Oyster transfers as a vector for marine species introductions: a realistic approach based on the macrophytes

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ABSTRACT

Transfer of livestock is a common practice in shellfish aquaculture. As part of the EU Program ALIENS 'Algal Introductions to European Shores' and the Programme National sur l'Environnement Côtier (PNEC) "Lagunes Méditerranéennes", an assessment of the efficiency of oyster transfers as vector of unintentional species introduction was carried out, focused on the marine macrophytes. This investigation included a field study of the exotic flora of two major French aquaculture sites: the Thau Lagoon (Mediterranean) (58 exotic species identified) and the Arcachon Basin (NE Atlantic) (21 exotic species identified), a bibliographical analysis of the exotic marine flora of 34 Mediterranean coastal lagoons (68 exotic species listed) and finally an experimental study of the vector efficiency by simulation of oyster transfers. The results confirmed the high degree of efficiency of the importation, transfer and farming of non-indigenous and native commercial shellfish especially oysters, as a vector of primary introduction and secondary dispersal of marine macrophytes. The importation of non-indigenous oysters, in particular the Japanese oyster *Crassostrea gigas,* involved massive quantities between 1964 and about 1980, and the regular transfers between aquaculture sites have been responsible for the introduction and the dispersal of several dozens of exotic macrophytes. When compared to the other major vectors of introduction (hull fouling, ballast waters, Suez Canal), the shellfish trade is by far the main vector of introduction of exotic macrophytes into the Mediterranean and the NE Atlantic. These results are discussed and recommendations for action are proposed.

INTRODUCTION

Unintentional introductions of non-indigenous species are a growing concern in environmental management, especially for marine ecosystems. Each introduction involves at least one vector of transfer. Major vectors include shipping (fouling on hulls, ballast water), trans-oceanic canals and aquaculture activities. As far as living resources are concerned, a great number – in terms of species and individuals – of living organisms are deliberately transported around the world for direct consumption, aquaculture purposes or "freshening" in the marine environment (Carlton, 2001; Wolff and Reise, 2002). The movement of live marine organisms by mechanisms other than shipping has increased dramatically in recent decades, and the trend will likely continue (Ribera and Boudouresque, 1995; Weigle *et al*., 2005). Should an important aquaculture activity suffer a decline following a serious disease or parasite outbreak, a separate exotic strain or species will be imported in large number to rapidly replace this decline in production. Such direct transplants of stock almost inevitably lead to the presence of escapees in the wild (Volpe *et al.*, 1999) or the introduction of unwanted species (Minchin and Gollasch, 2002). Likewise certain environmental crisis can induce collapse of the shellfish livestock, which consequently need to be renewed by massive imports. For example, the Thau Lagoon is regularly subject to a severe summer anoxic crisis, called *"malaigue"*, that can destroy large quantities of shellfish. For example, in 2006, the losses reached 3,455 metric tons of oysters and 4,000 metric tons of mussels on a livestock of 20,000 – 25,000 and 4,000 – 6,000 metric tons, respectively. In addition, because of the ease of transplanting livestock using modern transport, unauthorised movements may regularly occur. Occasionally such illegal shellfish movements are intercepted with a great number of pests associated (Minchin and Rosenthal, 2002). Consequently, aquaculture has become a leading vector of aquatic invasive species worldwide and international and inter-regional transfers of livestock for aquaculture pose high ecological risks given the absence of strong policies in most countries (Wasson *et al.*, 2001).

Among the marine organisms involved in aquaculture transfers, shellfish (especially oysters) have long been *a posteriori* associated with the introduction of marine organisms (Druehl, 1973; Gruet, 1976; Grizel and Héral, 1991; Zibrowius, 1994; Ribera and Boudouresque, 1995; Barber, 1997; Verlaque, 2001; Goulletquer *et al.*, 2002; Minchin and Gollasch, 2002; Ribera-Siguan, 2002; Wolff and Reise, 2002; Weigle *et al.*, 2005). Transport and transplantation of commercially important exotic oysters have resulted in numerous unintentional introductions of pathogens, parasites and pest species either carried in the packing materials, attached to shells or as parasites and disease agents in the living oyster tissues (Carlton, 1992; Sindermann, 1992; Minchin, 1996; Galil and Zenetos, 2002; Minchin and Eno, 2002; Minchin and Gollasch, 2002).

A majority of exotic marine species were discovered in, or close to, shellfish aquaculture areas (Cabioch and Magne, 1987; Rismondo *et al.*, 1993; Curiel *et al.*, 1995, 1999a and b; Cabioch *et al.*, 1997; Farnham, 1997; Stegenga, 1997; Maggs and Stegenga, 1999; Reise *et al.*, 1999; De Montaudoin and Sauriau, 2000; Wolff, 2005). Along the French Atlantic coast, the main area of species introduction (88 % of the primary introductions, 84 % of the secondary introductions) extends from Normandy to the Basin of Arcachon, i.e. in the areas with extensive oyster farming. There 28 % of the introduced species are presumed to have been brought in association with oyster shipments, and mainly *Crassostrea gigas* in the 1970s, (Goulletquer *et al.*, 2002). In the USA, many species of polychaetes were probably imported with the oyster seed stocks (Blake, 1999). According to Ruesink *et al.* (2005), 46 % of the introduced marine species in northern Europe and 20 % in Australia likely entered with oyster aquaculture. In the USA, the percentage varies by region: 10 % on the Gulf Coast, 20 % on the East Coast and 49 % on the West Coast: the regions where a wider variety of oyster species have been cultured tend to have a greater number and percentage of "hitchhiking" non-native species.

According to Elton (1958): 'The greatest agency of all that spreads marine animals to new quarters of the world must be the business of oyster culture'. Into the North Sea area, the introductions due to the oyster imports would be slightly more important than those due to the transport on ship hulls, and clearly more important than the introductions through ballast waters (Reise *et al.*, 2002). For others (Grizel and Héral, 1991; Grizel, 1994; Goulletquer *et al.*, 2002; Wolff, 2005), shellfish transfers arrive in second position right after shipping activities.

As far as macrophytes are concerned, shellfish transfer is considered to be the most important vector of introduction (Eno *et al.*, 1997; Maggs and Stegenga, 1999; Reise *et al.*, 1999; Verlaque, 2001; Ribera Siguan, 2002, 2003). According to Wallentinus (2002), the transfers of oysters and other molluscs may be responsible for 44 % of the introductions of macrophytes, both intercontinentally and within Europe, with the northwest Pacific as the major donor area. However, a direct assessment of the efficiency of oyster transfers as vector of species introductions is lacking. As part of a Fifth Framework Program of the EU (ALIENS: 'Algal Introductions to European Shores') and the Programme National sur l'Environnement Côtier (PNEC) "Lagunes Méditerranéennes", this vector was analysed with a focus on marine macrophytes. The programmes encompassed: (i) a field study of the exotic flora of two major French aquaculture sites: the Thau Lagoon (Mediterranean) and the Arcachon Basin (NE Atlantic); (ii) a bibliographical analysis of the exotic marine flora of 35 Mediterranean coastal lagoons; and (iii) an experimental study of the vector efficiency by simulation of oyster transfers.

Shellfish aquaculture practices

Shellfish aquaculture (mussels, oysters and clams) constantly involves transport of livestock. Transfers of oysters date back to the Roman period (Héral, 1990). The modern European oyster industry depended for decades on the native oyster *Ostrea edulis* Linnaeus and a strain of *Crassostrea gigas* (Thunberg), called the "Portuguese oyster", which was probably introduced from Taiwan in the 16th century. In 1970, oyster farming in Europe faced a collapse due to disease, and required massive imports of *C. gigas* from the Pacific. To sustain future production, both adult oysters and spat were imported from British Columbia and Japan respectively. Around 10,000 metric tons (i.e. more than 5 billion small oysters) were imported between 1971 and 1977. Nowadays, such imports have been considerably reduced as a consequence of the self-sustaining spat production of *C. gigas* in Europe (see the review by Wolff and Reise, 2002) (Table 1). The European Union now restricts imports of oysters to those from a few countries around the Mediterranean (Croatia, Morocco, Tunisia, Turkey) and from the USA, Canada and New Zealand (EU 2003, 2004).

Table 1. European oysters aquaculture: History (based on Héral, 1990; Grizel and Héral, 1991; Wolff and Reise, 2002).

→ **19th century and early 20th century**:

- First attempts to restore natural beds and beginning of oyster farming.
- Accidental introduction of *Crassostrea angulata* into France.
- Regular imports of *Crassostrea virginica* (Britain).
- First trial of intentional introduction of *Crassostrea gigas* into Europe.

→ **1960s and 1970s**:

- Epidemics on *Crassostrea angulata.*
- Massive imports of *Crassostrea gigas* from Japan and British Columbia ("Résur" operation).

→ **During the 1970s**:

- French production relied mainly on Japanese spat.
- From 1971 to 1977, 10,000 metric tons of spat were imported (i.e. more than 5 billion small oysters) from Japan by air.
- Shipments were inspected and immersed in freshwater in order to avoid the introduction of exotic organisms.

→ **From 1977 to present:**

- *Crassostrea gigas* is the main oyster cultured in Europe.
- Imports from Japan have officially stopped.
- Transfers occur inside Europe and abroad.
- In the Mediterranean Sea, production is wholly dependent on the importation of spat or adults. The only *C. gigas* officially authorized in the French lagoons are that produced in the Atlantic.

In the Mediterranean, the European *C. gigas* production is wholly dependant on the importation of spat or adults. In France, only the spat produced in the NE Atlantic is authorized in the Mediterranean.

In France, oyster-farming areas are allocated to different activities, for example:
- spat production (Arcachon, Marennes-Oléron);
- growth (Brittany, Normandy, Thau Lagoon);
- "greening" (Marennes-Oléron).

Continuous transfers of livestock between areas occur to ensure optimal growth conditions for each part of the rearing cycle (Goulletquer and Le Moine, 2002; Girard *et al.*, 2005). Furthermore, additional transfers can occur between areas dedicated to the same activity, or other European areas.

The Thau Lagoon (Mediterranean Sea)

With 2500 farming tables, more than 3.5 million ropes, a standing stock reaching 25,000 metric tons and an annual production up to 12,000 – 13,000 metric tons of *C. gigas,* respectively, the Thau Lagoon is by far the leading site of oyster farming in the Mediterranean sea (Verlaque, 2001).

Massive importations of *Crassostrea gigas* occurred from 1971 to 1977 ("Résur" operation; Grizel and Héral, 1991). Since 1977, the only spat officially authorized to enter Thau Lagoon is that produced in the Atlantic. As a result of the failure in decontamination processes and/or quarantine of these imports, an increasing number of species introductions have occurred in Thau Lagoon. When compared to the previous checklist (Verlaque, 2001), the exotic flora of the Thau Lagoon saw the recent addition of 13 taxa, giving a total of 58 exotic macrophytes (i.e. 25 % of the total flora) (Verlaque, 2005, amended) (Table 2).

Table 2. Exotic taxa recorded in Thau (*) and in other Mediterranean coastal lagoons. All the suspected introductions have been considered. For each taxon, the class, possible origin and vector of primary introduction and secondary dispersal are mentioned. Phyla: **R** = Rhodophyta; **O** = Ochrophyta; **C** = Chlorophyta. Origin: **A** = Atlantic; **C** = cosmopolite; **I** = Indian Ocean; **P** = Pacific; **T** = Tropical seas. Vectors: **C** = Antic and Suez Canals; **FB** = fishing baits; **Shell** = shellfish transfer; **Ship** = shipping (hull fouling, ballast waters). An exotic macrophyte can have several possible origins and vectors (from Verlaque, 2005, amended).

Species	Phyla	Origin	Vector
Acanthophora nayadiformis (Delile) Papenfuss	R	I	C – Ship
* *Agardhiella subulata* (C. Agardh) Kraft & M.J. Wynne	R	A – P (?)	Ship - Shell
* *Ahnfeltiopsis flabelliformis* (Harvey) Masuda	R	P	Shell
* *Antithamnion nipponicum* Yamada *et* Inagaki	R	P	Shell
Antithamnionella elegans (Berthold) J.H. Price *et* D.M. John	R	P	Ship - Shell
* *Antithamnionella spirographidis* (Schiffner) E.M. Wollaston	R	P	Ship - Shell
* *Asparagopsis armata* Harvey, as "*Falkenbergia*" life history phase	R	P	Ship - Shell
* *Ceramium* sp.	R	P	Shell
* *Chondria coerulescens* (J. Agardh) Falkenberg	R	A	Shell
* *Chondrus giganteus* Yendo f. *flabellatus* Mikami	R	P	Shell
* *Chrysymenia wrightii* (Harvey) Yamada	R	P	Shell
* *Dasya sessilis* Yamada	R	P	Shell
* *Grateloupia asiatica* Kawaguchi *et* Wang	R	P	Shell
* *Grateloupia lanceolata* (Okamura) Kawaguchi	R	P	Shell
* *Grateloupia minima* P.L. Crouan & H.M. Crouan	R	A	Shell
* *Grateloupia subpectinata* Holmes	R	P	Shell
* *Grateloupia patens* (Okamura) Kawaguchi & Wang	R	P	Shell
* *Grateloupia turuturu* Yamada	R	P	Shell
* *Griffithsia corallinoides* (Linnaeus) Batters	R	A - P	Shell
* *Herposiphonia parca* Setchell	R	P	Shell
* *Heterosiphonia japonica* Yendo	R	P	Shell
Hypnea cornuta (Kützing) J. Agardh	R	I	C – Ship - Shell
Hypnea spinella (C. Agardh) Kützing	R	T	Ship - Shell
* *Hypnea valentiae* (Turner) Montagne	R	P	Shell
* *Laurencia okamurae* Yamada	R	P	Shell
* *Lithophyllum yessoense* Foslie	R	P	Shell
* *Lomentaria flaccida* Tanaka	R	P	Shell
* *Lomentaria hakodatensis* Yendo	R	P	Shell
* *Nemalion vermiculare* Suringar	R	P	Shell
* *Neosiphonia harveyi* (Bailey) M.-S. Kim, H.-G. Choi, Guiry & G.W. Saunders	R	P	Ship - Shell
* *Nitophyllum stellato-corticatum* Okamura	R	P	Shell
* *Polysiphonia atlantica* Kapraun *et* J. Norris	R	A - P	Ship - Shell
* *Polysiphonia fucoides* (Hudson) Greville	R	A	FB - Shell
* *Polysiphonia morrowii* Harvey	R	P	Shell
* *Polysiphonia paniculata* Montagne	R	P	Ship
* *Polysiphonia stricta* (Dillwyn) Greville	R	A	Ship - Shell
* *Porphyra yezoensis* Ueda	R	P	Shell
* *Pterosiphonia tanakae* Uwai *et* Masuda	R	P	Shell
* *Rhodophysema georgii* Batters	R	A - P	Shell
* *Rhodothamniella codicola* (Børgesen) Bidoux *et* F. Magne	R	P	Ship - Shell
Solieria filiformis (Kützing) Gabrielson	R	A	Ship - Shell
Bonnemaisonia hamifera Hariot	R	P	Ship - Shell
* *Acrothrix gracilis* Kylin	O	A - P	Shell
Botrytella parva (Takamatsu) Kim ?	O	P	Shell
* *Chorda filum* (Linnaeus) Stackhouse	O	A - P	Shell
* *Cladosiphon zosterae* (J. Agardh) Kylin	O	A	Shell
* *Colpomenia peregrina* (Sauvageau) Hamel	O	P	Ship - Shell
* *Desmarestia viridis* O.F. Müller	O	A - P	Shell
**Dictyota okamurae* (E.Y. Dawson) I. Hörnig, R. Schnetter *et* W.F. Prud'homme van Reine	O	P	Shell
Ectocarpus silicosus var. *hiemalis* (Crouan *frat. ex* Kjellman) Kjellman	O	A	Ship - Shell
Fucus spiralis L.	O	A	FB
* *Halothrix lumbricalis* (Kützing) Reinke	O	A - P	Shell
* *Laminaria japonica* Areschoug	O	P	Shell
* *Leathesia difformis* (Linnaeus) Areschoug	O	C	Shell
* *Microspongium tenuissimum* (Hauck) A.F. Peters	O	A	Shell
* *Pilayella littoralis* (Linnaeus) Kjellman	O	A - P	Shell
* *Punctaria tenuissima* (C. Agardh) Greville	O	A	Shell
* *Sargassum muticum* (Yendo) Fensholt	O	P	Shell
* *Scytosiphon dotyi* Wynne	O	P	Ship - Shell
* *Sphaerotrichia firma* (E. Gepp) Zinova	O	P	Shell
* *Undaria pinnatifida* (Harvey) Suringar	O	P	Shell
Caulerpa racemosa var. *cylindracea* (Sonder) Verlaque, Huisman *et* Boudouresque	C	I	? - Ship
* *Cladophora hutchinsioides* Hoek *et* Womersley	C	P	Shell
* *Codium fragile* (Suringar) Hariot	C	P	Ship - Shell
* *Derbesia rhizophora* Yamada	C	P	Shell
* *Monostroma obscurum* (Kützing) J. Agardh	C	P	Ship - Shell
* *Ulva fasciata* Delile	C	P	Ship - Shell
* *Ulva pertusa* Kjellman	C	P	Shell

The majority of these taxa may originate from the Pacific region (89 % of the total), having been introduced either directly with Japanese oyster imports or by shellfish transfers (oysters, mussels and clams) from other aquaculture areas (attached to shells or on the packing materials). An introduction or co-introduction by shipping, *via* the harbour of Sète, is considered possible for only few species. Although no extensive study was carried on the fauna, several exotic invertebrates have also been identified in the Thau Lagoon (Zibrowius, 1991, 1994, and pers. comm.).

Approximately thirty years after the accidental introduction of a first contingent of Pacific macrophytes along with massive importations of *Crassostrea gigas* from Japan, new Asiatic species are still discovered. This provides evidence that importations of oysters (spat or adults) from the NW Pacific have occurred in Europe after 1977. In 1994, illicit imports of Korean oysters have effectively spread in Europe (Verlaque, 1996). The high number of oyster farms and the difficulty in controlling the origin of the oysters did probably increase the risk of this type of importation.

The Thau Lagoon is one of the world's hot spots of marine macrophyte introduction, as it comes far before other major introduction sites, such as New Zealand (21 introduced marine macrophytes), Australia (20) and San Francisco Bay (6) (Ribera and Boudouresque, 1995; Carlton, 1996). This result is a cause for worry as the Thau Lagoon is also an important exportation site of living bivalve molluscs (*C. gigas, Ostrea edulis, Mytilus galloprovincialis, Tapes* spp.) towards other French regions and abroad and, in light of the legislation currently in force, the introduced algae present in the Thau Lagoon have a high-probability of being spread throughout Europe and other Mediterranean countries.

The Arcachon Basin (N.E. Atlantic)

The Arcachon Basin is another important oyster-farming area of France. Since the massive *C. gigas* imports from Japan and British Columbia in 1970s, oyster transfers with the other European and extra-European shellfish basins regularly occur (Auby, 1993), for example:

Arcachon (spat) → Thau (or) Ireland (or) Brittany (ou) Normandie → Arcachon.

Arcachon (spat) → The Ebro Delta (Mediterranean, Spain) → Thau.

During the PNEC Program, 21 exotic macrophytes have been identified (Verlaque *et al.*, 2006, amended). The main possible vector of introduction and the main donor region are the shellfish transfers (oysters, mussels and clams) and the Pacific, respectively (Table 3). Among the 16 taxa that also occur in the Thau Lagoon, two Rhodophyta, *Pterosiphonia tanakae* and *Herposiphonia parca* are known only from these two localities in Europe, and three other species, *Dasya sessilis, Heterosiphonia japonica* Yendo and *Ulva pertusa,* have been identified close to other European oyster-farming areas in Brittany and in Holland (Stegenga, 1997; Maggs and Stegenga, 1999; Stegenga and Mol, 2002; Pe_a and Bárbara, 2006; Christine Maggs and Frédéric Mineur, unpublished data).

As for the Thau Lagoon, excepting the oldest introductions for which shipping cannot be excluded, the shellfish transfer (oysters, mussels, clams and the packing materials) and the Pacific appear as the most probable vector and origin of introduction respectively (Table 3).

The Mediterranean coastal lagoons

Stressed environments are easily colonized by alien species. Coastal lagoons exhibit at once natural stress (variable salinity), lower diversity, abrupt changes in dominant species and high human-induced disturbances through organic enrichment, pollution, physical habitat alterations, ship traffic and extensive aquaculture (Occhipinti-Ambrogi and Savini, 2003). Consequently, a bibliographical analysis of the flora of 34 Mediterranean coastal lagoons has been carried out to inventory the exotic species. For each lagoon, we considered both the presence of shellfish farming and the number of introduced species.

Table 3. Exotic taxa of the Arcachon Basin. All the suspected introductions have been considered. For each taxon, the class, possible origin and vector of primary introduction and secondary dispersal are mentioned. Phyla: **R** = Rhodophyta; **O** = Ochrophyta; **C** = Chlorophyta. Origin: **A** = Atlantic; **I** = Indian Ocean; **M** = Mediterranean; **P** = Pacific. Vectors: **Shell** = shellfish transfers; **Ship** = shipping (hull fouling, ballast waters). An exotic macrophyte can have several possible origins and vectors (from Verlaque *et al.,* 2006, amended).

Species	Phyla	Origin	Vector
Anotrichium furcellatum (J. Agardh) Baldock	R	P	Ship - Shell
Antithamnionella spirographidis (Schiffner) E.M. Wollaston	R	P	Ship - Shell
Antithamnionella ternifolia (J.D. Hooker & Harvey) Lyle	R	P	Ship - Shell
Caulacanthus okamurae Yamada	R	P	Ship - Shell
Centroceras clavulatum (C. Agardh)	R	A - M - IP	Ship - Shell
Dasya sessilis Yamada	R	P	Shell
Gracilaria vermiculophylla (Ohmi) Papenfuss	R	P	Shell
Grateloupia subpectinata Holmes	R	P	Shell
Herposiphonia parca Setchell	R	P	Shell
Heterosiphonia japonica Yendo	R	P	Shell
Hypnea valentiae (Turner) Montagne	R	P	Shell
Lomentaria hakodatensis	R	P	Shell
Neosiphonia harveyi (Bailey) M.-S. Kim, H.-G. Choi *et al.*	R	P	Ship - Shell
Pterosiphonia tanakae Uwai *et* Masuda	R	P	Shell
Rhodothamniella codicola (Børgesen) Bidoux & F. Magne	R	P	Ship - Shell
Colpomenia peregrina (Sauvageau) Hamel	O	P	Ship - Shell
Sargassum muticum (Yendo) Fensholt	O	P	Shell
Codium fragile (Suringar) Hariot	C	P	Ship - Shell
Kornmannia leptoderma (Kjellman) Bliding	C	P	Shell
Monostroma obscurum (Kützing) J. Agardh	C	P	Ship - Shell
Ulva pertusa Kjellman	C	P	Shell

Table 4. Shellfish farming activities (Yes/No) and number of exotic macrophytes in 34 Mediterranean coastal lagoons. In bold: lagoons with introduced macrophytes (from Verlaque, 2005, amended).

COUNTRY	COASTAL LAGOON	SHELLFISH FARMING	NUMBER OF EXOTIC MACROPHYTES
CROATIA	Veliko and Malo Jezero	N	0
FRANCE	Arnel	N	0
	Bages Sigean	**N**	**1**
	Berre	**N**	**2**
	Biguglia	N	0
	Diane	**Y**	**2**
	Ingril	N	0
	Mauguio	N	0
	Palo	N	0
	Pérols	N	0
	Prévost	**Y**	**1**
	Salses-Leucate	**Y**	**11**
	Thau	**Y**	**58**
	Urbino	Y	0
	Vic	N	0
GREECE	Agiasma, Eratino, Fanari, Keramoti and Vassova	N	0
ITALY	Lesina	N	0
	Mar Piccolo	**Y**	**10**
	Orbetello	Y	0
	Stagnone di Marsala	**N**	**1**
	Vendicari	N	0
	Venice	**Y**	**25**
MOROCCO	**Mar Chica**	**Y**	**1**
SPAIN	Addaia Bay	N	0
	Buda (Ebro delta)	Y	0
	Mar Menor	**N**	**1**
TUNISIA	**Bizerte**	**Y**	**3**
	Ghar El Melh	N	0
	Lac of Tunis	N	0

Table 5. Vectors of introduction and donor regions of the exotic macrophytes reported in Mediterranean coastal lagoons. An exotic species can have several possible vectors and donor regions, which explains a sum of percentages > 100 % for each category (from Verlaque, 2005; amended).

		N	%
Vectors of primary introduction and secondary dispersal	Canals	2	3.0
	Fishing baits	2	3.0
	Shellfish transfers	**64**	**94.0**
	Shipping	21	31.0
Donor regions	Atlantic	19	28.0
	Cosmopolite	1	1.5
	Indian Ocean	3	4.4
	Pacific	**53**	**78.0**
	Tropical seas	1	1.5

Using present-day taxonomy, the exotic flora reported in these lagoons reaches a total of 68 taxa (42 Rhodophyta, 19 Ochrophyta and 7 Chlorophyta) (Table 2). Exotic taxa have been reported from twelve Mediterranean coastal lagoons (Table 4). The exotic flora is the lowest (one or two taxa) in the lagoons without aquaculture activities, whereas its richness is maximum in the leading Mediterranean shellfish-farming areas as the Thau Lagoon and the Lagoon of Venice, with 58 and 25 exotic taxa, respectively.

After a primary introduction in Europe, the Asiatic taxa were probably secondarily dispersed with the frequent shellfish transfers between the Atlantic and the Mediterranean Sea and between the different Mediterranean shellfish-farming areas like Thau and Venice (Occhipinti Ambrogi, 2000).

An additional mode of transportation is with the direct importation of marketable products from a source country to a host country where the shellfish is sold in local markets (Blake, 1999; Carlton, 2001; Weigle *et al.*, 2005). For example, France regularly imports large livestock of adult mussels for the seafood trade from Spain, Italy and other Mediterranean regions. Although the re-immersion ("*retrempage*") in coastal waters of such livestock is strictly prohibited, this practice is frequent. Moreover, when quarantine tanks exist, the effluent seawater discharged is rarely sterilized.

THE EXPERIMENTAL EVIDENCE

Despite the presumed importance of oyster transfer in species introductions, only a few studies were devoted to the epibionta of shells (Schodduyn, 1931; Korringa, 1951; Gruet *et al.*, 1976; Haydar and Wolff, 2004). As part of the ALIENS Program, the risk of transferring native and non-indigenous macrophytes in association with oysters, from one farming site to another, was assessed (Mineur *et al.*, 2007a). Several transfers of oysters were simulated. The experimental donor area was the Thau Lagoon. The simulation involved conditions likely to be experienced during surface transport (by road) to most other European oyster farming sites. Several durations of emersion of the shells were tested. We also tested two realistic methods (i.e. immersion in hot seawater and immersion in brine) to reduce potential risks of macrophyte transfers. Immersion in freshwater was not tested because it is inefficient (Gruet *et al.*, 1976). After a simulated transfer, the oyster shells were maintained in culture tanks until the epiflora reached a suitable size for identification.

The simulation showed that oysters visually cleaned of epibionts can still bear a high diversity of viable macrophyte propagules. A total of 57 taxa belonging to 17 orders were recorded across all treatments and experiments, including 16 exotic species. By comparison, only seven macrophyte orders were found during a survey in the same area (harbour of Sète) from the hull fouling of 23 large standard commercial ships coming from all over the world (Mineur *et al.*, 2007b). The period of aerial emersion did not reduce the number of taxa nor the total 'propagule pressure' measured as the cumulative number of shells fouled by each taxon. The abundance of macrophyte propagules on the shells may be due to the fact that after cleaning, the oysters are re-immersed for two weeks in plastic net bags in order to decrease stress and to allow removal of moribund individuals prior to transport.

Immersion for short periods (3 seconds) at high temperatures (80 to 85 °C) had a lethal effect on nearly all macrophyte propagules, except for tubular *Ulva* spp. Under brine treatment, the reduction of macrophyte propagules was significantly less and some resistant Chlorophyta (*Cladophora* spp. and tubular *Ulva* spp.), Ochrophyta (ectocarpalean species and *Scytosiphon lomentaria*) and Rhodophyta (*Porphyra* sp. and *Stylonema alsidii)* were able to survive.

DISCUSSION AND CONCLUSION

In 1994, a bibliographic review identified the Suez Canal as the major vector of introduction of macrophytes into the Mediterranean Sea (Verlaque, 1994). The ALIEN and PNEC Programs showed that the oyster transfer is a more efficient vector. This is especially true when one considers that the list of introduced macrophytes recognized likely represents the "tip of the iceberg". Indeed, the number of introduced species is probably underestimated since one introduction can remain undetected when it concerns a cryptic species that is similar to a native one (Carlton, 2001). Likewise, when native species are present in a large but fragmented area (e.g. the Atlantic and the Mediterranean), gene introductions from remote populations must occur. Such types of introduction, which are very difficult to detect, constitute an important biological pollution to be considered. Thus, when compared to the Mediterranean lagoons without aquaculture activities, the diversity of the Thau Lagoon flora is abnormally high; this situation might reflect undetected introductions from the Atlantic.

Consequently, the remark by Elton (1958) "The greatest agency of all that spreads marine animals to new quarters of the world is the business of oyster culture" also holds true for marine macrophytes.

The ALIENS and PNEC Programs demonstrated the high efficiency of oyster transfers as vector of macrophyte introduction. When compared to the constraints imposed by other major vectors like hull fouling and ballast waters (e.g. long travel, changes in latitude, darkness, anti-fouling paintings, pollutants, etc.), the conditions of marine livestock transfers appear very soft, non selective and favorable to the survival of many organisms (Weigle *et al.*, 2005). Aquaculture acts as a "low-cost" vector for the hitchhiker species, particularly for the macrophytes.

The ALIEN experiment involved four simulated transfers of 320 oyster valves each (i.e. only 160 oysters, more or less equivalent to 15 kg), a very small quantity compared to those transferred every year by European oyster farmers (e.g. 205 million of juvenile *C. gigas* at Thau in 2001; Girard *et al.*, 2005). Likewise, in France the oysters frequently change rearing basins before their marketing. In 2001, these transfers represented several tens of thousand metric tons of young and adult *C. gigas* and 2,000 metric tons of *Ostrea edulis* (Girard *et al.*, 2005).

Before the 1960s, the ecological consequences of the large-scale, deliberate introduction of exotic shellfish species were in general disregarded. But the growing awareness that shellfish imports could be accompanied by the import of pests, parasites and devastating diseases as well as the observed effects on native communities, led to a number of measures since. Codes of conduct were introduced in several countries (see Utting and Spencer, 1992, for the United Kingdom). Quarantine measures have been introduced as well. In addition, hatchery production of marine bivalves became technically and economically feasible, thus diminishing the necessity to import seed shellfish from the wild and often from other parts of the world. However, large quantities of shellfish are still being transported from one culture area to another within Europe. The European Common Market even encourages this practice (Wolff and Reise, 2002). The inadequacy of current legislation is such that these transfers occur with accidental primary introduction and secondary dispersal of marine species (Martel *et al.*, 2004; Verlaque *et al.*, 2005, this study).

Relatively simple changes to the shellfish transfer practice can reduce the risk of species introductions. Heat treatment is an efficient way to kill macrophyte propagules (Mountfort *et al.*, 1999; Mineur *et al.*, 2007a). Certain French oyster farmers already commonly use such a treatment to remove small oyster spat and other fouling organisms from medium-sized oysters. Immersion in saturated brine for a short period is another effective method of control of various invasive organisms such as *Crepidula fornicata* (Linnaeus, 1758) and *Sargassum muticum* (Hancock, 1969; Franklin, 1974; Lewey, 1976; Ruellet, 2004; Mineur *et al.*, 2007a). Other preventative methods involve toxic chemicals (MacKenzie and Shearer, 1959; Barber, 1997; McEnnulty *et al.*, 2001; Ruellet, 2004). However, the use of toxic substances is not suitable for shellfish production aimed at human consumption. Hitchhiking species, pests, parasites and diseases are not confined to the shell exterior alone but also occur within the shell, the mantle cavity and tissues as well as within the vacant spaces of dead oysters.

Table 6. Guidelines to reduce the unintentional introductions by aquaculture.

- Awareness of farmers concerning the risks associated with uncontrolled importation has to be increased.
- Aquaculture should be based on native, local stock whenever possible. Imports and transfers of stock should be minimized, thoroughly inspected, and quarantined for an appropriate observation period.
- Special attention would have to be paid during aquaculture trials with new exotic species (even with livestock from hatcheries).
- Non-native livestock for introduction has to be produced in hatcheries.
- Live products destined for consumption, processing, and aquarium or display should not be placed into the natural environment.
- In the case of livestock transfers (including interregional ones), decontamination processes and/or quarantine as proposed by the ICES (2005) have to be followed.
- Efficient treatment (e.g. hot-seawater for oysters) to avoid introduction or secondary dispersal of exotic or native species would have to be carried out prior to each transfer that is to say after the period of re-immersion preceding the transfer and would have been repeated on arrival.

Comprehensive guidelines for preventing introductions of exotic species are available through IUCN (Shine *et al.*, 2000) and ICES (2005) (Table 6). Widespread adoption of these policies is urgently needed to stem the rising tide of aquatic invasions (Naylor *et al.*, 2001; Occhipinti Ambrogi, 2001; Cohen, 2005; Weigle *et al.*, 2005).

Acknowledgements

This study was supported by grants from the Programme National sur l'Environnement Côtier (PNEC) "Lagunes Méditerranéennes" and a Fifth Framework Program of the European Community (ALIENS: 'Algal Introductions to European Shores'). We thank Isabelle Auby, Martin Plus and the IFREMER Station of Arcachon for their collaboration during the study at Arcachon, and Michèle Boudouresque for bibliographical assistance.