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Short communication

Styela clava (Tunicata, Ascidiacea) – a new threat to the Mediterranean shellfish industry?

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Abstract

The solitary ascidian *Styela clava* Herdman, 1882 has recently been found in the Bassin de Thau, France, an area of intensive oyster and mussel farming. The shellfish are grown on ropes suspended in the water column, similar to the technique employed in Prince Edward Island (PEI), Canada. *S. clava* is considered a major threat to the mussel industry in PEI but, at present, it is not considered a threat to oyster production in the Bassin de Thau. Anoxia or the combined effect of high water temperature and high salinity may be constraining the growth of the *S. clava* population in the Bassin de Thau. Identification of the factors restricting the population growth may provide clues to potential control methods.

Key words: *Styela clava*, shellfish farming, Bassin de Thau

Introduction

The solitary ascidian *Styela clava* is native to the north-west Pacific, particularly Japanese and Korean waters. It has a club-shaped body that is attached to firm substratum by a stem-like stolon with an expanded membranous base. Adults may grow to 220 mm total length. *S. clava* was first found in European waters in 1953. Since its initial discovery, it has spread up the North Sea coast as far as Denmark and south along the Atlantic coast to Portugal (Davis et al. 2007). Despite extensive searches of harbours and marinas along the northern Mediterranean coastline (Figure 1), no populations could be located in the Mediterranean Sea. In 2004 oyster fishermen in the the Bassin de Thau, in southern France, recognised pictures of *S. clava* and claimed that it occurred on the shellfish ropes, but was not a serious pest. We surveyed the accessible edges of the lagoon but could find no specimens that year.

Six specimens of a solitary ascidian resembling *S. clava* were collected from ~5m depth in the southern end of the Bassin de Thau, near Sète, on June 1, 2005 by David Luquet (L'Observatoire Océanologique de Villefranche-sur-Mer). Subsequent examination confirmed that the specimens were *Styela clava* Herdman, 1882 (Davis and Davis 2008).

In June 2007 we found a few specimens in the Canal de Sète, in the centre of the town of Sète, so the population appears to be sustainable and expanding. The present paper aims to raise awareness of the presence of *S. clava* in the Mediterranean region and of the threat that it poses to the shellfish farming industry.

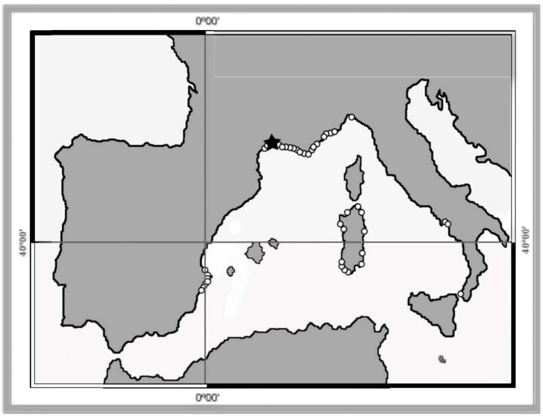

Figure 1. Location of sites searched for *Styela clava* in the Mediterranean Sea.

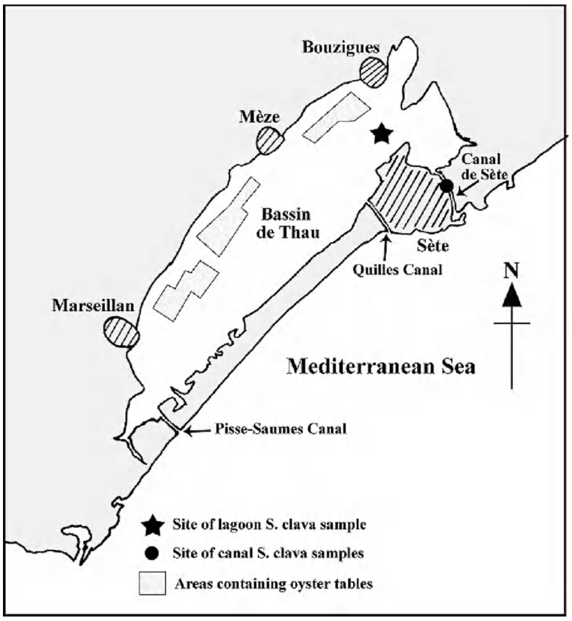

Figure 2. The Bassin de Thau showing the sites where *Styela clava* was found.

The site

The Bassin (or Étang) de Thau is a 21 km long, 5 km wide lagoon situated between the towns of Beziers and Montpellier, in southern France (Figure 1). The mean depth of the lagoon is 4.5m, but the depth can exceed 30 m in places. The tidal range is small, generally less than 30 cm in amplitude (La Jeunesse and Elliot 2004), and the tidal currents are weak (Souchu et al. 2001). The lagoon is slightly eutrophic. Gangnery et al. (2003) reported total chlorophyll *a* concentrations averaging 1.2 µg l^{-1} and total particulate matter averaging 2.2 mg l^{-1} in 2002; the organic content of the particulate matter was approximately 50%.

Salinity ranges from 19.9 - 40.4 psu (FOGEM 2006), but spot measurements taken during our surveys only ranged from 28-37 psu. The 7,500-hectare expanse of water is separated from the Mediterranean Sea by a narrow sand bar with openings at either end (Figure 2). The western opening, at Marseillan-Plage, is the Pisse-Saumes canal; the eastern opening, just to the west of Sète, is the Quilles Canal. To the east of Sète, the Canal de Sète links the lagoon with Sète marina and the Mediterranean Sea. Flow rates in these canals varied from <0.5 to ~2 ms^{-1} during our surveys.

The threat

In Prince Edward Island, where mussels are grown on fabric "socks" suspended in the water column, *Styela clava* is considered to be a major pest by the mussel farming industry (Thompson and MacNair 2004). It settles on the socks and competes with the mussels for food, costing the mussel industry several million C$ per year in reduced mussel production. The recent discovery of *S. clava* in New Zealand (Davis and Davis 2006) caused considerable alarm since the green lipped mussels are also grown suspended in the water column. The mussel industry in New Zealand, worth millions of NZ$ per year, is now considered to be at risk.

The Bassin de Thau is an area of intensive oyster and mussel farming. Oysters, mainly *Crassostrea gigas* (Thunberg, 1793), are farmed using oyster tables (Figure 3) and a line cultivation technique called "Collées" rather than traditional oyster beds. In the Collées method, young oysters are fixed individually to a rope with a type of fast setting mortar. The "oyster lines" are then hung from large wooden racks which form the oyster table (Figure 4). When the oysters reach marketable size, they are cut from the line and the remaining cement is ground off. The suspended lines terminate just above the bottom, thus keeping the oysters safe from predators and preventing fouling by mud. Approximately 750 producers farm 2,750 oyster tables and harvest 13,000 tonnes annually.

Figure 3. The Bassin de Thau viewed from Bouzigues, showing the oyster tables. Photo: M.E. Davis.

Figure 4. Oyster tables showing the culturing ropes. Photo: M.E. Davis.

Mussels, *Mytilus galloprovincialis* (Lamarck, 1819) are grown from naturally settled juveniles, packed in net 'socks' that are suspended in the water column (Gangnery et al. 2001); approximately 3,000 tonnes are produced every year.

Currently both *Styela plicata* (Lesueur, 1823) and *Ascidiella aspersa* (Müller, 1776) grow in abundance on the shellfish culturing ropes but, as they die back in summer, both are perceived as a nuisance rather than an economic threat. *S. clava* has been observed attached to the ropes, but at present is also considered to be merely a nuisance. Given that it has been present in the Bassin de Thau for at least three years, should it be considered a serious threat to the shellfishery?

There are many similarities between shellfish farming in the Bassin de Thau and mussel farming in Prince Edward Island, yet *S. clava* has failed to spread throughout the lagoon or develop large populations. We could find no evidence of predation during our short sampling visits. In the absence of predators, water quality (temperature, salinity and dissolved oxygen) food and substrate availability are the main constraints to population growth. Water temperature must exceed 15°C for several weeks for reproduction to occur, and salinity should be between 22 and 34.5 psu for successful recruitment. These criteria should not constrain population growth in the lagoon. Suitable settlement substrate is often the main resource limitation for growth of *S. clava* populations. The natural substrate available in Prince Edward Island is mainly soft, typically mud (Bourque et al. 2007), so *S. clava* exploits the mussel farming equipment there. The abundant hard substrate in the Bassin de Thau may be preferentially colonised by *S. clava*, mitigating the threat to oyster production; alternatively, the shellfish ropes may offer the potential for greater population growth.

The population of *S. clava* in the lagoon does not appear to have grown rapidly in the three years since its discovery. Maximum water temperature may be an important factor controlling population growth; local fishermen claim that the solitary ascidians that blanket the shellfish in spring die back in the summer months as the water temperature rises. Surface water temperatures midway between Bouzigues and Sète varied during 1993-1994 from 6°C in February to 26°C in August (Souchu et al. 2001); they reached a maximum of 29.1°C in July 2006 but only 24.3°C in August 2007 (Ifremer 2007). As yet we have no information regarding the maximum water temperature that *S. clava* can tolerate. Boothroyd et al. (2002) reported that *S. clava* larvae were able to survive in water temperatures as high as 30°C, but it is possible that the summer 2006 water temperature in the Bassin de Thau may have exceeded the temperature tolerance of adults. However, the maximum recorded bottom water temperatures at the site were 26.6°C in July 2006 and 22.9°C in August 2007, temperatures that can occur in southern European marinas where *S. clava* has been found. Thus *S. clava* could survive at depth to repopulate the lagoon in cooler summers.

It is also possible that the effect of temperature may be exacerbated by the high salinities that occur during the summer months, typically 37-38 psu (Ifremer 2007). Brunetti et al. (1980) studied the combined effects of temperature (from 3°C to 28°C) and salinity (from 16 to 44 psu) on colonies of *Botryllus schlosseri* and *Botrylloides leachi* from the Venetian Lagoon;

they found that colonial regression increased when high temperature was combined with high salinity. However, no similar data could be located for *S. clava*.

The development of anoxic conditions in the lagoon could also explain the limited development of the *S. clava* population in the Bassin de Thau. Mazouni et al. (1996) noted anoxia in the bottom waters of the Thau lagoon in July which they thought could be responsible for a massive mortality of the benthos. Oysters may survive periods of anoxia because they can adapt to anaerobic conditions (Shumway 1981) but ascidians, which are more sensitive to hypoxia (Osman et al. 1989), would die. Thus anoxia could explain the summer decline of ascidians in the Bassin de Thau. However, Mazouni et al. (2001) reported the coexistence of oysters and ascidians (*Ciona intestinalis*, *Phallusia mammillata* and *Botryllus* sp.) throughout 1992 although in August, five weeks after a bottom anoxia event, only young ascidians were present; the water temperature during this period only reached 26°C. Incidentally, Mazouni et al. (2001) monitored the composition of biofouling communities that recruited onto suspended oyster cultures during 1992 and did not record the presence of *S. clava*.

The probable introduction vector

Non-indigenous species may be introduced by natural dispersal or human-aided vectors. Natural dispersal is mainly dependent on water movement, which in a Mediterranean marina or harbour has a maximum range of little more than the tidal excursion. *Styela clava* is oviparous; the eggs hatch after approximately 12 hours. The pelagic lecithotrophic larvae rarely swim more than a few centimetres, and are only active for approximately 12 h (Davis 1997). The nearest recorded populations are in Portugal (Davis and Davis 2005), approximately 1200 miles away by sea. Given the short planktonic period, colonization by the natural dispersal of larvae is unrealistic. Therefore, introduced sessile adults must have established the Bassin de Thau population. Although natural dispersal of settled animals attached to floating debris is feasible, the long distance to the nearest population and the narrow entries into the Bassin de Thau from the Mediterranean Sea render this vector unlikely.

Human-aided ascidian introduction may occur if animals are transported attached to ships' hulls (Gollasch 2002) or in sea chests (Coutts et al. 2003, Davis and Davis 2004). Large ships cannot enter the Bassin de Thau, so small fishing boats and pleasure craft represent the most likely hull transport vectors. We have found no *S. clava* populations in the adjacent Mediterranean marinas and harbours (Annex 1), and such small boats are unlikely to venture further afield. It is possible that recreational boats from the Atlantic coast could visit the Bassin de Thau via the 240 km long Canal du Midi or be transported there by trailer. However, *S. clava* is unlikely to withstand the prolonged exposure to fresh water in the former or to air in the latter.

Long distance dispersal of *S. clava* can occur if juvenile animals are transported attached to oyster shells when the oysters are re-laid. It is probable that this was the vector by which *S. clava* was introduced into the Limfjord (Lützen 1999). The Bassin de Thau has a thriving commercial shellfishery, the most important product being oysters - mainly *Crassostrea gigas*. Stocks of *C. gigas* were imported from Japan to the Bassin de Thau from the late 1960s until at least the mid 1970s. It is possible that some juvenile oysters have been imported during the last few years and grown to marketable size, although the fishermen claim that there have been no oyster imports in the last twenty five years.

The proximity of commercial shellfisheries to the discovered populations, the lack of commercial shipping routes into the lagoon and the absence of *S. clava* from other harbours and marinas along the coast, suggests that the species may have been introduced by shellfish transfer. Indeed, more than 20% of the macrophyte species in the lagoon are introduced species that are thought to have arrived as a consequence of shellfish transfer (Vincent et al. 2006).

The potential for further spread

The important prerequisites for a fouling organism to spread from one site to another are that:

- there is a mobile phase,
- the donor and receiving ecosystems are connected by a dispersal vector;
- there is suitable settlement substrate in the receiving ecosystem.

The eggs and larvae of *Styela clava* provide a planktonic dispersal phase that lasts approximately 24 hours. Therefore natural spread within the lagoon is likely to be slow and, given the low exchange rate with the Mediterranean, spread outside the lagoon is likely to take several years. Man-aided dispersal by small fishing boats and pleasure craft that leave the Bassin de Thau, after being anchored there for sufficiently long periods to become fouled, may enhance the spread of *S. clava* into the Mediterranean; there are numerous harbours and marinas adjacent to the Bassin de Thau that contain suitable hard substrate. In June 2007 we found a few specimens (Figure 5) in the Canal de Sète in the centre of Sète (Figure 6), so the population appears to be expanding - although the vector is uncertain. Consequently, it is important to follow this population through several more years to determine if it persists and spreads.

Figure 5. *Styela clava* collected from the Canal de Sète. Photo: M.E. Davis.

Figure 6. Sampling site in the Canal de Sète. Photo: M.E. Davis.

Conclusions

Styela clava has been recorded in the Bassin de Thau, near Sète. This is the first record of its presence in the Mediterranean region. No populations of *S. clava* could be found in harbours and marinas along the adjacent Mediterranean coastline. The proximity of commercial shellfisheries to the discovered populations, the lack of commercial shipping routes into the lagoon and the absence of *S. clava* from other harbours and marinas along the coast, suggest that the species may have been introduced by shellfish transfer.

The number of *S. clava* in the lagoon does not appear to have grown rapidly in the three years since its discovery. It is possible that this is because summer water temperatures exceed the temperature tolerance of *S. clava*, or a combination of high temperature and salinity may kill a large proportion of the population. However, some individuals could survive at depth to repopulate the lagoon in the cooler summers. The development of anoxic conditions in the Bassin de Thau could also explain the limited development of the *S. clava* population. Adult ascidians disappeared after one bottom anoxia event in the lagoon, but no *S. clava* were present so it is not possible to predict how they would be affected.

Fishermen have observed *S. clava* attached to the shellfish culturing ropes used in the Bassin de Thau but, as the ascidians die back in summer, they are perceived as a nuisance rather than an economic threat at present. It is important to follow this population of *S. clava* through several more years to determine if it persists and if numbers increase. Temperature, salinity and dissolved oxygen should be monitored during this period.

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References

Boothroyd FA, MacNair NG, Landry T, Locke A, Davidson TJ (2002) Dealing with an aquatic invader: The clubbed tunicate (*Styela clava*) in Prince Edward Island waters. Aquaculture Canada 2002: First International Mussel Forum – International Perspective on Non-Indigenous Species Introduction

Bourque D, Davidson J, MacNair NG, Arsenault G, LeBlanc AR, Landry T, Miron G (2007) Reproduction and early life history of the invasive ascidian *Styela clava* Herdman in Prince Edward Island, Canada. Journal of Experimental Marine Biology and Ecology 342: 78-84

Brunetti R, Beghi L, Bressan M, Marin MG (1980) Combined effects of temperature and salinity on colonies of *Botryllus schlosseri* and *Botrylloides leachi* (Ascidiacea) from the Venetian Lagoon. Marine Ecology Progress Series 1980: 202-314

Coutts ADM, Moore KM, Hewitt CL (2003) Ships' sea-chests: an overlooked transfer mechanism for non-indigenous marine species? Marine Pollution Bulletin 46: 1510-1513

Davis MH (1997) Physical factors influencing larval behaviour in three species of solitary ascidian. PhD Thesis, Department of Biology, The Open University, 360 pp.

Davis MH, Davis ME (2004) The role of man-aided dispersal in the spread of the immigrant *Styela clava* Herdman, 1882. Journal of Marine Science and Environment 1: 18-24

Davis MH, Davis ME (2005) *Styela clava* (Tunicata: Ascidiacea) - a new addition to the fauna of the Portuguese coast. Journal of the Marine Biological Association of the United Kingdom 85: 403-404

Davis MH, Davis ME (2006) *Styela clava* (Tunicata: Ascidiacea) a new edition to the fauna of New Zealand. Porcupine Marine Natural History Society Newsletter 20: 23-28

Davis MH, Lützen J, Davis ME (2007) The spread of *Styela clava* Herdman, 1882 (Tunicata, Ascidiacea) in European waters. Aquatic Invasions **2:** 378-390

Davis MH, Davis ME (2008) *Styela clava* (Tunicata, Ascidiacea) – first record in the Mediterranean region. Aquatic Invasions 3: 125-132

FOGEM (2006) La surveillance FOGEM des zones humides côtières du Languedoc-Roussillon 2000-2005. http://www.languedoc-roussillon.ecologie.gouv.fr/eau/fogem/bilan_2000_2005/ Partie-1/28-Etang-Thau.pdf

Gangnery A, Bacher C, Buestel D (2001) Assessing the production and the impact of cultivated oysters in the Thau lagoon (Mediterranee, France) with a population dynamics model. Canadian Journal of Fisheries and Aquatic Sciences 58: 1012-1020

Gangnery A, Chabirand J-M, Lagarde F, Le Gall P, Oheix J, Bacher C, Buestel D (2003) Growth model of the Pacific oyster, *Crassostrea gigas*, cultured in Thau Lagoon (Méditerranée, France). Aquaculture 215: 267-290

Gollasch S (2002) The importance of ship hull fouling as a vector of species introductions into the North Sea. Biofouling 18: 105-121

Ifremer (2007) Suivi régulier de la lagune de Thau. http://www.ifremer.fr/lerlr

La Jeunesse I, Elliot M (2004) Anthropogenic regulation of the phosphorus balance in the Thau catchment-coastal lagoon system (Mediterranean Sea, France) over 24 years. Marine Pollution Bulletin 48: 679-687

Lützen J (1999) *Styela clava* Herdman (Urochordata, Ascidiacea), a successful immigrant to North West Europe: ecology, propagation and chronology of spread. Helgoländer Meeresunters 52: 383–391

Mazouni N, Gaertner J-C Deslous-Paoli J-M, Landrein S, Geringer d'Oedenberg M (1996) Nutrient and oxygen exchanges at the water-sediment interface in a shellfish farming lagoon (Thau, France). Journal of Experimental Marine Biology and Ecology 203: 92-113

Mazouni N, Gaertner J-C, Deslous-Paoli J-M (2001) Composition of biofouling communities on suspended oyster cultures: an *in situ* study of their interactions with the water column. Marine Ecology Progress Series 214: 93-102

Osman RW, Whitlatch RB, Zajac RN (1989) Effects of resident species on recruitment into a community: larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica.* Marine Ecology Progress Series 54: 61-73

Shumway SE (1981) Oxygen consumption in oysters: an overview. Marine Biology Letters 3: 1-23

Souchu P, Vaquer A, Collos Y, Landrein S, Deslous-Paoli J-M, Bibent B (2001) Influence of shellfish farming activities on the biogeochemical composition of the water column in Thau lagoon. Marine Ecology Progress Series 218: 141-152

Thompson R, MacNair N (2004) An overview of the clubbed tunicate (*Styela clava*) in Prince Edward Island. PEI Department of Agriculture, Fisheries, Aquaculture and Forestry Technical Report 234, 29 pp.

Vincent C, Mouillot D, Lauret M, Do Chi T, Troussellier M, Aliaume C (2006) Contribution of exotic species, environmental factors and spatial components to the macrophyte assemblages in a Mediterranean lagoon (Thau lagoon, Southern France). Ecological Modelling 193: 119-131

Annex 1

Sites examined along the Northern Mediterranean coast

Sites	Date	Coordinates	Salinity (psu)
Sites in France			
Marseille (Old Port)	26-Feb-04	43°17.76'N, 05°02.36'E	34.8
La Pointe-Rouge	26-Feb-04	43°14.58'N, 05°21.89'E	37.2
La Seyne	27-Feb-04	43°06.25'N, 05°53.05'E	37.6
Toulon (Darse du Mourillon)	27-Feb-04	43°06.88'N, 05°55.65'E	36.2
Toulon (Darse viéille)	27-Feb-04	43°07.21'N, 05°55.96'E	37.4
Frejus	28-Feb-04	43°25.25'N, 06°44.86'E	37.6
St Raphael (Port Santa Lucia)	28-Feb-04	43°24.72'N, 06°46.95'E	37.4
St Maxime	28-Feb-04	43°18.36'N, 06°38.43'E	36.2
Port Grimaud	29-Feb-04	43°16.39'N, 06°34.52'E	36.4
Cavalaire-sur-Mer	29-Feb-04	43°10.27'N, 06°32.15'E	37.8
Le Lavandou	29-Feb-04	43°08.26'N, 06°22.34'E	37.8
Port-de-Miramar	29-Feb-04	43°06.98'N, 06°14.85'E	37.5
Bandol	29-Feb-04	43°07.95'N, 05°45.15'E	37.9
La Ciotat	29-Feb-04	43°10.55'N, 05°36.70'E	37.8
La Ciotat (Old Port)	01-Mar-04	43°10.45'N, 05°36.41'E	37.8
Cassis	01-Mar-04	43°12.82'N, 05°32.17'E	27.3
Marseille	01-Mar-04	43°17.76'N, 05°22.36'E	37.8
Martigues	02-Mar-04	43°24.37'N, 05°03.00'E	10.6
Port-St-Louis	02-Mar-04	43°23.27'N, 04°48.57'E	37.7
Villefranche	31-May-05	43°41.87'N, 07°18.43'E	33.1
Menton (Old Port)	31-May-05	43°46.54'N, 07°30.64'E	35.1
Menton (Garavan)	01-Jun-05	43°47.05'N, 07°31.18'E	35.4
Cap D'Ail	01-Jun-05	43°43.50'N, 07°25.02'E	35.9
Beaulieu sur Mer	01-Jun-05	43°42.54'N, 07°20.15'E	34.8
Nice	01-Jun-05	43°41.74'N, 07°17.02'E	22.6
Martigues	24-Feb-06	43°24.10'N, 05°03.59'E	20.8
Port du Bouc	24-Feb-06	43°24.17'N, 04°59.01'E	25.3
Saint-Gervais	24-Feb-06	43°25.71'N, 04°56.48'E	34.9
Port-St-Louis du Rhone	24-Feb-06	43°23.28'N, 04°48.56'E	27.0
Méze, Bassin de Thau	25-Feb-06	43°25.29'N, 03°36.33'E	30.3
Marseillan, Bassin de Thau	25-Feb-06	43°21.13'N, 03°32.06'E	28.3
Sete Marina	25-Feb-06	43°23.75'N, 03°41.96'E	33.9
Le Barrou	25-Feb-06	43°24.72'N, 03°39.61'E	29.8
Cap D'Agde	25-Feb-06	43°17.03'N, 03°30.76'E	34.5
Palavas les Flots	26-Feb-06	43°31.57'N, 03°55.88'E	32.9
Grande Motte	26-Feb-06	43°33.40'N, 04°05.02'E	33.6
Port Camargue	26-Feb-06	43°31.13'N, 04°07.83'E	34.4
Sites in Monaco			
Monte Carlo (Port de Monaco)	31-May-05	43°44.16'N, 07°25.34'E	35.4
Monte Carlo (Port Fortville)	01-Jun-05	43°43.81'N, 07°25.15'E	35.8
Sites in North-East Spain			
Gandia	05-Feb-06	38°59.83'N, 00°09.38'W	33.1
Denia	03-Feb-06	38°50.71'N, 00°06.57'E	28.4
Moraira	03-Feb-06	38°41.18'N, 00°08.15'E	34.0
Calpe	03-Feb-06	38°38.34'N, 00°04.27'E	34.2
Mascarat	03-Feb-06	38°37.80'N, 00°00.17'W	22.5
Altea	03-Feb-06	38°35.49'N, 00°03.30'W	34.8