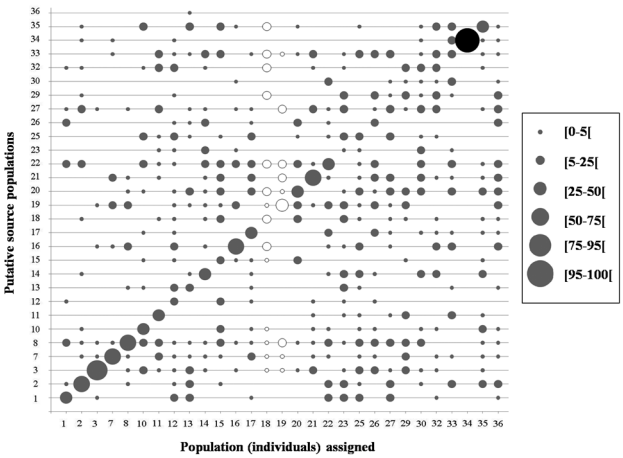

Figure 4. *Undaria pinnatifida.* Assignment test for individuals sampled in 29 sites in the St-Malo area. For each population (x-axis), the percentage of individuals assigned to one of the putative sources (y-axis) is represented by circles whose sizes correspond to different frequency categories as indicated in the right panel. Putative sources used were the same 29 samples. The populations are numbered as indicated in Table 1 and Figure 1. Samples from marinas, farms and spontaneous populations established in the wild are pictured by white, black and grey circles, respectively.

Figure 4. *Undaria pinnatifida.* Assignation génétique des individus échantillonnés dans 29 sites de la baie de St-Malo. Pour chaque population (axe des abscisses), le pourcentage d'individus assigné à une des sources potentielles (en ordonnée) est indiqué par des cercles représentant différentes proportions telles que décrites dans le panneau de droite. Les sources potentielles utilisées sont toujours le même ensemble de 29 localités. Les populations sont numérotées comme indiqué dans le Tableau 1 et la Figure 1. Les ports, la ferme et les sites en milieu naturel sont représentés respectivement par des cercles blanc, noir et gris.

& Farrell, 1999). This origin was later confirmed by molecular analyses (Voisin et al., 2005). The first survey of *U. pinnatifida* in the Bay of St-Malo, a decade after its initial introduction (Castric-Fey et al., 1993), revealed the occurrence of populations in several locations at various distances from the established farm. These populations were located in rocky habitats with limited overlap with the distribution of native kelps (e.g. *Laminaria digitata* (Hudson) J.V. Lamouroux, 1813, *Sacchoriza polyschides* (Lighfoot) Batters, 1902). In the absence of plausible alternative pathways, the most likely explanation put forward was the escape from farms as already documented in other bays in Brittany, e.g. Ushant Island (Floc'h et al., 1996) and Aber Wrac'h (Voisin, 2007). In the 2000s, recreational divers reported that *U. pinnatifida* was well-established in many diving sites in the Bay of St-Malo (Alain Cabioch, pers. com.). The lack of dedicated surveys nevertheless hampers our ability to analyse temporal changes in percentage cover or local density of these spontaneously established populations in the Bay of St-Malo. This study therefore now provides a baseline for future surveys of the spatial distribution of *U. pinnatifida* in the bay.

Almost every site (31 out of 37) where we looked for the presence of *U. pinnatifida* was found to be colonised by this kelp. Spontaneous populations were present over a large range of environments: intertidal, shallow subtidal, habitats made of rock, boulders or mixed-sediments, more or less exposed sites, fully marine to moderately brackish waters, human-made structures (marinas, buoys, etc.). The establishment of *U. pinnatifida* over a large range of habitats has been observed in other regions where it has been introduced (e.g. in New Zealand: Russell et al., 2008). *U. pinnatifida* is an opportunistic and pioneering species (Sliwa et al., 2006). This characteristic, combined with its ability to colonise various habitats and its tolerance to a large set of environmental conditions, has been cited as a reason why the introduction of *U. pinnatifida* has been so successful worldwide (Wallentinus, 2007 and references therein).

Spontaneous populations were found distributed over the whole bay. This observation fits with the pattern described 15-20 years before our survey (in 1986-1989 and 1990-1994) by *L'Association pour la Découverte du Monde Sous-marin* (ADMS) (a former regional naturalist diving association) and reported by Girard-Descatoire et al. (1997). Despite the lack of ecological data that would have allowed a qualitative assessment of the long-term evolution of the presence of *U. pinnatifida*, the comparison of our field observations and those of 20 years ago suggests that *U. pinnatifida* is well-established in the Bay of St-Malo. In addition, several populations observed during our survey in 2009, had been by chance already reported in the early 1990s by Girard-Descatoire et al. (1997), in the exact same site or in nearby sites (Table 1 & Fig. 1). Native *U. pinnatifida* populations have discrete annual cycles, whereas some introduced populations can be found year-round (e.g. New Zealand, Brittany) with more than one reproductive cycle per year. For instance, two discrete recruitment events have been observed in California (Thornber et al., 2004) and in Northern Brittany (see for example, Castric-Fey et al. (1999) for a study in the St-Malo area and Voisin (2007) for a study in two marinas in Brittany). Thus ca. 40 generations have transpired in the Bay of St-Malo since the surveys reported in Girard-Descatoire et al. (1997), highlighting the successful establishment of *U. pinnatifida* throughout the bay. Despite the above-mentioned limitations in terms of ecological data, our results suggest that this Asian alga is now a permanent and common member of the kelp community in the Bay of St-Malo.

Although farming activities initiated the spread of *U. pinnatifida* in the open Bay of St-Malo, it is unlikely that

they are the sole contributors to the rapid expansion documented in 1992 over several tens of kilometres, or to the annual renewal of the spontaneous populations found throughout the bay. Spontaneous populations far from the farming areas (in the Rance estuary) were indeed among the first to be recorded in natural habitats: at Pointe de la Varde (in 1986, Castric-Fey et al., 1993), which is near population # 30 in Fig.1. and in offshore locations (in 1992 at Huppée, Découverte, Courtis: Girard-Descatoire et al., 1997) near population # 12 (Fig. 1). The same holds for the present-day situation: our genetic data suggest that farming activities probably do not continue to play a major role in the annual renewal of the spontaneous populations. Farms were indeed genetically distinct from most of the spontaneous populations. Although based on different theoretical assumptions and statistical methods, every genetic analysis (assignment tests, Bayesian clustering, AMOVA and PCA based on *F*-statistics) demonstrated a significant genetic difference between the two farmed populations compared to the spontaneous populations. Rapid genetic divergence seems to have occurred between the farmed and spontaneous populations since the primary introduction in 1983, nearly 30 years ago (i.e. roughly 60 generations). This divergence may have been driven by two processes: (1) the reproductive season in farmed and spontaneous populations does not fully overlap, and (2) strong genetic drift that occurs in farms, an effect due to a limited number of reproductive individuals used for seedling production by farmers. The resilience of spontaneous populations, independent of farm inputs, has been observed in other bays in Brittany. For instance, in Aber Wrac'h (an estuary located several hundred kilometres west of Bay of St-Malo), farming activities were discontinued in the early 2000s, but *U. pinnatifida* is still a common alga in the wild (pers. obs.).

The "historical fingerprint" of the introduction process (i.e. establishment of spontaneous populations due to the escape of individuals from farms) is no longer visible in the spontaneous populations in the open bay, i.e. downstream of the dam. It is only slightly traceable in the spontaneous populations located in the estuary itself. Populations very close to the farm, i.e. upstream of the dam, and which are the most likely to receive migrants from the farm, did not form a distinct genetic group from the farmed population (Table 2). For these populations, the spores released each year during spring by farmed individuals could maintain the historical fingerprint longer. Some of these spores may successfully settle in the neighbouring habitats despite competition with spores released by local individuals (e.g. results of assignment test of population # 32 in Fig. 4).

Our genetic study also aimed at documenting the genetic diversity in spontaneous populations and its spatial distribution throughout the bay. High and similar genetic diversity was observed in every site sampled and allelic diversity was low only in farms. There was no evidence for departure from equilibrium. In particular, there was no evidence for recent bottleneck events in the spontaneous populations (Wilcoxon text implemented in Bottleneck software, data not shown). In addition, many populations did not show any departure from HWE, suggesting that local individuals originate from panmictic reproductive events. Only the population from the Vauban marina (# 19) showed a large heterozygote deficiency and probable asexual parthenogenetic reproduction. Finally, assignment tests showed in many spontaneous populations a high rate of self-assignment, particularly in the western part of the bay. These genetic results suggest that these populations are mostly self-seeded, at drift-migration equilibrium and demographically stable over time. Overall, the genetic data support the field observations that *U. pinnatifida* is a long-term member of the community established in the Bay of St-Malo.

Spore dispersal is unlikely to be the main dispersal mechanism. In *U. pinnatifida*, the lifespan of spores generally does not exceed a few hours to 24 h and leads to annual dispersal distance of the order of 10 m (Forrest et al., 2000; Sliwa et al., 2006; Grulois, 2010). At best, this limited spore dispersal is likely to generate a step-wise colonisation pattern and thus a correlation between genetic distance and spatial distance between populations. However, we did not observe any isolation-by-distance pattern to support this scenario. Conversely, in the bay, there was evidence for significant and patchy genetic structure (F_{st} = 0.10, P < 10^{-4}). Genetic structuring did not reflect any habitat (as described with the parameters retained) or population location effects. Computations of pairwise F_{st} estimates revealed that even very close populations were significantly differentiated. Genetic drift may overcome any migration effects. Assignment tests also showed that the individuals not assigned to the site where they were sampled were not preferentially assigned to a neighbouring population (Fig. 4). Rare and long-distance dispersal events are more likely to explain the genetic structure observed in the bay.

Rare and long-distance dispersal events may be due to two non-exclusive and interacting factors. First, detached mature thalli or transport of mature individuals attached to small rocks, cobbles or mollusc shells may be an important source of founding individuals. This mode of transport has been suggested to be efficient enough to disperse *U. pinnatifida* in Tasmania and New Zealand over distances of 10^3-10^4 m per year (Sliwa et al., 2006). In the Bay of St-Malo, current velocity is particularly high due to strong tide effects and we hypothesise that they facilitate the spread of drifting thalli over long distances. Second, we cannot neglect the role of recreational nautical activities (e.g. fishing and diving) which are particularly popular in the

Bay of St-Malo. Small water crafts and diving are increasingly recognized as important drivers of the spread of non-indigenous species (for a review, see Minchin et al., 2006). For instance, divers and anchors can easily and accidentally detach mature individuals. Moreover, marinas may be direct sources of migrants on a medium-scale (e.g. spores and gametophytes attached to hulls or ropes of small crafts). Accidental boat transportation has been suggested to be a vector to spread *U. pinnatifida* in California (Thornber et al., 2004). The genetic signature of the Bas Sablons marina (# 18) is noteworthy, because this site was found clustered with many other spontaneous populations (see the PCA analysis in Fig.2). Recreational boating activities and commercial maritime traffic are highly developed in this marina with regard to fishing, sailing, diving and ferries to England and to Jersey and Guernsey Islands. If these vectors play a significant role in the dispersal of *U. pinnatifida*, genetic similarities should be found at the regional (e.g. English Channel) level. All the above-mentioned vectors favour jump dispersal of individuals over tens of kilometres. A previous molecular study carried out at the European scale shows a common origin of spontaneous populations in England and France in the English Channel (Voisin et al., 2005). However, an increased number of localities and the use of markers with a higher polymorphism are needed to further test this jump dispersal scenario.

Conclusion

In the marine environment, where direct surveys are often difficult to implement, population genetics can help elucidate some of the mechanisms which drive the establishment and long-term settlement of non-native species. However, genetic studies on introduced algae are relatively scarce compared to those on aquatic and, especially marine animals (Roman & Darling, 2007). Here, we showed that, 30 years after its intentional introduction for farming, *U. pinnatifida* is conspicuous in the Bay of St-Malo. Despite limited natural dispersal of gametes and spores, gene flow has been well established throughout the bay over time creating genetically diverse populations. This is a typical outcome for a pioneer, semi-annual and highly fecund species. Rare and long-distance dispersal events are likely to be main factors for the foundation of new spontaneous populations.

In this context and given the large occurrence of the species within the bay, an eradication program is unlikely to be successful for the management of *U. pinnatifida* in the Bay of St-Malo. In addition, eradication attempts often involve canopy disturbance, which can even enhance the establishment of *U. pinnatifida* (Valentine & Johnson, 2003). Monitoring existing populations and preventing the founding of new populations are nevertheless important management strategies that should be considered. To our knowledge, there is no report that documents that *U. pinnatifida* cause serious damage to native communities in Europe. It is likely that this Asian kelp is being controlled just like other native macroalgae: by grazers, interspecific competition for space and light, pathogens etc. However, experimental ecology studies are needed to test this hypothesis. In addition, we cannot exclude the possibility for evolution towards more aggressive behaviour. In particular, each new population may demographically re-inforce the species' presence and, through genetic drift, may drive local evolution of a more aggressive type. Biological invasions are dynamic processes that require repeated spatial surveys to determine the long-term effects and dynamics of the invaders (Strayer et al., 2006). Furthermore, new farms should not be established unless the individuals can be harvested before reaching sexual maturity. The present survey can be used as a baseline for future surveys of *U. pinnatifida*. Particular attention should be paid to disturbed and artificial habitats for which this kelp has a predilection (Floc'h et al., 1996; Valentine & Johnson, 2003). Disturbances, either natural (e.g. winter storms) or human-based (e.g. anchoring, building new hard structures, such as seawalls) may promote the reinforcement of existing populations and founding of new populations of *U. pinnatifida*.

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