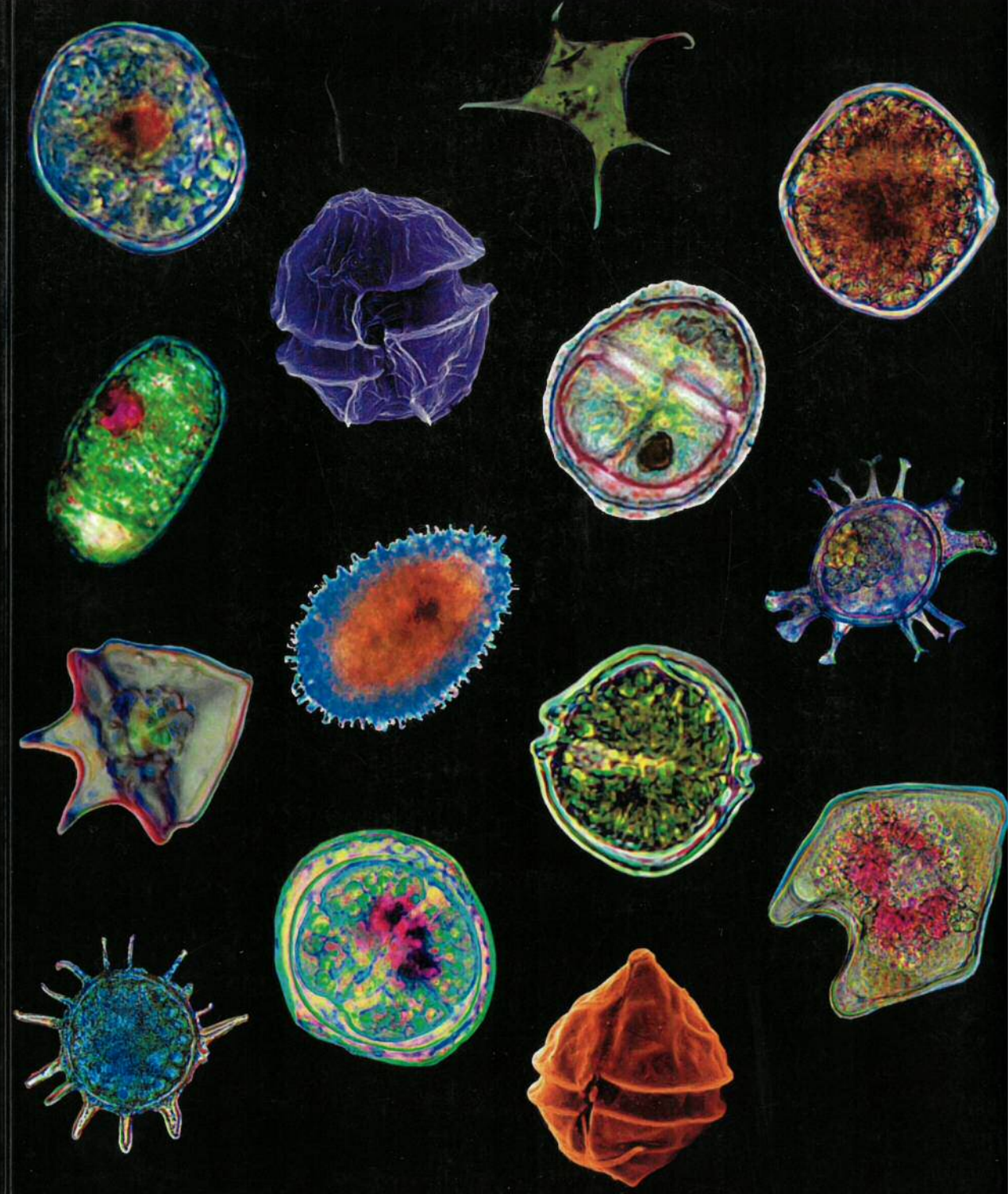


Role of life-cycle stages in dinoflagellate population dynamics

Sílvia Anglès

UNIVERSITAT DE BARCELONA

Institut de Ciències del Mar (CSIC)

Universitat de Barcelona

ROLE OF LIFE-CYCLE STAGES IN DINOFLAGELLATE POPULATION DYNAMICS

PAPER DELS ESTADIS DEL CICLE DE VIDA EN LA DINÀMICA DE POBLACIONS DE DINOFLAGEL·LADES

Sílvia Anglès i Calvo

Memòria presentada per optar al grau de Doctor
Programa d' Ecologia Fonamental i Aplicada
Departament d' Ecologia, Universitat de Barcelona
Bienni 1999-2001

Doctoranda	*Directora de la tesi*	*Tutora de la tesi*
Sílvia Anglès i Calvo	***Dra. Esther Garcés i Pieres***	***Dra. Pilar López Laseras***
Institut de Ciències del Mar	*Institut de Ciències del Mar*	*Universitat de Barcelona*

Barcelona 2010

Agraïments

Per fi estic en aquest punt, el moment dels agraïments! Això vol dir que la tesi arriba al seu final!! Realment, hi havia vegades on no el veia arribar...

Però no l'hauria acabat sense l'Esther. Els dies de la Marmota ja s'han acabat!!!! T'agraeixo de tot cor tot el teu suport, el qual no m'ha faltat mai, des del ben principi de la tesi i fins el seu final. Al llarg d'aquest temps, que no ha estat poc, m'has encomanat el teu entusiasme, i m'has anat transmetent els coneixements sobre les intimitats de les dinoflagel·lades, però sobretot sempre dins d'un embolcall d'humanitat. Espero que se'm "pegui" tot plegat encara que sigui una mica! Moltes gràcies Esther per ser com ets! I per tenir una família tan güai i comprensiva... i que encara tenen "fresca" l'ortografia!! (aquí tens els teus crèdits, Oriol!)

Darrera de l'Esther ha estat sempre el Jordi. Encara que aquesta tesi t'ha vingut de retruc, la teva ajuda ha estat inestimable! M'has aportat la teva visió global, i de vegades, pràctica, que falta em feia. Al final només pensava en cistos amunt i avall... I després, m'has donat suport anímic, que també és important! "Oh Leoncio, no lo conseguiremos..."

Aquesta tesi tampoc hagués estat possible sense els meus "peebles": Albert, Claudio, Eli, Eva, Laura, Magda i Nagore. Us agraeixo molt no només el vostre suport en el tram final de la tesi, sinó tota la vostra ajuda des de sempre en les campanyes, les mostres, els *Alexandriums*... els bons moments d'oci, els dinars, els "tea-times"... però sobretot el que sigueu els millors companys que una pugui tenir!

Tampoc em vull oblidar dels que ja no hi són al grup, però que sempre els trobarem a faltar: Kees (gracias por tu maña con las trampas y los cores!), Hassina (echo de menos nuestras charlas!), i Sonia (vaaaa, te llevaré en moto siempre que vengas a Barcelonaaaa). Especialment voldria agrair a la Memé haver confiat en mi en un primer moment i introduir-me en el intrigant món del fito. Qui ho anava a dir que acabaria així, oi? Recordo la teva frase: "No vols fer tesi?"

Gràcies també als IMEDEAs Gotzon i Benja. Gracias por tu ayuda, Gotzon, y por contar siempre conmigo. Y suerte de tí, Benja, y de tus dotes de McGiver! Gracias a los dos por hacer que las campañas no sean aburridas.

Dels integrants dels diferents projectes europeus en els que he tingut la sort de participar també n'he après molt. Isa, Santi, Rosa, qué suerte haberos conocido! Mención especial para las sardas, Antonella y Cecilia. Grazie Ceci por ser mi compañera de quistes y mi amiga! Me has ayudado a no sentirme sola en este mundo de sedimentos...

Thanks to Chris Gobler and the people from his Lab for their help and kindness during my stay. Gracias por supuestísimo a Ale y Jero, mi estancia hubiera sido muy diferente sin ustedes! Desde entonces llevo remera y voy a la pileta...

També vull agrair el suport de tots el companys de l'ICM, des dels tècnics, doctorands i científics, fins als que fan les tasques administratives i de manteniment. A tots gràcies!!! En especial, vull agrair als meus companys del "despacho molón" el seu bon rotllo: Claudio, Cristina, Juancho i Mireia. Vinga, que ara us toca a vosaltres! No em voldria oblidar dels companys del meu anterior despatx, que no han sigut pocs i segur que m'oblido d'algun... Ero (ánimo blondie!), Sergio, Andrés, José Antonio, Rodrigo (venga, te paso el testigo!), Irene, Clara, Pati...

Els meus amics han estat els millors en aguantar-me, tot i desaparèixer del mapa durant la recta final. L'Olga i el Quirino no només han patit la tesi, han patit exàmens i viscut feines vàries! Pero por suerte, hemos compartido mucho más que esto. Gracias por vuestra amistad, y por animarme en mis momentos de agobio. Una cenita con vosotros es la mejor terapia! Gràcies Blanca! El destí va fer que ens coneixèssim a l'ICM, i mostreig rera mostreig vam acabar sent amigues. Gràcies Diana pel teu carinyo infinit i per sempre donar-me suport, i per tots els moments viscuts! Gràcies Bibi i Amaia, mi abeja favorita. I gràcies als que m'ajuden en la distància, l'Elena i l'Ángel, la alegria del sur!!!

No puc oblidar tampoc agrair els bons moments als meus companys de facultat, amb els quals tinc la sort de mantenir el contacte, encara que sigui esporàdic: Cristina, Eva, Marta, Fanny, Tana, Marc (i Eli!), Magdala, Rosa...

Finalment, a la meva mare li vull agrair el que hagi confiat en mi sempre, fins i tot sense entendre ben bé què és el que faig amb aquests bitxos amb un nom tan raro. Mareta, sense el teu suport (i sacrifici en ocasions) no hagués aconseguit res d'això. I gràcies als meus germans, Sergi i Gerard, per ser tan ganàpies però alhora per tants bons moments compartits!

Agraïments també a la meva família "consort": gràcies per estar pendents d'aquesta segona tesi que us ha tocat passar!

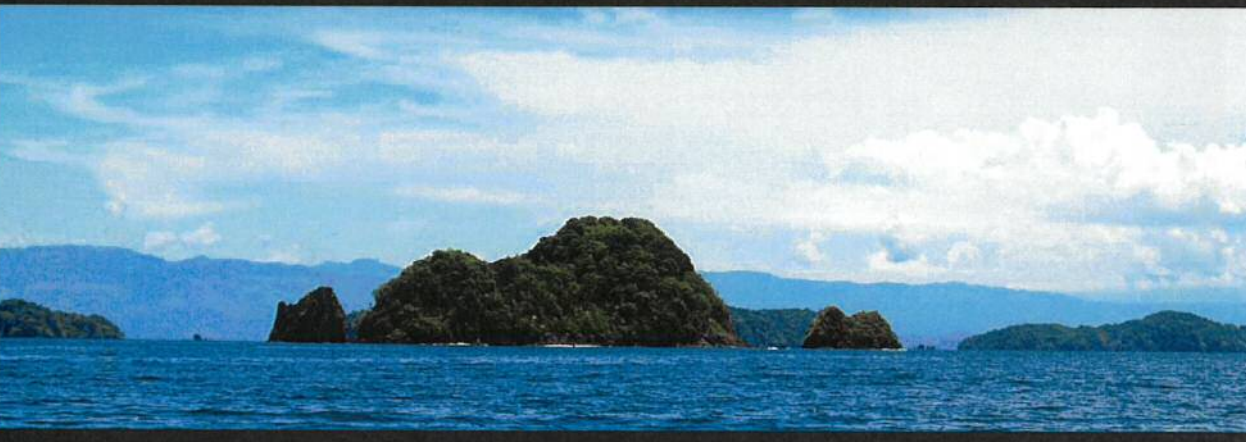
I, en últim lloc, last but not least, vull agrair-li al Toni tot el seu suport, amor i confiança. Aquesta tesi no existiria sense tu! Sort dels teus ànims... Sort de la teva ment matemàtica... Sort de que t'he conegut! Gràcies per aguantar-me, i per ajudar-me i estar amb mi en aquests últims dies estressants! Espero que continuïs aguantant-me molt de temps... et prometo que no faré més tesis!!!

Finançament: El treball desenvolupat en aquesta tesi ha estat finançat per un contracte dins del Projecte Europeu SEED (GOCE-CT-2005-003875) i del conveni amb l'Agència Catalana de l'Aigua (Programa de Vigilància de fitoplàncton nociu i tòxic a la costa catalana)

Funding: The work carried out during this thesis has been funded by a contract from de EU Project SEED (GOCE-CT-2005-003875) and from a collaboration agreement with the Agència Catalana de l'Aigua (Monitoring Programme for toxic and noxious phytoplankton in the Catalan coast)

Contents

Resum de la tesi



Resum de la tesi

Introducció

El fitoplàncton comprèn el conjunt d'organismes aquàtics unicel·lulars principalment autotròfics que realitzen la fotosíntesi, és a dir, produeixen matèria orgànica a partir de diòxid de carboni (CO_2) i aigua amb l'energia solar (producció primària). Constitueixen la base de la xarxa tròfica en el medi marí, proveint d'aliment a altres organismes de nivells tròfics superiors i també inferiors. El fitoplàncton és responsable d'aproximadament el 50% de la fotosíntesi total del planeta Terra (Field et al., 1998), contribuint a la producció d'oxigen (O_2) en el medi marí i a la captació de CO_2 de l'atmosfera. Són, per tant, una peça clau no només en els oceans sinó també en tot l'ecosistema planetari. De fet, la teoria actualment més acceptada sobre l'origen de la presència d'O_2 en l'atmosfera terrestre afirma que es deu a la fotosíntesi duta a terme pel fitoplàncton, concretament pels cianobacteris. El fitoplàncton es compon de diversos tipus d'organismes, des de procariotes (bacteris) fins a eucariotes (microalgues). Entre ells podem trobar grups importants com són les diatomees, els cianobacteris, els cocolitòfors i les dinoflagel·lades, grup en el qual es centra la present tesi.

Les proliferacions d'algues nocives

Les proliferacions són augments exagerats de l'abundància de cèl·lules d'una o vàries espècies de fitoplàncton respecte a la seva concentració basal quan les condicions ambientals són adequades. Aquestes proliferacions de fitoplàncton són part del funcionament normal dels ecosistemes, però les proliferacions de certes espècies poden ser perjudicials des del punt de vista humà. Aquest fet es coneix genèricament amb el nom de proliferacions d'algues nocives (PAN), un terme que no és gaire precís perquè no totes les espècies que formen PANs són algues, i no tots els episodis d'algues nocives impliquen el desenvolupament d'alts nivells de biomassa. És a dir, les espècies d'algues nocives (AN) pertanyen a grups de fitoplàncton diversos com dinoflagel·lades, diatomees i també cianobacteris, els quals no es consideren algues, sino procariotes. Per altra banda, l'abundància cel·lular necessària per donar lloc a episodis nocius pot ser des de només 100 fins a més de 1.000.000 de cèl·lules l^{-1}, depenent de cada espècie (Shumway, 1990; Smayda, 1997). De totes les espècies de fitoplàncton, es coneixen aproximadament 300 espècies d'AN, de les quals 80 són considerades tòxiques. En particular, les dinoflagel·lades són un dels principals grups d'AN, ja que un 75% de les espècies que causen proliferacions nocives pertanyen a aquest grup (Smayda, 1997b). Hi ha diversos tipus de PANs nocives, els quals generalment es poden agrupar en dos grups principals (GEOHAB, 2001):

1) Proliferacions d'espècies que produeixen toxines potents, les quals són acumulades per mol·luscs bivalves filtradors o per peixos, i es transfereixen a través de la xarxa tròfica a nivells superiors, com peixos, mamífers marins, i éssers humans. Una altra via d'intoxicació és per transport de toxines a través d'aerosols, afavorit per certes condicions hidrogràfiques i de vent. Els síndromes en humans associats a diferents tipus de toxines i d'espècies inclouen: l'enverinament paralitzant, diarreic, amnèsic, neurotòxic, i per azaspiràcids degut al consum de mol·luscs bivalves (PSP, DSP, ASP, NSP, i AZP, respectivament); l'enverinament per ciguatera pel consum de peixos (CFP); i problemes respiratoris i al·lèrgics provocats per la presència de toxines en aerosols. Per altra banda, existeixen altres tipus de toxines que són inofensives per als éssers humans però no per als peixos (ictiotoxines). Aquestes espècies d'AN tenen un impacte econòmic significatiu sobre l'aqüicultura degut a pèrdues en la producció de peixos o al tancament d'àrees de cultiu de marisc, i també suposen un perill pels recursos marins no comercials.

2) Proliferacions d'espècies que causen efectes nocius per a l'ecosistema, dins les quals s'inclouen proliferacions d'alta biomassa i proliferacions d'espècies productores d'escumes i mucílags. Els principals impactes ecològics d'aquestes proliferacions són la coloració de l'aigua, que causa una pèrdua de qualitat estètica, i l'anòxia, que causa la mort de la fauna marina que no pot escapar (per exemple, peixos en gàbies de piscifactories o invertebrats bentònics sèssils). Una gran abundància de cèl·lules fitoplanctòniques o la presència de mucílags també pot provocar la mort de peixos a gran escala per l'obstrucció de les seves brànquies. En conseqüència, a més dels efectes negatius sobre l'ecosistema, aquestes proliferacions també tenen un impacte econòmic en les activitats humanes (l'aqüicultura o el turisme).

Les PANs són fenòmens tant costaners com de mar obert, observats en una àmplia varietat d'ecosistemes com ara badies costaneres o sistemes d'aflorament. Les poblacions de fitoplàncton es veuen influïdes per interaccions físiques, químiques i biològiques que tenen lloc en un ampli rang d'escales temporals i espacials (GEOHAB, 2001). Factors físics rellevants inclouen tant processos de gran escala (el forçament climàtic, l'advecció o el transport de masses d'aigua), com processos de mesoescala (zones de convergència, fronts o surgències) o de petita escala (onatge o turbulència). Processos biològics inclouen la natació, l'agregació, la depredació, i les transicions en el cicle de vida dels organismes.

La zona costanera abasta una àmplia gamma d'entorns aquàtics oberts, tancats i semitancats al llarg de marges terrestres. Els sistemes costaners tancats o semitancats, que inclouen badies, estuaris, llacunes, platges semitancades i ports, són diversos en funció de la seva grandària, profunditat i règim hidrodinàmic, determinat pel grau d'aïllament físic del sistema respecte al mar obert. Tanmateix comparteixen característiques comunes, com una hidrodinàmica reduïda o les interaccions entre la terra i el mar, que les distingeixen

dels processos costaners a gran escala. A més, reben aportacions de material dissolt i partícules terrestres com ara nutrients, matèria en suspensió i oligoelements, tant de fonts naturals i com d'antropogèniques (Cembella et al., 2005).

Com a resultat de les limitacions físiques imposades per la costa i la baixa fondària, els processos de petita escala són fonamentals per determinar la circulació de l'aigua en aquests sistemes semi-tancats o tancats. La baixa hidrodinàmica combinada amb la morfologia del sistema típicament afavoreix la retenció de les masses d'aigua i redueix la dilució i l'intercanvi amb les aigües exteriors. Aquestes característiques ofereixen nínxols específics per a les espècies de fitoplàncton, incloent les espècies d'AN. Les espècies fitoplanctòniques són capaces d'aprofitar l'àmplia varietat de condicions ambientals que es troben en aquests sistemes semitancats o tancats mitjançant adaptacions (per exemple, estratègies consistint en canvis de fase en el cicle de vida o en comportament migratori) que permeten la seva supervivència, persistència i, en alguns casos, supremacia durant els episodis de proliferacions. Les interaccions físiques i biològiques a petita escala, com la interacció entre els organismes i la circulació de l'aigua, són essencials per l'advecció i l'acumulació de les poblacions, així com pel manteniment de certes proliferacions (Anderson i Stolzenbach, 1985; Basterretxea et al, 2005).

La recerca d'aquesta tesi es va dur a terme en sistemes costaners semitancats (dos ports, un golf i una platja), situats en el Mar Mediterrani nordoccidental i en l'Oceà Atlàntic nordoccidental.

Les dinoflagel·lades i el seu cicle de vida

Les dinoflagel·lades són uns dels grups de fitoplàncton més abundants, amb aproximadament 2.000 espècies reconegudes fins ara, de les quals més de 1.700 són marines (Graham i Wilcox, 2000; Smayda i Reynolds, 2003). Formen un grup divers en el qual la meitat de les espècies són fotosintètiques (autòtrofes) o combinen fotosíntesi amb depredació (mixòtrofes) i l'altra meitat s'alimenta d'altres organismes planctònics (heteròtrofes). A més, les dinoflagel·lades també inclouen espècies endosimbiòtiques d'invertebrats marins i protozous, i espècies paràsites (Gaines i Elbrächter, 1987; Taylor et al, 2008.).

Les primeres descripcions de dinoflagel·lades es van basar en observacions de mostres d'aigua vives o fixades obtingudes al camp. Per aquesta raó, la fase o l'estadi de les dinoflagel·lades més conegut és la cèl·lula vegetativa, que defineix la fase planctònica (Fig. 1). Les cèl·lules vegetatives són mòbils gràcies a la possessió de dos flagels, els qual són de diferent longitud. Un és el flagel transversal, que envolta la cèl·lula i permet la rotació, i l'altre és el flagel longitudinal, situat a la part posterior de la cèl·lula per propulsar-la cap endavant (Taylor, 1975). Les cèl·lules vegetatives són haploides i esdevenen divisió asexual per fissió binària, augmentant la població de manera exponencial.

Fins a les primeres observacions del cicle de vida de les dinoflagel·lades dutes a terme per Von Stosch (per exemple, Von Stosch, 1969, 1972, 1973), es creia en general que les dinoflagel·lades es reproduïen només de manera asexual. Fins i tot, les fases asexuals i sexuals d'una mateixa espècie es van descriure erròniament com a dues espècies independents. Actualment, es reconeix ampliament que les dinoflagel·lades també es reprodueixen sexualment i el nombre d'espècies reconegudes que presenten aquesta reproducció augmenta progressivament. Després d'un procés encara poc clar, les cèl·lules vegetatives es converteixen en gàmetes, dos dels quals es fusionen durant la reproducció sexual. La fusió de gàmetes pot implicar igual (isogàmia) o desigual (anisogàmia) mida de gàmetes i es pot produir entre cèl·lules d'idèntic (homotal·lisme) o diferent (heterotal·lisme) tipus de compatibilitat sexual. La fase resultant és el planozigot, una cèl·lula diploide amb dos parells de flagels (un de cada gàmeta fos) que roman mòbil durant diverses hores o pocs dies abans de convertir-se en un zigot sense mobilitat. Durant aquesta transformació, coneguda com a encistament, el planozigot perd tots els flagels i forma una paret gruixuda i resistent. Això dóna lloc al cist de resistència (o hipnozigot), que cau fins el sediment per entrar a la fase bentònica (Fig. 1). Després d'un període obligatori de latència que pot durar

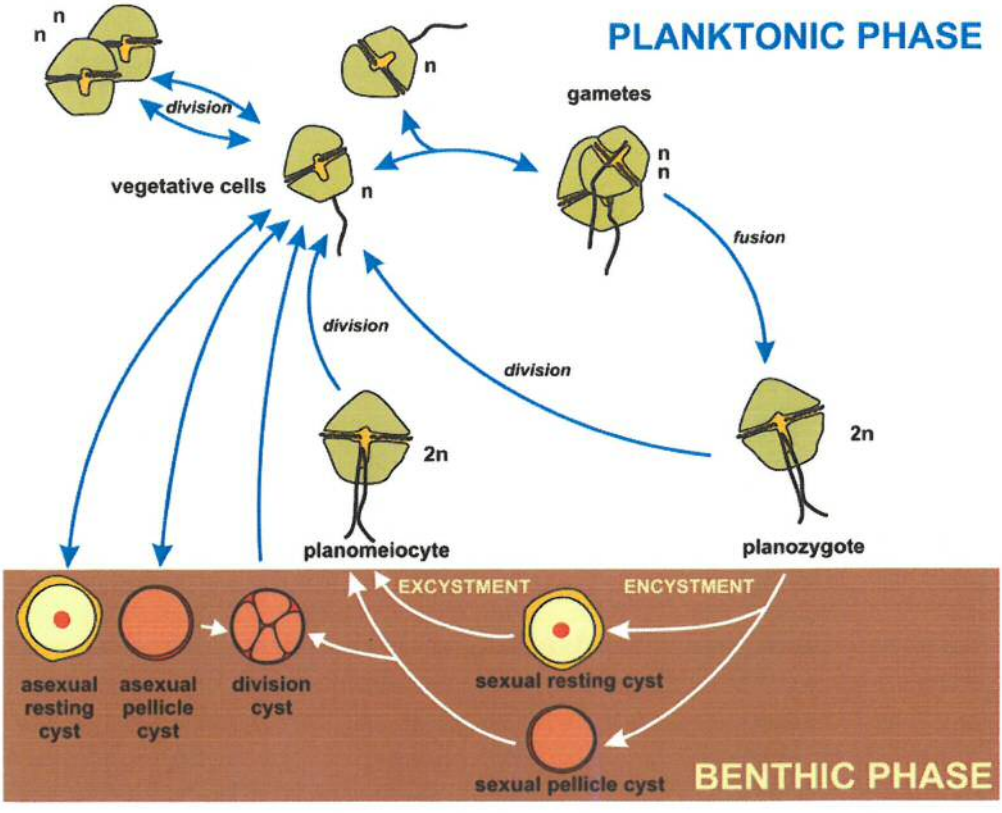


Fig. 1. Cicle de vida general de les dinoflagel·lades que produeixen cists (adaptat de Walker, 1984) actualitzat amb els nous estadis tal i com s'explica en el text.

dies o alguns mesos, la germinació del cist de resistència dóna lloc al planomeiozigot. Aquesta cèl·lula diploide mòbil es divideix per formar dues cèl·lules vegetatives haploides, restablint per tant la fase planctònica (Walker, 1984). La meiosi normalment es produeix en el zigot, però es desconeix el moment exacte en el que passa. Von Stosch (1973) va proposar la ciclosi nuclear com una característica de diagnòstic de la meiosi en les dinoflagel·lades, però aquesta característica no s'ha observat en moltes de les espècies estudiades.

Amb el desenvolupament de les tècniques de cultiu, els investigadors van ser capaços de cultivar espècies i seguir-ne el seu cicle de vida al laboratori. Com a resultat, un nombre creixent d'estudis científics han demostrat que els cicles de vida d'algunes dinoflagel·lades són relativament complexes. La figura 1 resumeix el cicle de vida general de les dinoflagel·lades formadores de cists i els principals resultats obtinguts fins ara:

1) Durant la fase planctònica, les cèl·lules vegetatives poden formar cists pel·liculars (també anomenats cists temporals) (Bravo et al, 2010; Dale, 1977; Walker, 1984). Aquest tipus de cist és diferent del cist de resistència: té una paret prima (pel·licle) i és capaç de germinar en poc temps (d'hores a dies) a causa de l'absència d'un període obligatori de latència. La formació del cist pel·licular s'ha associat tradicionalment a condicions d'estrès per a l'espècie, com ara canvis en temperatura o en llum, l'esgotament de nutrients, o també com a mitjà per evitar la depredació o l'atac de virus, bacteris i paràsits. Tanmateix, hi ha evidències de que els cists pel·liculars són una fase essencial en la dinàmica poblacional d'algunes espècies (veure revisió de Garcés et al., 2002). Altrament s'ha observat que en algunes espècies la divisió cel·lular es pot produir en cists pel·liculars (els anomenats cists de divisió), procés que dóna lloc a dues o més cèl·lules vegetatives (Figueroa i Bravo, 2005; Garcés et al, 1998; Kita et al, 1985).

2) Les cèl·lules vegetatives també poden produir cists de resistència asexuals (Kremp i Parow, 2006). Aquests cists tenen la mateixa morfologia i el mateix període obligatori de latència de diversos mesos que els cists de resistència sexuals produïts mitjançant la formació de planozigots. A més, s'han observat cèl·lules vegetatives que poden formar una fase temporal en forma de cist, encara no ben entesa, que pot donar lloc o bé a cèl·lules mòbils en uns pocs dies o bé romandre viables fins a dos anys (Rintala et al., 2007).

3) Encara que la transformació del planozigot en un cist de resistència és la via més comú en les espècies productores de cists, recentment s'ha observat que la fase de planozigot de certes espècies pot seguir múltiples rutes subseqüents: i) divisió i reversió de la fase asexual (cèl·lula vegetativa), ii) encistament com cist de resistència sexual, iii) encistament com cist pel·licular sexual que pot dividir-se (Uchida, 1991; Figueroa et al., 2006, 2006b).

4) Els gàmetes poden tornar a la fase asexual, dividint-se per fissió binària en lloc de fusionar-se (Figueroa et al., 2006a, 2006c).

El cicle de vida de les dinoflagel·lades i la dinàmica de poblacions

La dinàmica de poblacions descriu els canvis que experimenta la població en l'espai i en el temps al llarg de la seva vida i els factors que desencadenen aquests canvis. Una estratègia d'adaptació comú de les dinoflagel·lades, incloent les espècies d'AN, és un cicle de vida amb fases de resistència. En conseqüència, les espècies de fitoplàncton es poden dividir en dos grans grups en funció del seu cicle de vida: holoplanctòniques (presents en l'aigua durant tot l'any) i meroplanctòniques (amb un estadi de resistència documentat que habita al bentos). En les espècies meroplanctòniques, l'alternança de les fases planctònica i bentònica implica l'existència de diferents estadis amb diferents ploïdies que ocupen distints nínxols en l'espai i en el temps. Diferents factors físics, químics i biològics interactuen i regulen les diferents fases, així com les seves alternances, amb implicacions importants per a la supervivència de les espècies (Steidinger i Garcés, 2006; Valero et al., 1992.). Per tant, la recerca sobre la dinàmica de poblacions de dinoflagel·lades ha d'incorporar totes les fases del seu cicle de vida i els factors ambientals que les regulen. Aquest tipus de recerca es pot desenvolupar al llarg de tres línies principals:

1) Estudi de la dinàmica de la fase planctònica de les espècies objectiu en diversos sistemes semitancats.

2) Identificació i quantificació de la fase bentònica i estudi de la seva dinàmica. En aquesta tesi, el terme fase bentònica fa referència als cists de resistència sexuals.

3) Avaluació de les transicions entre les fases planctònica i bentònica (germinació i d'encistament) de les espècies objectiu en els sistemes semitancats seleccionats.

La recerca desenvolupada en aquesta tesi consisteix en una anàlisi de la dinàmica de les espècies objectiu en diversos sistemes semitancats. En aquests llocs, la dinàmica de les proliferacions varia tant en funció de les característiques topogràfiques i hidrogràfiques com també de les peculiaritats ecològiques i biològiques dels organismes que les causen. No obstant, és possible extreure conclusions generals sobre els processos importants que controlen la dinàmica de les proliferacions en ecosistemes similars (Anderson et al., 2005; GEOHAB, 2001). Les espècies objectiu de la present tesi pertanyen sobretot al gènere *Alexandrium*, un grup de dinoflagel·lades que formen cists i que depenen en gran mesura de la germinació d'aquests cists per a la iniciació i la recurrència de les seves proliferacions. A més, aquest gènere inclou 13 espècies nocives, 10 de les quals es troben entre les més tòxiques (Moestrup et al., 2004). També és un dels gèneres principals que causen episodis tòxics al Mar Mediterrani (Vila et al., 2001). Les espècies d'*Alexandrium* es poden estudiar en diferents hàbitats, on la importància relativa de les fases del cicle de vida pot variar segons la profunditat, l'advecció i altres factors.

A continuació, es mostra el marc general de l'estat actual de les tres línies de recerca mencionades anteriorment, alhora que s'indiquen les mancances en el coneixement científic actual, algunes de les quals s'han abordat en aquesta recerca doctoral.

La fase planctònica

La dinàmica de la fase planctònica és particularment interessant en el marc de les PANs perquè els episodis tòxics o nocius es deuen a l'estadi vegetatiu. Per tant, és essencial entendre perquè i quan es produeixen les PANs, així com, una vegada iniciada la proliferació, la durada i la intensitat de la fase planctònica. En les espècies que produeixen cists, els diferents estadis del cicle de vida juguen un paper específic i important en cada fase del desenvolupament de la proliferació, amb fortes implicacions per a la seva dinàmica:

1) Iniciació de la proliferació: La primera fase de la proliferació requereix un inòcul de cèl·lules vegetatives, que poden provenir de la germinació dels cists de resistència. Steidinger (1975) va proposar el terme botànic "planter de llavors" per referir-se als estadis de resistència que actuen com a subministradors de l'inòcul de proliferacions arreu del món, destacant la importància dels cists de resistència en la iniciació de proliferacions. L'inòcul pot provenir de cists de resistència germinats a la mateixa zona on la proliferació es desenvolupa o pot ser transportat des de planters de llavors de zones adjacents. Els factors que desencadenen l'inici de la proliferació són poc coneguts per a la majoria de dinoflagel·lades nocives, principalment a causa de la dificultat d'obtenir dades suficients durant el període anterior a la proliferació i de les condicions inicials d'aquesta.

2) Creixement exponencial i manteniment: Durant el creixement exponencial, la cèl·lula vegetativa respon a les condicions ambientals (com llum, fotoperíode, disponibilitat de nutrients o preses, temperatura, salinitat i turbulència), que determinen el creixement i l'augment de la biomassa de la població (Steidinger, 1975; Steidinger i Garcés, 2006). Durant la fase de manteniment, la població reflecteix l'equilibri entre el creixement vegetatiu i els factors de pèrdues, que inclouen la depredació, la mort cel·lular, el parasitisme i la dispersió, entre d'altres (Burkholder et al., 2006; Taylor, 1987). En aquesta fase de la proliferació, qualsevol estratègia d'adaptació per afavorir el manteniment de la població és crítica. Cèl·lules vegetatives de moltes dinoflagel·lades formen cists pel·liculars capaços de suportar condicions ambientals adverses (Garcés et al., 1999). A més, la migració vertical és una estratègia per arribar a les zones més riques en llum o nutrients, així com per a evitar la dispersió de cèl·lules (Basterretxea et al., 2005; Eppley et al., 1968). En aquest sentit, és necessari el coneixement sobre l'acoblament dels factors físics amb el comportament biològic per comprendre tant la distribució vertical i horitzontal com la dinàmica de la proliferació d'una espècie. No obstant, aquestes interaccions a petita escala sovint no s'han quantificat acuradament en el camp, ja que la distribució del fitoplàncton tendeix a ser desigual en l'espai i el temps. Per tant, els estudis de la dinàmica de proliferacions es poden millorar de forma significativa amb la utilització d'instruments capaços de mesurar amb una resolució espacial i temporal adequada (Babin et al., 2005, 2008).

3) Finalització: L'acabament de la proliferació es sol atribuir a condicions adverses pel creixement vegetatiu (per exemple, temperatures o salinitats desfavorables, llum insuficient i falta de nutrients). Altres factors ambientals poden ser la mortalitat cel·lular, la depredació, la competència entre espècies, la dispersió, i la transició de les cèl·lules vegetatives a la fase de resistència (Anderson et al., 1983; Burkholder et al., 2006; Calbet et al., 2003). En molts casos, les causes de la finalització de les proliferacions no s'han aclarit completament, probablement perquè impliquen una combinació de factors (Garcés et al., 2004; Van Lenning et al., 2007).

Al llarg d'aquesta tesi, es va investigar la dinàmica de la fase planctònica, en concret les fluctuacions temporals de les cèl·lules vegetatives a llarg termini (Articles 3 i 4) i les seves variacions espacials i temporals durant el desenvolupament de les proliferacions (Articles 3, 4 i 5). A més, es va avaluar la variabilitat de petita escala espacial i temporal de les cèl·lules vegetatives durant el manteniment d'una proliferació utilitzant tecnologies d'alta resolució (laser in situ scattering and transmissometry, LISST) per determinar l'acoblament físic-biològic subjacent en aquesta fase de la proliferació (Article 1).

La fase bentònica

Els primers estudis sobre els cists de dinoflagel·lades es van realitzar al segle XIX per palinòlegs que treballaven amb microfòssils dels sediments. No obstant, degut a la seva morfologia, tan diferent respecte a les cèl·lules planctòniques, aquests cists no es van relacionar ni amb dinoflagel·lades ni es van identificar com cists de resistència. La investigació paleontològica en "histricosferes", tal i com se'ls anomenava en aquell temps, va augmentar considerablement des de la dècada de 1930 per la seva importància com a indicadors paleoecològics. Tanmateix, els biòlegs interessats en estudiar les dinoflagel·lades planctòniques no van fixar-se gaire en els cists de resistència, llevat d'unes poques excepcions com ara els estudis sobre l'encistament de dinoflagel·lades per Huber i Nipkow (1922, 1923), Braarud (1945) i de Nordli (1951), qui va suggerir que les histricosferes eren cists de dinoflagel·lades. A més, un altre grup de palinòlegs dedicats a la recerca paleoclimàtica també trobaren histricosferes i les consideraren com restes d'organismes extints. En canvi, Erdtman (1949, 1950, 1954) va demostrar que no estaven extingits en proporcionar proves de representants vius d'histricosferes recuperats de trampes de sediment. Tot i així, el fet de l'existència d'un equivalent viu de les histricosferes no va ser reconegut per la comunitat de palinòlegs fins l'evidència definitiva aportada per Evitt (1961): la presència d'una obertura (arqueopil) en el cist i de característiques comunes entre les fases mòbils i els cists fòssils (paratabulació). Mentrestant, Wall i Dale van demostrar que les histricosferes mostrejades en els sediments produïen cèl·lules mòbils de dinoflagel·lades, proporcionant la primera evidència de germinació i del paper de les histricosferes com a cists de resistència de

dinoflagel·lades (Wall, 1965; Wall i Dale, 1966). Aquest descobriment de la connexió entre les histricosferes i els cists de resistència de les dinoflagel·lades va revolucionar els camps de la palinologia i de la biologia. Els palinòlegs van iniciar nombroses investigacions per trobar els cists fòssils i les seves corresponents cèl·lules vives, les quals van donar lloc a una extensa classificació taxonòmica de cists ("dinocists"). Al mateix temps, els estudis biològics van estar (i segueixen estant) més focalitzats en el paper ecològic dels cists de resistència com a zigots i en les seves espècies biològiques equivalents. Així, no sorprèn que com a resultat de les dues línies d'investigació independents hi hagi dues denominacions taxonòmiques diferents pels cists de les dinoflagel·lades: la palinològica i la biològica. Per tal d'unir les dues branques i compartir els coneixements entre els investigadors d'ambdós camps, Evitt va iniciar una sèrie de conferències ("The International Conference on Modern and Fossil Dinoflagellates"), la primera de les quals va tenir lloc a Colorado Springs el 1978 i que encara avui es segueix celebrant de forma regular. En aquesta tesi, els cists són examinats des del punt de vista biològic.

Avui en dia es coneixen aproximadament 200 espècies de dinoflagel·lades que produeixen cists de resistència (Head, 1996), una xifra que augmenta progressivament i que potser mai reflectirà amb exactitud la que hi ha en realitat. Els cists de resistència són fases essencials en l'ecologia de les dinoflagel·lades. Les funcions d'aquesta fase bentònica, modificades a partir de les cinc primeres funcions descrites per Wall (1971) i completades segons els coneixements més actuals, inclouen:

1) Variabilitat genètica: Els cists de resistència representen una de les etapes sexuals del cicle de vida de les dinoflagel·lades que contribueixen a la recombinació cromosòmica, amb implicacions òbvies per a la plasticitat, el *fitness*, i l'èxit de l'espècie (Figueroa et al., 2006c).

2) Recurrència: Els cists de resistència són una font de llavors per a l'inici i la recurrència de les proliferacions vegetatives. Prakash (1967) va ser el primer en introduir la hipòtesi de que la fase bentònica està involucrada en la recurrència de les PANs, i estudis posteriors van confirmar l'ocurrència de proliferacions recurrents en els llocs on els cists de resistència "hivernen" en els sediments (per exemple, Anderson i Wall, 1978; Dale, 1977).

3) Estacionalitat: Braarud (1962) va suggerir que l'estacionalitat observada en diverses espècies de fitoplàncton està relacionada amb l'alternança de fases del seu cicle de vida, i estudis posteriors van demostrar que els cists de resistència són responsables de la sincronització entre l'inici de la proliferació i els factors ambientals estacionals (Anderson i Wall, 1978; Anderson i Morel, 1979).

4) Supervivència: La durada de la fase bentònica és molt més llarga que la fase vegetativa i la seva resistència a entorns inadequats és molt més gran, cosa que permet la supervivència de les espècies quan les condicions ambientals són desfavorables.

5) Dispersió de les espècies: Els cists de resistència poden afavorir la dispersió geogràfica. Poden ser transportats pels corrents com a partícules passives i dispersats segons la dinàmica sedimentària, ampliant l'àrea de distribució geogràfica d'aquestes espècies a través de la colonització de nous territoris (Dale, 1983). A més, els cists de resistència són un vector important de dispersió d'espècies a través de l'aigua de llast de les embarcacions o durant la transferència de mol·luscs bivalves d'una zona a una altra (Hallegraeff et al., 1993).

6) Defensa: S'ha suggerit que els cists de resistència són un mecanisme per evitar la depredació tant en ambients marins com d'aigua dolça (Hansson, 1996; Rengefors et al., 1998).

7) Biodiversitat: Els cists de resistència formen un "banc de llavors" que es pot activar quan les condicions ambientals són adequades, garantint així la continuïtat de l'espècie en l'espai i el temps i constituint un reservori de biodiversitat (Boero et al., 1996; Margalef, 1994, 2002). En altres paraules, els cists de resistència representen una part de la memòria genètica d'un ecosistema.

Morfologia dels cists de resistència

Els cists de resistència de les dinoflagel·lades són fases bentòniques immòbils que tenen una paret gruixuda de diverses capes (Evitt et al., 1977; Von Stosch, 1973). La composició de les diferents capes de la paret no s'ha determinat del tot, i pot variar depenent de l'espècie. Les parets de la majoria dels cists de resistència es componen de matèria orgànica (cists orgànics). Alguns contenen cel·lulosa, mentre que en d'altres s'ha identificat un polímer complexe semblant a l'esporopol·lenina, anomenat dinosporina. Es considera que la dinosporina és responsable de la fossilització dels cists de resistència (Dale, 1983; Fensome et al., 1993). A més, els cists de resistència de vàries espècies estan mineralitzats amb calcita (cists calcaris). Les parets dels cists de resistència poden ser llises o presentar una gran varietat d'ornaments, com espines, protuberàncies, banyes o estructures calcàries. La forma dels cists de resistència pot ser esfèrica, el·lipsoïdal o ovoide, o poden assemblar-se a la de la cèl·lula vegetativa. El seu contingut es compon d'un nucli, un o diversos cossos d'acumulació pigmentats amb tonalitats de groc a vermell (com a resultat de l'acumulació de pigments carotenoides derivats de la degradació de plastidis) i una sèrie d'elements d'emmagatzematge, com ara grans de midó o gotetes d'oli (Chapman et al., 1981). En comparació amb les cèl·lules vegetatives, hi ha una reducció o desaparició de les estructures citoplasmàtiques, inclosos els cloroplasts i cossos de Golgi (Bibby i Dodge, 1972). Totes aquestes característiques morfològiques (forma, mida, tipus d'ornamentació i contingut) s'utilitzen com a criteri taxonòmic per a la classificació dels cists de resistència.

Una part fonamental d'aquesta tesi ha consistit en la identificació taxonòmica dels cists de resistència de les dinoflagel·lades en ecosistemes costaners (Articles 2, 3, 4 i 5).

Dinàmica dels cists de resistència

La reducció de l'activitat metabòlica (Bibby i Dodge, 1972) dels cists de resistència els permet sobreviure durant diversos anys soterrats en el sediment. Aquesta supervivència també es veu afavorida per la seva paret gruixuda i densa, que resisteix l'atac biològic (per exemple, bacteris i fongs) i químic (Dale, 1983). Encara que el temps de supervivència varia per a cada espècie, la mitjana pot ser de 2 a 10 anys (Keafer et al., 1992). Recentment, Ellegaard et al. (2008) han aconseguit fer germinar cists aïllats de sediments de fa 100 anys.

Els cists de resistència en els sediments es comporten com a partícules sedimentàries passives i, en conseqüència, estan controlats principalment pel règim sedimentari. Atès que el comportament d'aquestes fases bentòniques s'assembla a la de la fracció fina del sediment (llims i argiles), les zones amb major proporció de sediments fins tenen en general més abundàncies de cists de resistència (Dale, 1976). Així, els processos que determinen la dispersió i acumulació del sediment influencien també l'abundància i la distribució horitzontal i vertical de cists de resistència. Altres processos inclouen factors biòtics i abiòtics, com la germinació, la mortalitat, la depredació, la formació de cists de resistència, l'enterrament, la bioturbació per organismes bentònics i la resuspensió (Anderson et al., 1982; Giangrande et al., 2002; Persson, 2000).

El mostreig dels cists de resistència en els sediments proporciona informació sobre les espècies presents, la seva distribució i abundància. La major part de la recerca en aquest camp s'ha centrat en les espècies que conformen el conjunt de cists de resistència (banc de llavors) en els sediments. El principal objectiu d'aquests estudis ha estat avaluar la distribució biogeogràfica de les espècies actuals o passades (Amorim i Dale, 2006; Bolch et al., 1990; Joyce, 2004; Nehring, 1994) o determinar la seva relació amb la presència de cèl·lules vegetatives i les condicions ambientals en la columna d'aigua (An et al., 1992; Godha et al., 2003; Matsuoka et al., 2003). El mostreig dels cists de resistència també és una forma útil per detectar espècies rarament observades en fase planctònica i per avaluar el potencial de futures proliferacions (Bravo et al., 2006; Joyce, 2005). Malgrat tot, hi ha pocs estudis de camp sobre la dinàmica dels cists de resistència en els sediments que hagin considerat els mecanismes biològics, físics i ambientals que controlen l'abundància i la distribució espacial i temporal dels cists. A més, no es coneix com aquests mecanismes interactuen entre sí.

Es va avaluar i comparar la diversitat del banc de llavors de les dinoflagel·lades a dos llocs semitancats del Mar Mediterrani (Article 2). A més, es va determinar la distribució espacial i les variacions temporals dels cists de resistència per tal de: i) estudiar la influència de la resuspensió causada per processos físics en la distribució dels cists de resistència i la relació d'aquesta distribució amb la formació de cists de resistència a la columna d'aigua (Article 3), i ii) obtenir informació bàsica que proporcioni una millor comprensió de les transicions entre fases del cicle de vida (Articles 4 i 5).

Transicions entre les fases planctòniques i bentòniques

Les transicions entre les fases planctònica i bentònica són un component clau per a les espècies productores de cists. Els principals processos de transició en el cicle de vida d'aquestes espècies són la germinació i l'encistament (en aquesta tesi es consideren com la transició de cist de resistència a cèl·lula vegetativa i de cèl·lula vegetativa a cist de resistència, respectivament). Aquestes transicions entre les fases planctònica i bentònica estan marcades per característiques particulars i modulades per diversos factors tant endògens com exògens.

Germinació

La germinació dels cists de resistència es produeix després d'un període obligatori de latència, que es defineix com el temps necessari per a la maduració. Durant aquest període, hi ha una inhibició endògena de la germinació, fins i tot sota condicions ambientals favorables. La durada d'aquest període de latència és específic de cada espècie i pot estar afectat per condicions ambientals com la temperatura (Anderson, 1980) i factors genètics (per exemple, l'herència, Figueroa et al., 2005). També s'ha suggerit que la quantitat de productes de reserva emmagatzemats afecta la durada del període de latència (Steidinger i Haddad, 1981).

Una vegada que es supera el període obligatori de latència, els cists de resistència poden germinar si les condicions ambientals són favorables, o, en cas contrari, romandre en un estat de quiescència (inactius) a l'espera d'aquestes condicions. D'entre els factors ambientals que modulen la germinació dels cists de resistència de les dinoflagel·lades, la temperatura s'ha reconegut com a un d'important (Dale, 1983; Pfiester i Anderson, 1987). Les espècies tenen un rang de temperatura dins el qual els cists de resistència germinen (Anderson i Morel, 1979). Els estudis demostren que un desencadenant important de la germinació és un canvi en la temperatura cap a nivells favorables, com passa amb l'escalfament o refredament estacional en aigües temperades (Anderson, 1980). De forma similar a la temperatura, també hi ha un rang de salinitat òptim per a la germinació de cada espècie (Band-Schmidt et al., 2003; Canon, 1993; Kim et al., 2002). Les concentracions intracel·lulars de nutrients també poden ser un factor per acabar amb la quiescència (Steidinger i Haddad, 1981), encara que els efectes de les concentracions de nutrients ambientals només s'han estudiat en experiments de laboratori. D'acord amb aquests estudis, els nivells de nutrients necessaris per a la germinació depenen de cada espècie. Per exemple, la germinació dels cists de resistència d'algunes espècies d'*Alexandrium* no està influenciada per la concentració de nutrients (com *A. tamarense*, Anderson, 1980; o *A. minutum*, Canon, 1993), mentre que altres estudis han mostrat un efecte negatiu de l'abundància de nutrients en *A. catenella* (Figueroa et al., 2005). Per contra, l'esgotament de nutrients retarda o inhibeix la germinació d'altres espècies de dinoflagel·lades (per exemple, *Scrippsiella trochoidea*, Binder i Anderson, 1987; o *Ceratium hirundinella*, Rengefors i Anderson, 1998). Segons

Rengefors et al. (1996), com que els nivells de nutrients externs són un paràmetre crític que afecta la viabilitat i la supervivència de les cèl·lules germinades, el cists de resistència a punt de germinar poden ser capaços d'utilitzar els nutrients presents en l'aigua. Aquesta hipòtesi està recolzada per l'evidència de que els cists de resistència de *S. trochoidea* són capaços d'absorbir fòsfor inorgànic. No obstant, fins ara, la capacitat d'absorció de fòsfor no s'ha confirmat per altres dinoflagel·lades ni tampoc per altres nutrients, com el nitrogen. Es coneix que l'anòxia i la foscor, condicions que envolten els cists de resistència en els sediments, inhibeixen la germinació dels cists de resistència en la majoria d'espècies (Anderson et al., 1987). Aquesta inhibició de la germinació té diverses implicacions: la germinació de cists de resistència es limita als mil·límetres superiors de la superfície del sediment, mentre que els cists de resistència en les capes més profundes continuen sense germinar a menys que factors externs (com la bioturbació o la resuspensió) afavoreixin un entorn amb oxigenació i irradiància suficients. Pocs estudis de laboratori han tractat la influència de diferents intensitats de llum, però de nou els resultats mostren diferents efectes segons l'espècie. Per exemple, la germinació d'*A. minutum* varia segons les diferents intensitats de llum, amb taxes més altes observades a nivells de llum tan baixos com 20 $\mu E\ m^{-2}\ s^{-1}$ (Canon, 1993; Blanco et al., 2009), mentre que els cists de resistència d'*A. tamarense* germinen amb taxes similars independentment de la intensitat de llum. Finalment, es coneix l'existència d'un rellotge endogen anual que modula la germinació de cists de resistència de poblacions que habiten en zones profundes de l'oceà, on les senyals ambientals com ara la llum són pràcticament absents (cists d'*A. tamarense* en el Golf de Maine, Anderson i Keafer, 1987; Matrai et al., 2005).

Encistament

La fusió dels gàmetes dóna lloc al planozigot, una cèl·lula mòbil que sovint s'assembla a la cèl·lula vegetativa, una de les raons per les quals els planozigots han estat confosos freqüentment amb cèl·lules vegetatives. No obstant, el planozigot es pot distingir morfològicament pels seus dos flagels longitudinals i pel fet que normalment és més gran, més fosc, i es mou més lentament que les cèl·lules vegetatives (Anderson et al., 1983; Probert, 1999). Després de la seva formació, els planozigots conserven la seva mobilitat durant uns dies o diverses setmanes, fins a la finalització de l'encistament. Els processos pels quals el planozigot es converteix en un cist de resistència no es coneixen amb exactitud. La formació dels cists de resistència en dinoflagel·lades s'ha vinculat generalment a condicions inadequades pel creixement. En concret, la limitació de nutrients s'ha utilitzat àmpliament en estudis de laboratori per induir la sexualitat i la producció de cists de resistència. Conseqüentment, s'ha acceptat que l'esgotament de nutrients és el desencadenant principal que estimula la sexualitat i l'encistament. Malgrat aquestes troballes de laboratori, les observacions de formació de cists de resistència al camp no han recolzat aquesta relació entre la baixa disponibilitat de nutrients i l'encistament (Garcés et al., 2004; Kremp i Heiskanen, 1999; Pitcher et al., 2007; Paret, 1970). A més, Sgrosso et al. (2001) van observar la formació de cists de resistència amb nivells alts de nutrients

en experiments de laboratori. Per tant, alguns autors argumenten que unes condicions ambientals òptimes són necessàries per a l'encistament (Wall, 1970). Aquestes discrepàncies poden estar causades per deficiències en els nutrients intracel·lulars i no en els nivells de nutrients externs, ja que s'ha mostrat que hi ha una disminució dels nivells de nutrients interns durant la transició de les cèl·lules vegetatives a la fase sexual. D'altra banda, l'estat general en el metabolisme del carboni de les cèl·lules sembla que també influeix en la resposta sexual (Probert, 1999). Això implica la necessitat d'avaluar la limitació de nutrients en el context dels nutrients intracel·lulars i l'estat metabòlic enlloc de la disponibilitat de nutrients externs, però aquests paràmetres són difícils de mesurar en condicions de camp.

També s'han documentat altres factors que influeixen l'encistament, com els canvis de temperatura, la durada del dia o la presència de bacteris o paràsits (Adachi et al., 1999; Pfiester i Anderson, 1987; Sgrosso et al., 2001). La densitat cel·lular pot ser un paràmetre important. Wyatt i Jenkinson (1997) van suggerir que s'ha d'assolir un cert llindar en l'abundància de cèl·lules abans de procedir a la fase d'encistament. De fet, diversos estudis van mostrar que els planozigots de les espècies *S. trochoidea* i *Gyrodinium instriatum* no s'encisten si la densitat cel·lular està per sota d'un cert llindar (Uchida 1991; Uchida et al., 1996). En un estudi de camp, Garcés et al. (2004) també va observar un llindar en l'abundància abans de començar l'encistament. Uchida (2001) va concloure que el contacte cel·lular és un factor potencialment determinant per a l'encistament dels planozigots. De fet, l'èxit d'aparellament pot augmentar a mesura que les possibilitats de comunicació cel·lular augmenten, de manera que la necessitat de contacte cel·lular no pot ser descartada (Wyatt i Jenkinson, 1997). En experiments de laboratori, sovint s'ha observat en plaques de cultiu l'agrupació de gàmetes i la formació de grumolls de cists de resistència (Uchida et al., 1996). En el camp, encara que les observacions són més escasses, s'han detectat planozigots en convergències frontals dins de la columna d'aigua (Tyler et al., 1982). Els hipotètics senyals de comunicació podrien ser substàncies químiques com les feromones, estímuls mecànics, o molècules de "detecció de quòrum" anàlogues a les utilitzades pels bacteris (Persson et al., 2008; Uchida 2001; Wyatt i Jenkinson 1997).

En resum, els processos de germinació i encistament poden influir en la dinàmica de les poblacions de les espècies productores de cists mitjançant la determinació de l'inòcul per a la iniciació de la proliferació i la definició de la mida de la població de cists, respectivament. Dades in situ d'aquestes transicions i dels factors endògens i exògens que les regulen són necessaris per millorar el nostre coneixement del cicle de vida de les espècies individuals. Aquesta informació permetrà la comparació entre espècies i generar i parametritzar models de la dinàmica poblacional.

En aquesta tesi s'ha quantificat in situ la germinació i l'encistament de certes espècies objectiu, i els resultats obtinguts s'han integrat amb els que descriuen la dinàmica de les fases planctònica i bentònica per tal d'aprofundir en el coneixement de la dinàmica poblacional d'aquestes espècies (Articles 4 i 5).

Objectius

Aquesta tesi vol aprofundir en el coneixement sobre la dinàmica poblacional de les dinoflagel·lades mitjançant la integració de les fases planctònica i bentònica. L'objectiu principal és l'avaluació del paper de les diferents fases del cicle de vida utilitzant metodologies que impliquen l'observació i l'experimentació en condicions de camp. Atès que els estudis sobre la fase bentònica són més escassos, una gran part de la recerca desenvolupada en aquesta tesi s'ha centrat en aquesta fase del cicle de vida.

D'acord amb les línies de recerca principals exposades en la Introducció general, els objectius específics d'aquesta tesi són:

Estudi de la dinàmica de la fase planctònica de les espècies objectiu en diversos sistemes semi-tancats:

Investigar la variabilitat espacial i temporal a curt termini de la fase planctònica durant el manteniment de la proliferació i els factors físics i biològics que la causen utilitzant la tecnologia LISST (Article 1)

Avaluar les fluctuacions temporals a llarg termini de l'estadi vegetatiu (Articles 3 i 4)

Caracteritzar i quantificar les diferents fases del cicle de vida durant el desenvolupament de la proliferació: les cèl·lules vegetatives (Articles 3, 4 i 5) i planozigots planctònics (Article 5)

Identificació i quantificació de la fase bentònica i recerca sobre la seva dinàmica:

Avaluar i comparar la diversitat dels cists de resistència en els sediments de sistemes semitancats (Article 2)

Determinar la distribució temporal i espacial de cists de resistència de les espècies objectiu en els sediments en determinats llocs semitancats (Articles 3, 4 i 5)

Investigar el paper del procés físic dominant (resuspensió forçada per seixa) i del procés biològic de producció de fases de resistència (l'encistament) en la dinàmica de la distribució de cists de resistència.

Avaluació de les transicions entre les fases planctònica i bentònica de les espècies objectiu en els sistemes semitancats seleccionats:

Quantificar el flux dels estadis del cicle de vida des de i cap als sediments (Articles 4 i 5), i estimar in situ les taxes de germinació (Article 4) i d'encistament (Articles 4 i 5)

Investigar els factors que influeixen en la germinació i l'encistament, i la contribució d'aquestes transicions al desenvolupament de les proliferacions (Articles 4 i 5)

La recerca desenvolupada en la present tesi per abordar aquests objectius es presenta en cinc articles científics, resumits a continuació.

Article 1: Distribució espacial i temporal d'alta resolució d'una proliferació costanera de fitoplàncton amb l'instrument *laser in situ scattering and transmissometry* (LISST)

Els recents avenços tecnològics han permès el desenvolupament de noves tecnologies d'observació òptica que poden proporcionar noves dades de camp sobre espècies fitoplanctòniques a unes escales espacials i temporals fins ara pràcticament desconegudes. Per avaluar la capacitat d'un dels instruments recentment desenvolupat a l'hora de detectar PANs i d'aportar noves mesures que facilitin la comprensió sobre la dinàmica de les espècies d'algues nocives es va utilitzar un instrument analitzador de mides de partícules, el *laser in situ scattering and transmissometry* (LISST-100X) a la platja de La Fosca (Mediterrani nordoccidental).

En primer lloc, el potencial del LISST-100X per quantificar les espècies de fitoplàncton típicament involucrades en les proliferacions de la platja de La Fosca es va comprovar en proves preliminars de laboratori. L'èxit significatiu d'aquestes proves va promoure la utilització de l'instrument al camp per mesurar els canvis espacials i temporals a curt termini en la distribució de les mides de partícules durant una proliferació recurrent d'*Alexandrium taylori*. Es va proposar un mètode de fraccions per mides per discriminar espècies concretes a partir de les mesures del LISST-100X perquè la proliferació mesurada no va ser monoespecífica (juntament amb l'*A. taylori* es van detectar altes concentracions de *Gymnodinium* sp. i altres espècies de fitoplàncton en concentracions baixes). La correlació entre les mesures obtingudes per les dues metodologies (LISST-100X amb el mètode de fraccions per mides) i per microscòpia va ser significativament positiva, indicant la validesa del mètode.

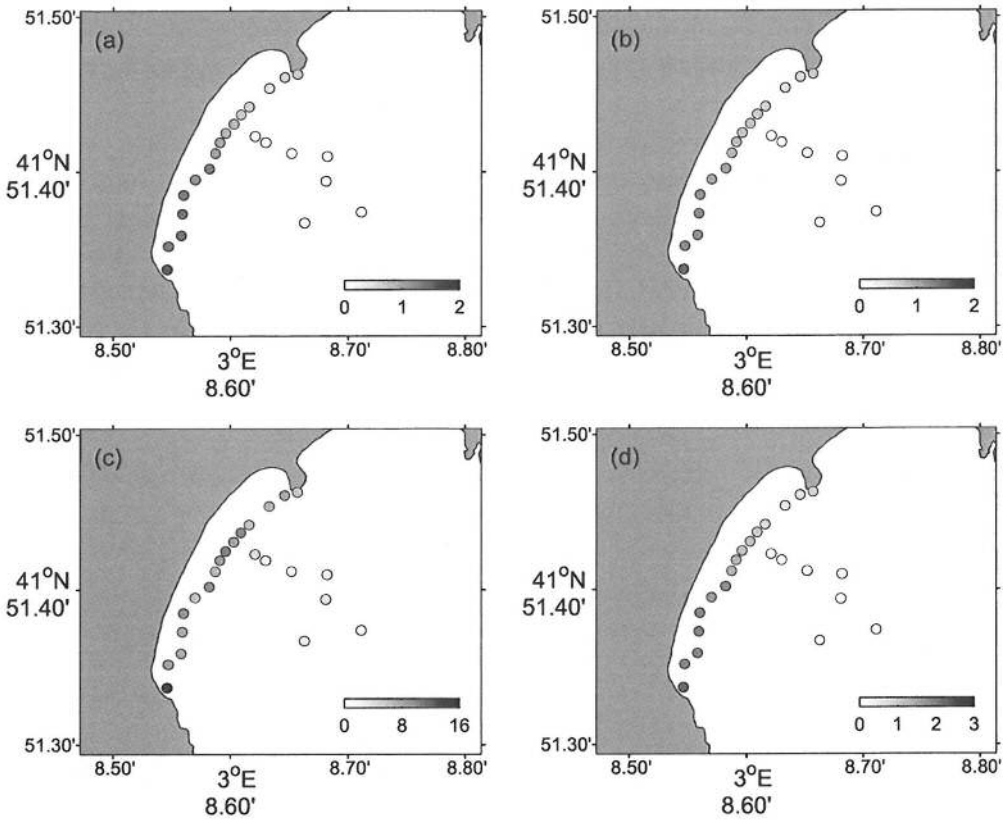


Fig. 2. Distribució espacial de (a) *A. taylori*, (b) *Gymnodinium* sp., (C) nanoplàncton, i (d) microplàncton mesurada fent ús del LISST-100X. Les unitats són 10^5 cèl·lules l^{-1}.

El LISST-100X obté dades in situ a una alta resolució temporal, i, per tant, és una alternativa millor que el microscopi tradicional a l'hora de mesurar l'evolució temporal de les proliferacions fitoplantòniques. A més, aquesta capacitat de mesurar a alta resolució temporal va permetre l'aplicació de l'instrument per esbrinar la distribució espacial de la proliferació. Els resultats obtinguts van mostrar una acumulació d'*A. taylori*, de *Gymnodinium* sp. i de la població total de fitoplàncton (nanoplàncton i microplàncton) prop de la costa i cap a la zona sud de la platja (Fig. 2). A més, també es va observar una alta variabilitat a escales espacials petites associada amb la migració del fitoplàncton.

Aquesta distribució es va comparar amb els resultats d'un model numèric de circulació marina per tal d'esbrinar la influència de la hidrodinàmica en la variabilitat horitzontal observada. Les simulacions mostren els corrents dirigits cap al sud durant els dies que es va realitzar el mostreig espacial, amb vents del nord-est. Aquests corrents són coherents amb una acumulació de les cèl·lules a la zona sud de la platja, tal i com es va observar a partir de les mesures del LISST-100X. Per tant, la circulació marina juga un paper important en la distribució d'*A. taylori* en la zona costanera i, en especial, modula les zones d'acumulació i de divergència.

Article 2: Cists de dinoflagel·lades en sediments recents de dues àrees semitancades del Mediterrani occidental subjectes a un alt impacte humà

Per tal d'investigar la diversitat d'espècies de fitoplàncton al Mediterrani occidental, es va realitzar un estudi sobre el repertori de cists de dinoflagel·lades. A més, es varen seleccionar dues àrees semitancades subjectes a un alt impacte humà perquè és en les àrees semitancades de baixa hidrodinàmica on es sol acumular una major quantitat de cists i perquè la comparació de les àrees permetria distingir entre característiques comunes del Mediterrani nord-occidental i trets específics de cada una de les zones. Les dues àrees seleccionades varen ser el port d'Arenys de Mar (Catalunya) i el Golf d'Òlbia (Sardenya, Itàlia).

En total es van prendre testimonis de sediment de set estacions (desembre de 2006 i agost de 2007) a Arenys i de vuit (octubre de 2006) i deu (maig de 2007) estacions a Òlbia. Els

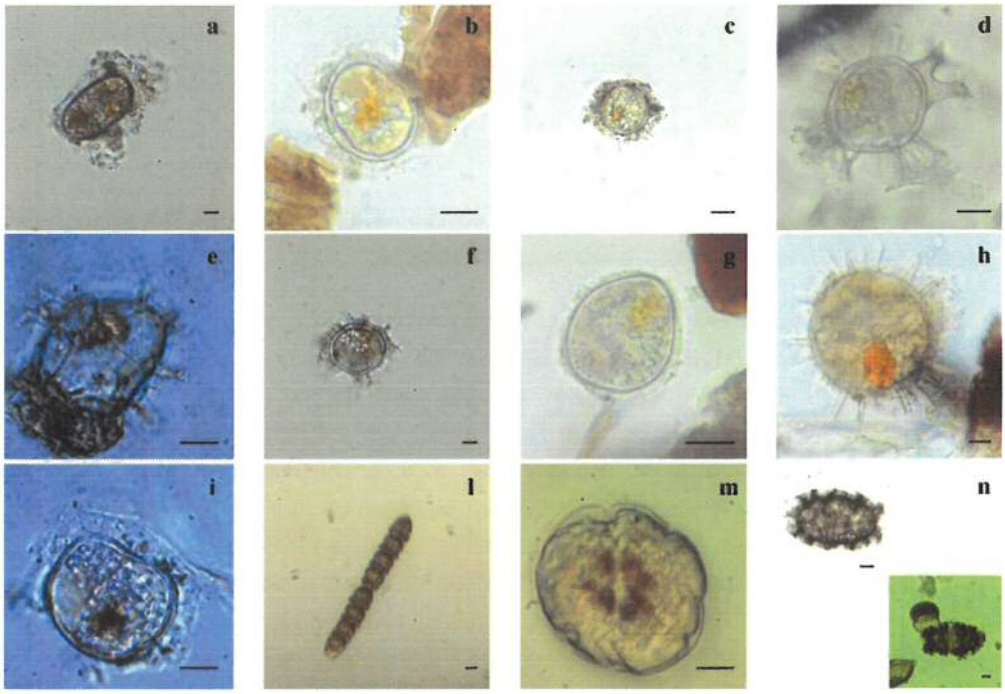


Fig. 3. Cists aïllats de dinoflagel·lades de sediments recents del Mar Mediterrani Occidental. Fotografies a–h: Gonyaulacals (a) *Alexandrium catenella/tamarense*. (b, c) *Alexandrium minutum*: (b) cist de sediments d'Arenys, (c) cist de sediments d'Òlbia. (d) *Gonyaulax* cf *membranacea*. (e) *Gonyaulax* cf *scrippsae*. (f) *Gonyaulax* cf *spinifera*. (g) *Gonyaulax verior*. (h) *Lingulodinium polyedrum*. Fotografies i–n: Gymnodinials (i, l) *Gymnodinium impudicum*: (i) cist viable, (l) cadena de cèl·lules mòbils. (m) *Gyrodinium instriatum*. (n) *Polykrikos* complex. Totes les barres d'escala són de 10 µm.

testimonis es van processar amb una metodologia que permet separar els cists de les partícules de sediment, i posteriorment es van classificar taxonòmicament els cists de resistència de dinoflagel·lades. Dels sediments recollits en els dos llocs es van trobar 42 morfotipus, 27 dels quals es van identificar a nivell d'espècie, representant 10 gèneres. A més, alguns d'aquests morfotipus no havien estat descrits prèviament a la literatura. Com a exemple, a la Fig. 3 es mostren alguns dels cists trobats.

Els cists més freqüents van ser els de *S. trochoidea*, *Scrippsiella* sp. 2, Gymnodiniales tipus 1 i *S. precaria*. L'abundància total de cists va variar substancialment entre els dos llocs, amb una major densitat total a Arenys. A nivell de grup i dintre de l'Ordre de les Peridinials calcàries, el gènere *Scrippsiella* va dominar el repertori de cists a ambdós llocs, encara que a certes estacions es van trobar major nombre de Gymnodinials (Òlbia) i Gonyaulacals (Arenys). També es van detectat cists de les espècies tòxiques *A. minutum* i *A. catenella/tamarense*. L'*A. minutum* va estar present als dos llocs, mentre que l'*A. catenella/tamarense* només es va trobar a Òlbia. En canvi, el *Peridinium quinquecorne* es va detectar en els sediments d'ambdós llocs. A més, a Òlbia, els cists d'aquesta espècie van estar presents en altes densitats i es van detectar fins i tot en capes de sediment profundes. Altres espècies com *Pentapharsodinium* cf *tyrrhenicum*, *S. crystallina*, *S. lachrymosa*, *S. precaria*, *S. trochoidea*, *Protoperidinium avellanum*, *P. claudicans*, *P. compressum*, *P. conicum*, *P.* cf *minutum*, *P. oblongum*, *P. pentagonum*, *P. subinerme*, i *Zygabikodinium lenticulatum* no s'havien detectat mai en fase planctònica a les àrees d'estudi, indicant la importància d'aquest tipus d'estudis per detectar la presència de possibles espècies d'AN. Els resultats d'aquest estudi, el primer sobre el repertori de cists de dinoflagel·lades a aquests dos llocs, va confirmar la importància de les àrees semitancades i amb baixes taxes de renovació de l'aigua com a reservoris de dinoflagel·lades.

Article 3: Dinàmica de la distribució del cist de resistència de l'*Alexandrium minutum* en un lloc confinat

El cicle de vida de la dinoflagel·lada tòxica *A. minutum* es compon d'una fase asexual, caracteritzada per cèl·lules vegetatives mòbils, i una fase sexual, el cist de resistència, que una vegada format roman latent en el sediment. El coneixement dels factors que determinen la distribució i abundància de cists de resistència és fonamental per comprendre la dinàmica de la fase vegetativa. Per adquirir aquest coneixement, es van estudiar els patrons de distribució espacial i temporal dels cists de resistència d'*A. minutum* i dels sediments durant diferents etapes de proliferació de la població vegetativa entre gener de 2005 i gener de 2008 al port d'Arenys de Mar.

La part més interna del port va ser on es van quantificar les majors abundàncies de cists, mentre que les menors abundàncies sempre es van registrar prop de l'entrada del port

(Fig. 4). A més, l'abundància de cists en els sediments va augmentar després dels períodes de proliferació vegetativa i va disminuir durant els períodes d'absència de proliferació, indicant que la formació de cists nous durant les proliferacions és un factor important en la distribució de cists.

D'altra banda, la distribució i l'evolució temporal dels cists de resistència en el sediment va concordar amb la distribució i l'evolució de les fraccions de sediments de llims i argiles. Les excepcions a aquesta tendència es van trobar a les estacions dominades per deposicions de sediments gruixuts. Tot i així, l'alta correlació entre la presència de cists i argiles durant els períodes d'absència de proliferació indica que els cists es comporten com partícules passives de sediment i que estan influenciats pels mateixos processos hidrodinàmics que les argiles.

Atès que el principal forçament físic que afecta a la resuspensió de sediments a Arenys de Mar és la seixa, es va estudiar la hidrodinàmica induïda per aquest procés mitjançant mesures in situ i models numèrics per tal d'interpretar els patrons de distribució de cists observats. Durant els períodes d'absència de proliferació, les pèrdues de cists van ser menors quan la seixa va ser més activa i als llocs on les corrents induïdes per la seixa van ser majors. Per tant, la resuspensió forçada per la seixa sembla reduir les pèrdues de cists mitjançant la recol·locació dels cists de nou a la superfície del sediment evitant el seu

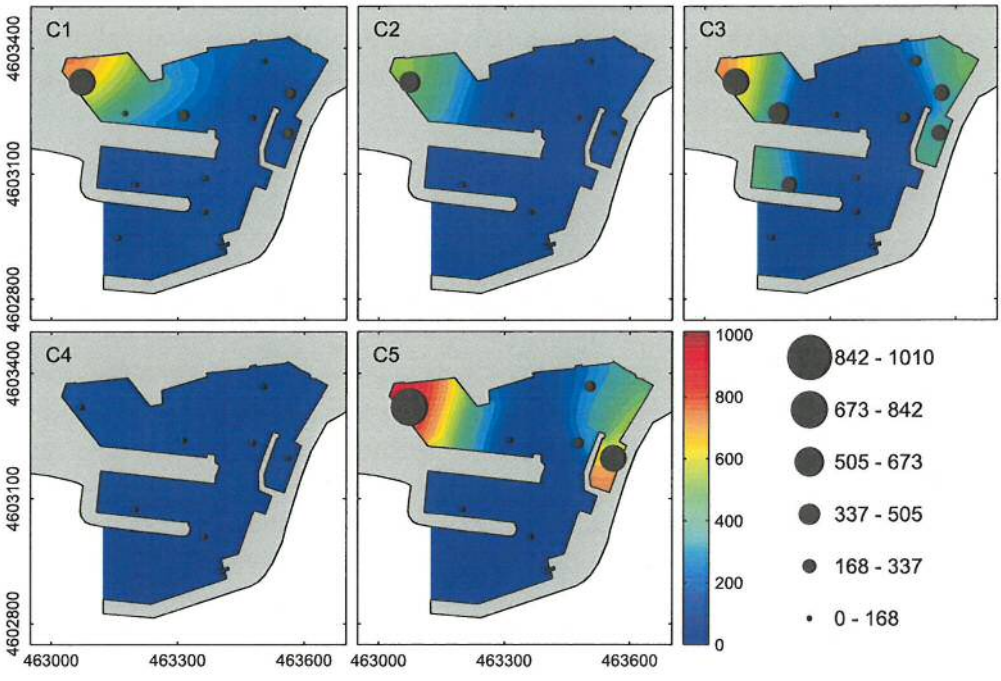


Fig. 4. Distribució i abundància de cists de resistència d'*A. minutum* (cists ml^{-1}) al port d'Arenys de Mar. Les coordenades són UTM (m)

soterrament en el sediment. També els perfils verticals d'abundància de cists observats van ser coherents amb aquest procés.

En resum, els cists de resistència actuen com a partícules fines de sediment i, per tant, estan afectats pels mateixos processos hidrodinàmics, que en el port d'Arenys de Mar, el més important és la seixa. Aquests processos hidrodinàmics determinen la distribució i evolució temporal dels cists excepte quan hi ha formació de cists nous durant les proliferacions de cèl·lules vegetatives.

Article 4: Alternances del cicle de vida de les poblacions naturals d'*Alexandrium minutum*

Amb l'objectiu d'investigar els factor biològics i ambientals que influencien les transicions en el cicle de vida d'*A. minutum*, es van mesurar els fluxos in situ de cèl·lules germinades i de formació de cists de resistència des d'octubre de 2005 fins a agost de 2008 durant proliferacions recurrents d'*A. minutum* al port d'Arenys de Mar. A més, es van quantificar les cèl·lules vegetatives a l'aigua i els cists de resistència en els sediments.

Els resultats respecte a la germinació de cists de resistència mostren que aquesta va ocórrer contínuament, encara que la germinació va ser major durant les èpoques amb condicions favorables pel creixement de les cèl·lules vegetatives (Fig. 5). Aquestes condicions que van estimular major germinació semblen estar relacionades amb factors ambientals, en concret amb un augment net de la irradiància i de la temperatura de l'aigua. D'altra banda, l'encistament va augmentar en funció d'una major abundància de cèl·lules vegetatives a l'aigua, iniciant-se quan l'abundància de cèl·lules va superar els 2×10^3 cèl·lules l^{-1}.

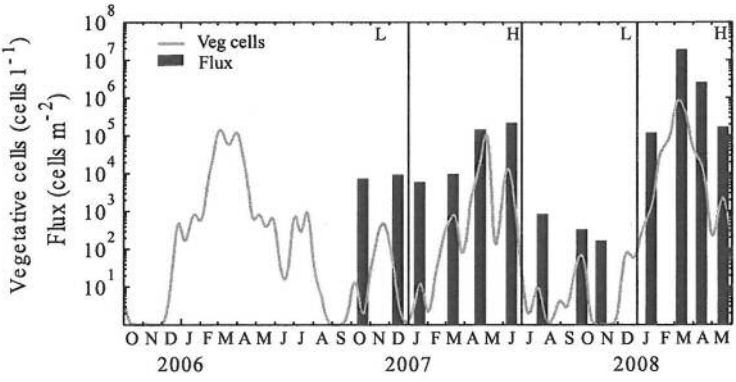


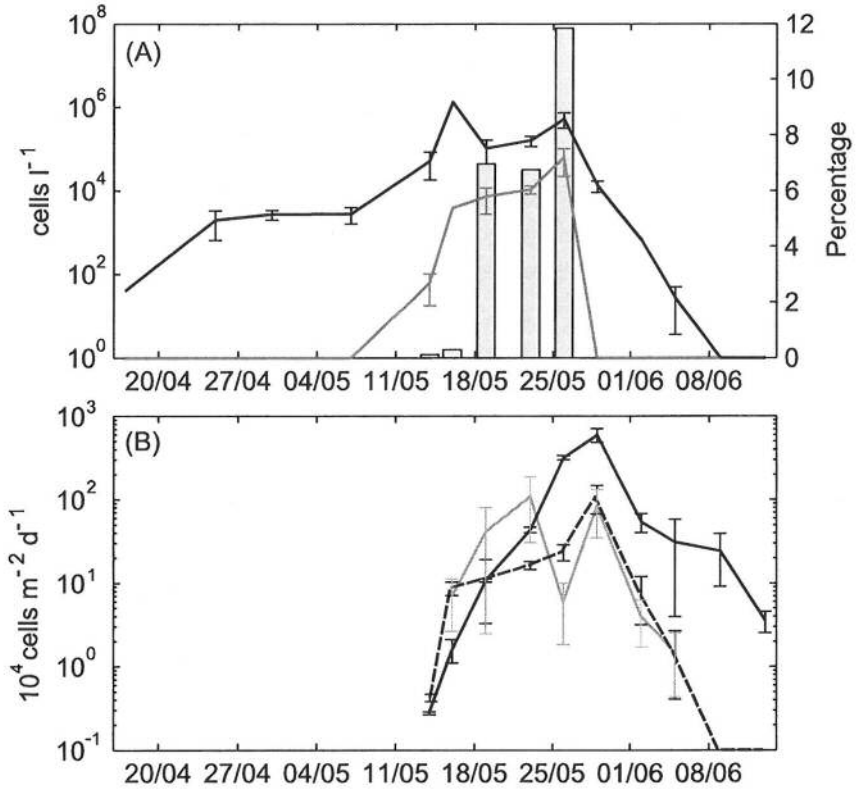
Fig. 5. Fluxos de les cèl·lules germinades d'*A. minutum* i cèl·lules vegetatives a la columna d'aigua. Les línies verticals delimiten els períodes amb fluxos baixos (L) i i fluxes alts (H) de germinació. No hi ha dades de germinació d'octubre de 2005 a setembre de 2006.

A més, els processos d'alta germinació i els d'encistament es superposaven durant 2 mesos degut a la llarga durada de les proliferacions. Aquest solapament es pot explicar gràcies als freqüents canvis entre fases del cicle de vida. Per una part, la germinació dels nous cists de resistència formats durant la mateixa proliferació explicaria els fluxos sostinguts d'alta germinació observats. Aquests cists, amb el període obligatori de latència ja superat, podrien subministrar cèl·lules vegetatives a la mateixa proliferació en què van ser produïts. Les cèl·lules germinades contribuirien al manteniment de la proliferació mitjançant divisió mitòtica i a la vegada entrarien a la fase de reproducció sexual de forma continuada, el qual explicaria la llarga durada del període de formació de cists de resistència. Així, encara que els cists de resistència en el sediments es van esgotar ràpidament durant els períodes d'alta germinació, la seva producció (encistament), tot i que només va involucrar una petita fracció de la població vegetativa, va compensar de llarg les seves pèrdues. Aquests resultats mostren la importància dels canvis entre fases del cicle, els quals són una estratègia d'assegurança per al manteniment de la proliferació d'*A. minutum* i la seva recurrència al llarg del temps.

Article 5: Fases in situ del cicle de vida del complex *Alexandrium tamarense* durant una proliferació a Long Island (EUA)

Per entendre l'aparició i l'evolució de proliferacions, i poder predir futurs episodis, és necessari conèixer en detall el cicle de vida de les espècies. Amb aquest objectiu es van caracteritzar i quantificar in situ les fases del cicle de vida del complex *A. tamarense* des de l'inici fins l'acabament d'una proliferació a Northport Harbor (Long Island, EUA) durant els mesos d'abril a juny de 2008. L'estudi es va dur a terme tant en la columna d'aigua com en els sediments, i es van quantificar l'excistament i encistament mitjançant trampes dissenyades especificament per a cada un dels processos.

La proliferació va durar 6 setmanes i va atènyer una abundància màxima de cèl·lules vegetatives de 1.3×10^6 cèl·lules l^{-1} (Fig. 6). L'excistament dels cists de resistència presents als sediments superficials, tot i que eren poc abundants, varen donar lloc a l'inòcul de les cèl·lules vegetatives per al desenvolupament de la proliferació. Tant escassa era la seva abundància que els cists es van esgotar després de subministrar l'inòcul. Durant la fase exponencial de la proliferació, amb abundàncies de cèl·lules vegetatives de $\sim 10^4$ cèl·lules l^{-1}, la detecció de planozigots va indicar que es va iniciar el procés de reproducció sexual. El fet de detectar planozigots a partir d'aquesta abundància suggereix l'existència d'un llindar de densitat de cèl·lules vegetatives per assolir una reproducció sexual amb èxit. Durant el primer pic en abundància de cèl·lules de la proliferació es van detectar al mateix temps els planozigots planctònics, i els planozigots immòbils i els cists de resistència en les trampes d'encistament. Posteriorment, les seves respectives abundàncies van anar incrementant progressivament. Aquest fet va reflectir l'acumulació de planozigots que



(A) Fluctuacions de les fases del cicle de vida del complex *A. tamarense* a la columna d'aigua: cèl•lules vegetatives (línia negra), planozigots (línia gris), i percentatges de planozigots respecte cèl•lules vegetatives (barres). Les dades són abundàncies mitjanes de les estacions 2, 7, i 8 (n=3). Les barres d'error representen l'error estàndard. (B) Fases del complex *A. tamarense* a les trampes d'encistament: cists de resistència (línia negra), planozigots (línia negra ratllada), i cists pel•liculars (línia gris). Les dades són abundàncies mitjanes (n=2). Les barres d'error representen l'error estàndard.

s'havien anat formant progressivament durant la proliferació degut a que els planozigots són capaços de nedar en la columna d'aigua durant 1-2 setmanes abans de dipositar-se en els sediments. Tant els percentatges de planozigots respecte les cèl•lules vegetatives com les taxes estimades d'encistament van ser baixes, fet que indica que només una petita proporció de la població vegetativa va estar involucrada en la reproducció sexual. Tot i així, es van emplenar sobradament les existències de cists de resistència del complex *A. tamarense* en els sediments superficials de l'àrea. L'ús de les trampes d'encistament va permetre observar per primera vegada la formació de cists pel•liculars en el camp coincidint amb una alta densitat de cèl•lules vegetatives durant la proliferació.

Discussió

En sistemes semitancats caracteritzats per un baix hidrodinamisme i una estabilitat de la columna d'aigua, els processos biològics poden prevaler sobre els processos físics. Els estudis sobre les característiques del cicle de vida, incloent la dinàmica de les fases planctònica i bentònica, el mostreig de cists de resistència i la mesura in situ de l'encistament i la germinació, són per tant més manejables en aquests sistemes semitancats.

Estudi de la dinàmica de la fase planctònica de les espècies objectiu en diversos sistemes semitancats

Per a una millor comprensió de la dinàmica de la fase bentònica, és necessària una recerca acurada sobre l'acoblament plantònic-bèntic. Els estudis sobre la dinàmica de la fase planctònica es basen normalment en el mostreig i l'anàlisi de diversos paràmetres físics, químics i biològics. Però l'esforç que requereix aquest tipus d'anàlisi, en particular les tècniques de microscòpia per identificar les espècies de fitoplàncton, usualment obstaculitza la capacitat d'obtenir dades a una resolució adequada per fer front a l'alta variabilitat espacial i temporal de l'abundància de cèl·lules vegetatives.

En l'actualitat s'està explorant l'aplicació de diverses tecnologies per resoldre aquesta manca de resolució (Babin et al., 2005, 2008). En aquesta tesi, l'analitzador de mides de partícules LISST-100X es va utilitzar per examinar l'alta variabilitat espacial i temporal d'una proliferació de fitoplàncton dominada per l'espècie *A. taylori* a la platja de La Fosca (Article 1). L'alta resolució temporal de les dades obtingudes va permetre relacionar l'alta variabilitat en l'abundància amb les migracions verticals del fitoplàncton. Les mesures del LISST-100X de cèl·lules vegetatives juntament amb les simulacions obtingudes a partir d'un model numèric físic van mostrar que la circulació de les aigües superficials, determinada pel règim de vents predominants, influencia la distribució de cèl·lules vegetatives a la platja. Aquest resultat té importants implicacions en termes de la distribució dels cists de resistència després d'una proliferació, com es discutirà més endavant.

La bona concordança entre les mesures del LISST-100X i els recomptes amb microscòpia va demostrar la utilitat de l'instrument per mesurar l'abundància de cèl·lules en les poblacions de fitoplàncton. No obstant, el LISST-100X no proporciona informació autònoma sobre la composició d'espècies i es necessita una anàlisis complementària de microscòpia. En el nostre estudi a La Fosca, el mètode de fraccions per mides va servir per discriminar, a partir de les mesures del LISST-100X, entre les dues espècies dominants de fitoplàncton. Aquests resultats inicials van encoratjar la utilització de l'aparell a altres llocs estudiats en aquesta

tesi, en concret, al port d'Arenys de Mar. No obstant, la comunitat de fitoplàncton que va acompanyar a l'espècie objectiu a Arenys (*A. minutum*) va ser molt diversa i algunes de les cèl·lules vegetatives no tenien forma esfèrica. Aquests fets van fer que les mides de moltes de les cèl·lules es solapessin entre elles, dificultant la discriminació d'espècies amb el LISST-100X. A més, la presència de partícules de sediments a la columna d'aigua, cosa relativament freqüent en els ports, va plantejar una font d'error addicional.

Per futurs estudis dirigits a la identificació d'espècies, l'instrument *Imaging Flow-Cytobot* és força prometedor, encara que a dia d'avui no està disponible comercialment. Aquest instrument és un analitzador submergible de partícules i d'imatges que combina aspectes de microscòpia i citometria de flux (Olson i Sosik, 2007; Sosik i Olson, 2007), que proporciona una detecció autònoma, a alta freqüència i en temps real d'espècies. L'ús de l'*Imaging Flow-Cytobot* ha permès recentment l'observació i l'anàlisi a nivell d'espècie de la dinàmica d'una proliferació de fitoplàncton (Campbell et al., 2010).

Els estudis de la dinàmica de la fase vegetativa que es descriuen en els articles 3 i 4 estan realitzats mitjançant recomptes per microscòpia a una freqüència suficient per avaluar la dinàmica de les proliferacions de les espècies objectiu. La dinàmica de les cèl·lules vegetatives detectades en les estacions monitoritzades rutinàriament proporcionen una bona base per a la coordinació dels mostreigs espacials de cists de resistència i de cèl·lules vegetatives (Article 3) i per intensificar la freqüència de mostreig en situacions de proliferació (Articles 3 i 4).

Identificació i quantificació de la fase bentònica i estudi de la seva dinàmica

Diversitat de cists de resistència

El coneixement del conjunt de cists de resistència present en els sediments és un prerequisit indispensable per la comprensió de la fase bentònica. L'estudi del banc de llavors en els sediments del Mediterrani nordoccidental d'aquesta tesi ha contribuït a l'escàs coneixement de la diversitat de cists de resistència en aquesta àrea (Article 2). Estudis anteriors desenvolupats en ports del litoral català enfocats en el gènere *Alexandrium* van mostrar la presència de cists de resistència de vuit espècies (Bravo et al., 2006, 2008; Garcés et al., 2004). Però la diversitat potencial de cists de resistència d'altres espècies de dinoflagel·lades no s'havia abordat mai fins aquesta tesi.

Les estimes de diversitat basades en cists de resistència en els sediments són superiors a les estimes basades en la presència de cèl·lules vegetatives (Boero et al., 1996). La comparació dels dos sistemes costaners (el port d'Arenys de Mar i el Golf d'Òlbia) va mostrar una alta diversitat d'espècies productores de cists en els seus respectius bancs de llavors, presentant un nombre major d'espècies respecte el nombre registrat en la població

vegetativa d'ambdós llocs. A més, va permetre identificar diversos morfotipus no descrits prèviament en la literatura. Les cèlul·les vegetatives que van germinar d'alguns d'aquests morfotipus corresponen probablement a noves espècies, les descripcions de les quals s'estan duent a terme.

Les abundàncies de cists de resistència va ser notablement més grans en el port d'Arenys que a Òlbia, fet que pot estar relacionat amb una hidrodinàmica més restringida al port d'Arenys respecte a Òlbia. Això recolzaria la hipòtesi que els sistemes semitancats són potencials reservoris de cists de resistència, i que els ports poden ser àrees més propenses a l'acumulació d'estats de resistència. En base a aquests resultats, el mostreig de la distribució dels estadis bentònics d'espècies de fitoplàncton en els sediments de zones semitancades, principalment ports, pot ésser una eina molt útil en combinació amb la presa de mostres planctòniques tradicionals. Això permetria el seguiment de la dispersió d'espècies al llarg del litoral, la detecció de noves espècies, i l'avaluació de risc potencial de PANs.

A més de l'anàlisi clàssica per microscòpia, des de fa poc també es poden utilitzar les sondes PCR a l'hora de detectar i classificar cists de resistència. Les mostres de sediments d'Arenys i Òlbia analitzades per microscòpia (Article 2) van ser utilitzades per validar un nou mètode de detecció de cists de resistència amb sondes PCR (Penna et al., 2010). Aquestes tècniques moleculars donen resultats comparables o fins i tot més acurats que els obtinguts per microscòpia per certes espècies i, per tant, poden proporcionar una metodologia útil per una anàlisi més ràpida de la composició d'aquestes espècies, sense necessitat d'experiència prèvia en identificació taxonòmica. Però, de moment encara no es pot quantificar els cists de resistència amb sondes PCR en la majoria d'espècies de dinoflagel·lades, amb l'excepció d'algunes espècies d'*Alexandrium* (Erdner et al., 2010; Kamikawa et al., 2005).

Dinàmica dels cists de resistència

El mostreig dels cists de resistència d'espècies objectiu, tant en l'espai com en el temps, a una freqüència relativament alta permet obtenir un coneixement detallat sobre la dinàmica d'aquests cists. Els cists es comporten com a partícules passives, de manera que són influenciats pels mateixos processos que determinen la dinàmica sedimentària. Els cists de resistència es dispersen des d'una "font puntual", que influeix en la seva distribució i per tant cal tenir-la en compte en els estudis de les poblacions de dinoflagel·lades i, sobretot, en l'estudi de les PANs (Wyatt, 2002). La distribució espacial de les proliferacions de cèl·lules vegetatives durant tota la seva durada (integrada en el temps) s'anomena "traça de la proliferació". Es coneix que les fases bentòniques hi són presents en els llocs on té lloc la proliferació, però pot ser que no s'acumulin principalment sota la traça de la proliferació. Així, els cists de resistència es troben en conques profundes del talús continental o en zones costaneres protegides (Joyce, 2005; Villanoy et al., 2006; Wang et al., 2004). Malgrat

la influència dels processos sedimentaris en la dinàmica dels cists, queden per clarificar alguns dels mecanismes que determinen la seva distribució. Aquesta tesi va analitzar la distribució dels cists de resistència d'*A. minutum* en el port d'Arenys de Mar en relació amb l'origen de la formació dels cists (la distribució de cèl·lules vegetatives), la resuspensió induïda per la seixa, i la granulometria dels sediments. L'àrea en la que els cists de resistència es dipositaren inicialment va estar determinada per la distribució espacial de la proliferació de cèl·lules vegetatives durant la formació dels cists. A Arenys, les regions preferents per l'acumulació de cèl·lules vegetatives són les parts del nord-oest i nord-est del port (Article 3, Garcés et al., 2004; Lenning Van et al., 2007; Vila et al., 2005), que coincideix amb les dues àrees on es distribuixen els cists de resistència després d'una proliferació (Article 3). Com les corrents induïdes pel vent influeixen la distribució de cèl·lules vegetatives a la superfície de l'aigua (Article 1), la distribució de cists de resistència també ha de ser el resultat de l'acció del vent acumulant o dispersant les cèl·lules vegetatives durant el desenvolupament de la proliferació (Article 3).

Durant els períodes en què no es van formar cists de resistència, aquests es van acumular a les mateixes àrees que les fraccions fines de sediment, principalment argiles, confirmant que processos hidrodinàmics i sedimentaris controlen la dispersió secundària dels cists (Dale, 1976). Per primera vegada, es va identificar la seixa com un mecanisme important per la resuspensió de sediments i cists en sistemes marins semitancats. Això és una contribució significativa cap a una millor comprensió de la dinàmica de les proliferacions en sistemes semitancats, ja que la resuspensió pot afavorir la germinació de cists de resistència i el creixement de cèl·lules vegetatives. D'altra banda, es va comprovar la influència de la resuspensió per la seixa en l'abundància i la distribució horitzontal i vertical de cists de resistència en el sediment.

Estudis addicionals (que no formen part d'aquesta tesis) basats en mostreigs de sediments a la platja de La Fosca mostren la presència de cists de resistència d'*A. taylori* en els sediments de la platja just després d'una proliferació de cèl·lules vegetatives (Article 1). Però mesos després, aquests cists no estaven als sediments de la platja. L'absència de cists de resistència en els sediments de la platja de La Fosca és consistent amb el fet que la fracció de sediment dominant és la sorra. Llavors, els cists de resistència deuen acumular-se als mateixos llocs que els sediments fins, com ara conques profundes en mar obert o zones protegides properes a la costa. A diferència del port d'Arenys de Mar, la platja de La Fosca es veu afectada per l'onatge que pot resuspendre les partícules fines de sediments i els cists, afavorint el seu transport a zones més protegides.

En aigües més obertes, els cists de resistència es poden acumular en zones allunyades de la seva font planctònica com a conseqüència del transport pels processos hidrogràfics i sedimentaris. Per contra, zones semitancades de circulació reduïda, com ara ports, poden presentar un cicle repetitiu on cists de resistència en els sediments formen planters de llavors que afavoreixen proliferacions, les quals novament produeixen cists.

La línia de costa del litoral català i moltes altres zones costaneres han sigut, i encara són, modificades mitjançant la construcció de ports, platges semitancades, o barreres de protecció. A més, la creixent tendència en la construcció de sistemes semitancats, com ara ports, està afavorint l'existència de reservoris de cists de resistència, inclosos els d'espècies tòxiques (Article 2).

Avaluació de les transicions entre les fases planctònica i bentònica de les espècies objectiu en els sistemes semitancats seleccionats

L'estudi in situ de la germinació i l'encistament ha demostrat que aquests processos tenen un paper clau en la recurrència anual de les proliferacions, així com en la seva iniciació i manteniment.

Les estratègies de germinació de les dinoflagel·lades són específiques de cada espècie. En un estudi amb trampes de germinació, Ishikawa i Taniguchi (1996, 1997) van identificar tres patrons bàsics de germinació per les espècies productores de cists en una mateixa comunitat: "esporàdic", "incessant", i "síncrona". Segons el seu estudi, les espècies amb un patró esporàdic de germinació no tenien una clara estacionalitat en la germinació i la seva taxa de germinació sempre era baixa. El patró de germinació incessant va consistir en una germinació continua caracteritzada per pics marcats en una temporada en particular, però que no contribuí notablement a la població planctònica. Finalment, el patró de germinació síncrona es va limitar a una estació particular depenent de l'espècie. Aquests autors suggereixen que l'estratègia de germinació síncrona resulta en una contribució síncrona a les cèl·lules vegetatives, mentre que les altres estratègies són oportunistes per mantenir la població planctònica durant tot l'any.

En aquesta tesi, els patrons de germinació de les dues espècies d'*Alexandrium* van ser de tipus incessant (*A. minutum*, Article 4) i síncrona (complex *A. tamarense*, Article 5). En el cas d'*A. minutum*, el pic de germinació va coincidir amb un augment en la irradiància i en la temperatura de l'aigua. En contrast amb les observacions de Ishikawa i Taniguchi (1996, 1997) per a les espècies amb germinació incessant, el període de major germinació va coincidir amb la proliferació de cèl·lules vegetatives, encara que la contribució d'aquesta alta germinació no va tenir cap efecte sobre les dimensions de la proliferació. En canvi, la germinació incessant sí que va afavorir el manteniment d'una certa població planctònica durant tot l'any. Aquests resultats suggereixen que l'estratègia de la germinació incessant d'*A. minutum*, que inclou pics de germinació en una època en particular, serveix per mantenir la població i actua com un mecanisme de seguretat per reinocular cèl·lules vegetatives. La germinació sincrònica del complex *A. tamarense* va estar influenciada per la temperatura de l'aigua, com s'ha demostrat en diversos estudis previs (Anderson et al., 1979; Anderson, 1980). A més, el període de germinació va coincidir amb la proliferació de

cèl·lules vegetatives (Article 5). En aquesta espècie, la limitació del període de germinació a l'ocurrència de condicions favorables pel creixement vegetatiu augmenta les possibilitats d'èxit de proliferació, però només durant un període limitat.

El procés d'encistament es va iniciar al principi de la proliferació de les dues espècies d'*Alexandrium*, amb fluxos d'encistament observats durant les fases de creixement exponencial, manteniment i declivi de la proliferació. La proporció de cèl·lules vegetatives que van participar en l'encistament va ser baixa. Encara que no es va detectar la influència de factors fisico-químics en l'encistament, hi va haver una alta correlació entre els cists de resistència i les cèl·lules vegetatives en el cas d'*A. minutum* (Article 4). Per tant, la magnitud dels fluxos d'encistament determina l'abundància de cists de resistència d'*A. minutum* que es troben en els sediments del port d'Arenys (Article 3). Aquesta relació entre cists de resistència i cèl·lules vegetatives no es va observar pel complex *A. tamarense*. Una explicació d'això seria l'acumulació de planozigots a la columna d'aigua, que, un cop formats, poden romandre fins a 1 o 2 setmanes abans de l'encistament (Article 5; Anderson et al., 1983). En canvi, els planozigots d'*A. minutum* només romanen a la columna d'aigua entre 2 i 3 dies després de la seva formació (Figueroa et al., 2007; Probert, 1999), facilitant la bona correlació entre cists i cèl·lules vegetatives.

Una condició prèvia per a que es produeixi reproducció sexual pot ser un cert llindar en la densitat de cèl·lules vegetatives (Wyatt i Jenkinson, 1997). Aquests autors suggereixen un llindar de més de 10^4 cèl·lules l^{-1}. El llindar de cèl·lules vegetatives observat en aquesta tesi per la formació de cists de resistència va ser de 10^3-10^4 cèl.lules l^{-1} per a ambdues espècies. Els sistemes més tancats es caracteritzen per aigües molt calmades i amb temps de residència llargs, factors que afavoreixen l'agregament de cèl·lules i per tant faciliten llindars baixos (el cas d'*A. minutum* al port d'Arenys de Mar, Article 4). En canvi, el llindar de densitat cel·lular pot ésser més difícil d'assolir en sistemes amb major hidrodinamisme (el cas del complex *A. tamarense* a Northport Harbor, Article 5). En aquests darrers sistemes, les estratègies d'agregament cèl·lular dels organismes poden tenir una major importància a l'hora d'assolir la densitat de les cèl·lules vegetatives necessàries per garantir la formació de cists de resistència.

Durant aquesta tesi s'han descrit altres estadis del cicle de vida en els experiments de camp. La formació de cists pel·liculars en condicions naturals es va observar tant en *A. minutum* com en el complex *A. tamarense*. Aquests cists pel·liculars es van observar durant la proliferació quan l'abundància de cèl·lules vegetatives va ser màxima (>10^5 cèl·lules l^{-1}). Segons la revisió recent de Bravo et al. (2010), aquesta tesi descriu per primera vegada la formació de cists pel·liculars del comlex *A. tamarense* en el camp. S'haurien de desenvolupar estudis més detallats per esbrinar la possible funció d'aquests cists en la dinàmica de poblacions de totes dues espècies .

Per analitzar el paper dels cists de resistència en la dinàmica poblacional de dinoflagel·lades, les dades in situ de germinació i encistament (Article 4) i l'abundància de cists de resistència (Articles 3 i 4) es van utilitzar per parametritzar models numèrics de la dinàmica poblacional d'*A. minutum* al port d'Arenys de Mar (Estrada et al., 2010). La majoria dels models sovint relacionen l'abundància d'espècies de fitoplàncton només amb els paràmetres físics i químics de la columna d'aigua, sense tenir en compte el paper dels estadis del cicle de vida. Els resultats obtinguts per Estrada et al. van remarcar la necessitat d'incorporar les característiques del cicle de vida en els futurs models de la dinàmica de poblacions de fitoplàncton, principalment les destinades a la gestió de les PANs.

Integració del cicle de vida en la dinàmica de poblacions de dinoflagel·lades

L'estudi combinat de la dinàmica de les fases planctònica i bentònica i de les seves transicions al camp ha proporcionat nous coneixements sobre la dinàmica de poblacions de les dinoflagel·lades i ha permès descriure una nova estratègia del cicle de vida d'una espècie d'*Alexandrium* (Article 4).

En espècies amb un abundant reservori de cists com l'*A. minutum*, la germinació contínua és un mitjà per aprofitar la possible aparició de condicions ambientals favorables per al creixement amb pèrdues només de pocs cists de resistència. En canvi, en espècies com el complex *A. tamarense*, on l'abundància de cists pot ser escassa, és millor reservar els cists per als períodes òptims per al creixement. La plasticitat de l'organisme també és un factor a tenir en compte, perquè les espècies d'alta plasticitat (com l'*A. minutum*) són capaces de créixer en unes condicions ambientals més amplies i variades. En qualsevol cas, per a les espècies que depenen dels cists, l'èxit serà major si la germinació coincideix amb unes condicions ambientals favorables pel creixement vegetatiu. Per tant, un cert grau de sincronia (períodes d'alta germinació en les espècies amb germinació contínua) resulta avantatjós. Aquesta sincronia pot implicar algun tipus de senyal de germinació que tendeixi a fer coincidir la germinació amb els factors ambientals de creixement (irradiància i temperatura en *A. minutum*, i temperatura en el complex *A. tamarense*).

Una vegada que s'inicia un creixement exponencial, la germinació addicional té poc efecte sobre la magnitud de la proliferació, però pot contribuir a l'augment i manteniment de l'abundància de cèl·lules vegetatives. Això és important perquè la població vegetativa ha d'assolir una densitat cel·lular suficient per produir cists de resistència. De fet, una funció essencial de la fase planctònica de les espècies d'*Alexandrium* podria ser la formació de cists de resistència per l'abastiment del planter de llavors (Wyatt i Jenkinson, 1997). En *A. minutum*, el període obligatori de latència relativament curt del cists de resistència recentment formats (un mes, Figueroa et al., 2007) implica que, donada la llarga durada de la proliferació vegetativa, aquests cists formats poden germinar dins de la mateixa

proliferació en la que es formen. Les cèl·lules vegetatives germinades es divideixen i entren de nou en la reproducció sexual, el que implica una estratègia d'alternància entre les fases planctònica i bentònica durant una mateixa proliferació. En el cas del complex *A. tamarense*, el període obligatori de latència llarg (3-6 mesos, Anderson, 1998) és indicatiu d'una estratègia adaptada a la supervivència durant les condicions ambientals menys favorables per al creixement.

L'estoc de cists de resistència pot veure's afectat per factors físics i biològics (resuspensió, soterrament, bioturbació, germinació, deposició, mortalitat, degradació, i depredació; Anderson et al., 1982; Giangrande et al., 2002; Persson, 2000). L'estudi d'Estrada et al. (2010) abans esmentat va posar de manifest la importància de conèixer la taxa de pèrdua de cists de resistència en els planters de llavors.

En un sistema més tancat i amb un estoc de cists elevat, les pèrdues de l'abundància de cists de resistència es van poder explicar en part per la germinació, mentre que la resuspensió causada per la seixa en canvi impedia el soterrament dels cists (Article 3 i 4). Per contra, en un sistema semitancat amb un estoc de cists baix, les pèrdues de cists en els sediments superficials van ser degudes principalment a la germinació sincrònica (Article 5). Aquest sistema està exposat a corrents de marea i d'onatge, les quals generen turbulència a la capa de sediment i podrien per tant tenir un paper important en la resuspensió de cists de capes de sediment més profundes.

Els cists de resistència presenten uns caràcters morfològics i fisiològics dissenyats per augmentar la seva resistència (és a dir, parets gruixudes, protuberàncies, espines, capes de mucosa, i productes d'emmagatzematge intracel·lular). No obstant, poden ésser vulnerables a factors encara no ben coneguts com ara la mortalitat natural, la depredació, bacteris, fongs, anòxia i compostos tòxics presents en els sediments. S'haurien de realitzar més estudis en aquest context, sobretot a causa de les implicacions de l'abundància de cists en el potencial de germinació del planters de llavors.

A través dels seus cicles de vida, les espècies de dinoflagel·lades han desenvolupat diferents estratègies en funció de l'entorn al que s'adapten. A través de la reproducció sexual, les dinoflagel·lades complementen els avantatges de la recombinació genètica amb la capacitat d'ajustar el seu cicle de vida als factors ambientals que són òptims pel seu creixement. Si les condicions ho permeten, aquestes espècies formaran proliferacions intenses i recurrents- una clara indicació de la perfecció del seu cicle de vida.

Conclusions

1) L'analitzador de mides de partícules LISST-100X ofereix dades in situ d'alta freqüència que permeten descriure la variabilitat temporal i espacial de les proliferacions de fitoplàncton. No obstant això, l'ús de la LISST-100X queda limitat a les característiques específiques de la comunitat fitoplanctònica i del sistema a estudiar.

2) Els sistemes semitancats són reservoris importants de diversitat biològica, on es pot trobar un major nombre d'espècies que produeixen cists que les registrades en la població vegetativa.

3) Es varen identificar deu morfotipus de cists de resistència no descrits anteriorment a la literatura. Alguns d'ells corresponen a noves espècies de dinoflagel·lades que encara no s'han descrit.

4) En sistemes tancats, els corrents impulsats pel vent van influir en la distribució de cèl·lules vegetatives a la superfície de l'aigua i com a conseqüència la distribució dels cists de resistència en el sediment.

5) La distribució espacial i temporal dels cists de resistència en els sediments es va relacionar amb el comportament biològic (producció de cists de resistència) i els processos físics (per exemple, la resuspensió per la seixa, els corrents induïts per marea i l'onatge). La producció biològica és de major rellevància en els sistemes tancats.

6) *Alexandrium minutum* i el complex *A. tamarense* van presentar dos tipus d'estratègies de germinació diferents: incessant i síncrona, respectivament. La germinació incessant és una estratègia oportunista per al manteniment de la població planctònica durant tot l'any, mentre que el patró de germinació síncrona limita la presència de la proliferació a una estació en particular.

7) El procés d'encistament, i per tant la reproducció sexual, que es va iniciar en les etapes inicials de la proliferació en les dues espècies d'*Alexandrium*, es va observar durant les fases de creixement exponencial, manteniment i declivi de la proliferació.

8) La proporció de cèl·lules vegetatives que va participar en l'encistament va ser baixa. Les taxes màximes d'encistament van ser 16% i 9% per *A. minutum* i pel complex *A. tamarense* respectivament.

9) L'encistament va començar amb abundàncies de cèl·lules vegetatives de 10^3–10^4 cèl·lules l^{-1}. La magnitud dels fluxos d'encistament va determinar l'abundància de cists de resistència d'*A. minutum* en els sediments, mentre que en el complex *A. tamarense* aquesta relació entre el cists de resistència i l'abundància de cèl·lules vegetatives no es va observar.

10) La formació de cists pel·liculars d'*A. minutum* i el complex *A. tamarense* va coincidir amb altes abundàncies de cèl·lules vegetatives durant la proliferació. El possible paper d'aquest estadi del cicle de vida en la dinàmica poblacional de les dues espècies és poc conegut.

11) Les característiques morfològiques van permetre la quantificació de planozigots planctònics del complex *A. tamarense* i va permetre seguir la transició de la cèl·lula vegetativa a l'etapa de cist de resistència d'una manera més precisa. No obstant això, aquest enfoc no va ser adequat per l'*A. minutum*.

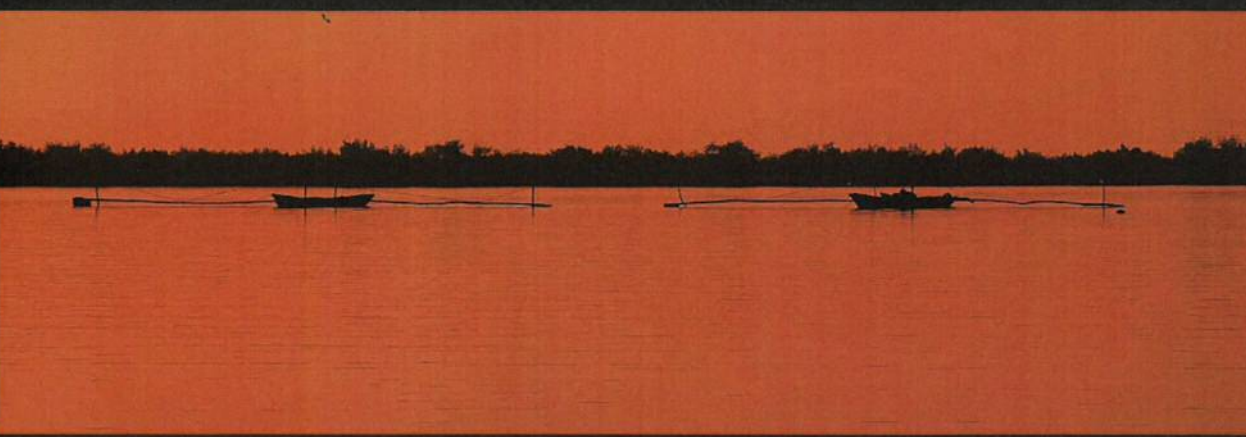
12) Les pèrdues de cists de resistència en els sediments superficials d'una àrea tancada amb un estoc alt de cists es va explicar, en part, per la germinació, tot i que aquesta fóra ser incessant. Per contra, la germinació síncrona va ser un factor significatiu de pèrdua de cists de resistència en una zona semi-tancada amb un estoc baix de cists.

13) Els cists de resistència acabats de formar d'*A. minutum* van ser capaços de germinar dins de la mateixa proliferació, i aquestes cèl·lules vegetatives germinades es van dividir i van entrar de nou en la reproducció sexual. Aquesta característica no es va observar durant la proliferació del complex *A. tamarense*.

14) Les transicions planctòniques-bentòniques en dinoflagel·lades són específiques de les espècies. Mitjançant aquestes transicions, les dinoflagel·lades desenvolupen la seva estratègia ecològica, afegint a les avantatges que proporciona la recombinació genètica l'ajust del cicle de vida als factors ambientals, a més de la capacitat de persistència.

15) Les característiques del cicle de vida permeten a les espècies colonitzar nous nínxols, com les construccions artificials que estan éssent creats pels éssers humans.

Informe de la directora de la tesi



INFORME DE LA DIRECTORA DE LA TESI

La Dra. Esther Garcés i Pieres, directora de la tesi titulada: **"Role of life-cycle stages in dinoflagellate population dynamics" - "Paper dels estadis del cicle de vida en la dinàmica de poblacions de dinoflagel·lades"** realitzada per Silvia Anglès i Calvo,

Informa de la implicació de la doctoranda en cada article científic desenvolupat per la present tesi i de que cap dels articles, ni de les dades aquí presentades, han estat usades per a la memòria d'una altra tesi.

ARTICLE 1: "High-resolution spatio-temporal distribution of a coastal phytoplankton bloom using laser in situ scattering and transmissometry (LISST)". Autors: S. Anglès, A. Jordi, E. Garcés, M. Masó, G. Basterretxea.

Article publicat a la revista "Harmful Algae", amb índex d'impacte de 2.69 a l'any 2008 i situada al primer quartil (categoria "Aquatic Sciences", posició 22 de 37). El disseny experimental es va fer conjuntament entre la directora i la doctoranda. La doctoranda va intervenir en el mostreig, la posada a punt del mètode de calibració i de l'aparell, i del tractament de dades. El tractament de les dades físiques i del model numèric de circulació marina correspon al Dr. A. Jordi, actualment investigador del IMEDEA (UIB-CSIC, Mallorca). La doctoranda va treballar les dades en conjunt, va interpretar els resultats, i va redactar l'article, sota l'assessorament dels coautors (E. Garcés, M. Masó, G. Basterretxea).

ARTICLE 2: "Dinoflagellate cysts in recent sediments from two semienclosed areas of the Western Mediterranean Sea subject to high human impact". Autors: C. T. Satta, S. Anglès, E. Garcés, A. Lugliè, B. M. Padedda, N. Sechi.

Article publicat a la revista "Deep-Sea Research II", amb índex d'impacte de 1.96 a l'any 2009 i situada al primer quartil (categoria "Oceanography", posició 11 de 24). El disseny experimental es va fer conjuntament entre la directora, la doctoranda i la investigadora C. T. Satta del Dipartimento di Scienze Botaniche, Ecologiche e Geologiche, de la Universitat de Sassari (Sardenya). El mostreig, processament de les mostres de sediment, i la posterior identificació i quantificació dels cists de resistència en una de les àrees pilot va ésser dut a terme per la doctoranda. La doctoranda va intervenir en el tractament de dades, la interpretació dels resultats, i la redacció de l'article, assessorada pels coautors E. Garcés, A. Lugliè, B. M. Padedda, N. Sechi).

ARTICLE 3: "*Alexandrium minutum* resting cyst distribution dynamics in a confined site ". Autors: S. Anglès, A. Jordi, E. Garcés, G. Basterretxea, A. Palanques.

Article publicat a la revista "Deep-Sea Research II", amb índex d'impacte de 1.96 a l'any 2009 i situada al primer quartil (categoria "Oceanography", posició 11 de 24). El disseny experimental es va fer conjuntament entre la directora i la doctoranda. La doctoranda va participar en els mostrejos, va identificar i quantificar els cistos de resistència de l'espècie objectiu en el sediment, va analitzar les dades i va redactar l'article. El tractament de les dades físiques i del model numèric de circulació marina correspon al Dr. A. Jordi. La granulometria del sediment va ser analitzada pel Dr. Palanques i el seu laboratori. La doctoranda va redactar l'article sota l'assessorament dels coautors (E. Garcés, G. Basterretxea, A. Palanques).

ARTICLE 4: "Life-cycle alternations in *Alexandrium minutum* natural populations". Autors: S. Anglès, E. Garcés, A. Reñé, N. Sampedro.

Article sotmès a la revista "Marine Ecology Progress Series", amb índex d'impacte de 2.63 a l'any 2009 i situada al primer quartil (categoria "Aquatic Sciences", posició 15 de 37). El disseny experimental es va fer conjuntament entre la directora i la doctoranda. La doctoranda va participar en els mostrejos, va identificar i quantificar els estats de resistència de l'espècie objectiu en el sediment, va quantificar el fluxos de germinació i encistament de les cèl·lules al camp, i va analitzar les dades en conjunt. La doctoranda va redactar l'article sota l'assessorament dels coautors (E. Garcés, A. Reñé, N. Sampedro).

ARTICLE 5: "In situ life-cycle stages of *Alexandrium tamarense* complex during a bloom development in Long Island (USA)". Autors: S. Anglès, E. Garcés, C. J. Gobler.

Article no publicat, la versió que es presenta en la memòria de tesi es la versió revisada i consensuada pels diversos coautors i en preparació per enviar a una revista especialitzada internacional. L'estudi va ésser dissenyat per la doctoranda, la directora de tesi i pel Dr. C. J. Gobler. El treball experimental es va realitzar durant una estada de la doctoranda en el laboratori del Dr. C. J. Gobler a la Universitat de Stony Brook University-Southampton (EUA). La doctoranda es va encarregar de dur a terme l'experiment al camp, de la quantificació de les mostres, de l'anàlisi de les dades i de la seva interpretació, així com de la redacció de l'article, assessorada pels coautors (E. Garcés, C. J. Gobler).

General introduction

GENERAL INTRODUCTION

Phytoplankton comprise unicellular aquatic organisms that carry out photosynthesis, using solar energy to transform CO_2 and water into organic matter (primary production). These organisms are also the basis of the marine food web and as such provide nutrients to organisms of higher and lower trophic levels. Phytoplankton account for approximately 50% of the total annual photosynthesis on Earth (Field et al., 1998), thereby contributing to O_2 production within the marine environment and to the uptake of CO_2 from the atmosphere. Therefore, they are key elements not only for the oceans but also for Earth's ecosystem overall. Indeed, the most accepted theory to date regarding the origin of O_2 in Earth's atmosphere cites oxygen production resulting from the photosynthesis carried out by phytoplankton, specifically by cyanobacteria.

Phytoplankton include both prokaryotic (bacteria) and eukaryotic (microalgae) organisms. Among them, some of the more important groups are diatoms, cyanobacteria, coccolithophores, and dinoflagellates. This thesis is centered on the latter group.

HARMFUL ALGAL BLOOMS

Under favorable environmental conditions, one or a few phytoplankton species can increase in cell abundance with respect to their basal concentration to form **blooms**. Phytoplankton blooms are part of normal ecosystem functioning, but the proliferations of certain phytoplankton species can be harmful from a human point of view. These events are referred to generically as **harmful algal blooms** (**HABs**), a term that is not precise since not all HAB-producing species are algae, and not all harmful events involve the development of abundant biomass. In fact, **harmful algal** (**HA**) species encompass a wide range of phytoplankton groups, including dinoflagellates, diatoms, and cyanobacteria, which are not considered algae. Additionally, harmful episodes can occur at different cell abundances depending on the species, ranging from 100 to more than 1,000,000 cells l^{-1} (Shumway, 1990; Smayda, 1997a). Of all phytoplankton species, approximately 300 are known HA species, and 80 of them are considered to be toxic. Particularly, dinoflagellates are one of the main causative groups, comprising 75% of HA species (Smayda, 1997b). There are several types of HABs; these can be generally assigned to one of the following two main classes (GEOHAB, 2001):

1) Proliferations of species that produce potent **toxins**, which are accumulated by filter-feeding bivalves or fish and transferred through the food web to higher trophic levels, such as fish, marine mammals, and humans, via consumption. Another route of intoxication is by the transport of toxins in aerosols, favored by certain hydrographic and wind conditions. Human disease syndromes associated

with the different types of toxins and species include paralytic, diarrheic, amnesic, neurotoxic, and azaspiracid shellfish poisoning (PSP, DSP, ASP, NSP, and AZP, respectively), ciguatera fish poisoning (CFP), and the respiratory and allergic effects provoked by the presence of the toxins in aerosols. Other types of toxins are harmless to humans but not to fish (icthyotoxins). These HA species economically impact aquaculture through losses of fish stocks, the closures of shellfish farms, and the endangerment of non-commercial marine resources.

2) Proliferations of species that produce deleterious effects to the ecosystem, which include **high-biomass blooms** and blooms of foam- or mucilage-producing species. The mainly ecological impacts of such blooms are water discoloration, which cause a loss of esthetic quality, and anoxia, which can result in the death of marine fauna unable to escape (e.g., caged fish in aquaculture farms or benthic sessile invertebrates). The high abundance of cells or the presence of mucilage can in themselves provoke both the large-scale death of fish, by clogging their gills. Consequently, in addition to the negative effects on the ecosystem, these blooms have economic impacts on human activities (e. g., aquaculture or tourism).

HABs in the coastal zone

HABs are coastal and open-ocean phenomena observed in a wide variety of ecosystems, such as coastal embayments or upwelling systems. All phytoplankton populations, including those of HA species, are influenced by physical, chemical, and biological interactions occurring over a broad range of temporal and spatial scales (GEOHAB, 2001; Fig. 1). Relevant physical factors consist of large-scale processes (climate forcing, advection, or transport) as well as mesoscale (convergence zones, fronts, or upwelling) and small-scale (turbulence or surface waves) ones. Biological processes include swimming, aggregation, grazing, and life-history transitions.

The coastal zone comprises a broad range of open, enclosed, and semi-enclosed aquatic environments along land-mass margins. Enclosed or semi-enclosed coastal systems, which are encompassed by the term **embayments**, include bays, estuaries, lagoons, semi-enclosed beaches, and harbors. Coastal embayments are diverse in terms of their size, depth, and hydrodynamic regimes, determined by the varying degrees of physical isolation from the open coast. However, they share several features, most notably, constrained hydrodynamics and land-sea interactions clearly distinguishable from large-scale coastal processes. In addition, they receive inputs of dissolved and particulate material from the land, e.g., nutrients, suspended material, and oligoelements from both natural and anthropogenic sources (Cembella et al., 2005).

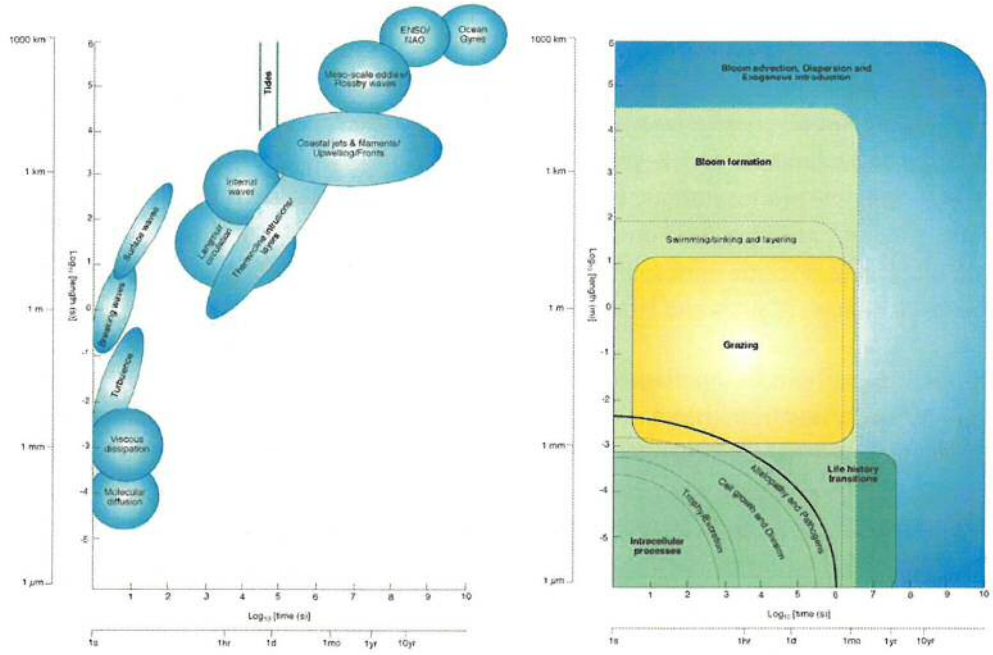


Fig. 1. Temporal and spatial scales of the physical and biological interactions that influence blooms. Source: GEOHAB (2001). Note that life-history transitions extend over a time scale ranging from seconds to years.

As a result of the physical constraints forced by land boundaries and shallow embayment bathymetry, small-scale processes are highly relevant in determining water circulation within these systems. The typically restricted hydrodynamics combined with the morphology of the basin enhance water-mass retention and reduce dilution and exchange with external waters. These characteristics of embayments provide specific niches for phytoplankton species, including HA species. Phytoplankton species are able to exploit the wide variety of environmental conditions found in these semi-enclosed or enclosed systems through adaptations (e.g., multiform life-cycle strategies or migratory behavior) that enable their survival, persistence, and, in some cases, dominance during bloom events. Physical-biological interactions at small scales, such as the interaction between organisms and water circulation, are essential for population advection and accumulation as well as the maintenance of certain blooms (Anderson and Stolzenbach, 1985; Basterretxea et al., 2005).

The research for this thesis was carried out in semi-enclosed coastal systems (two harbors, a gulf, and a beach) located in the NW Mediterranean Sea and in the NW Atlantic Ocean.

Dinoflagellates and their life cycle

Dinoflagellates are one of the most abundant phytoplankton groups, with approximately 2000 living species recognized so far, of which more than 1700 are marine (Graham and Wilcox, 2000; Smayda and Reynolds, 2003). They form a mixed group in which half of the species are photosynthetic (autotrophic) or combine photosynthesis with predation (mixotrophic), and half feed on other planktonic organisms (heterotrophic). In addition, dinoflagellates include endosymbiotic species of marine invertebrates and protozoa, and parasitic species (Gaines and Elbrächter, 1987; Taylor et al., 2008).

Earlier descriptions of dinoflagellates were based on observations in living or fixed water samples obtained from the field. For that reason, the most well-known dinoflagellate stage is the **vegetative cell**, which defines the **planktonic phase** (Fig. 2). Vegetative cells are motile due to the possession of two flagella. These are of unequal lengths and consist of a transversal flagellum, which encircles the cell and enables rotation, and a longitudinal flagellum, located at the posterior part of the cell for forward propulsion (Taylor, 1975). Vegetative cells are haploid and undergo asexual division by binary fission, thereby increasing the population exponentially.

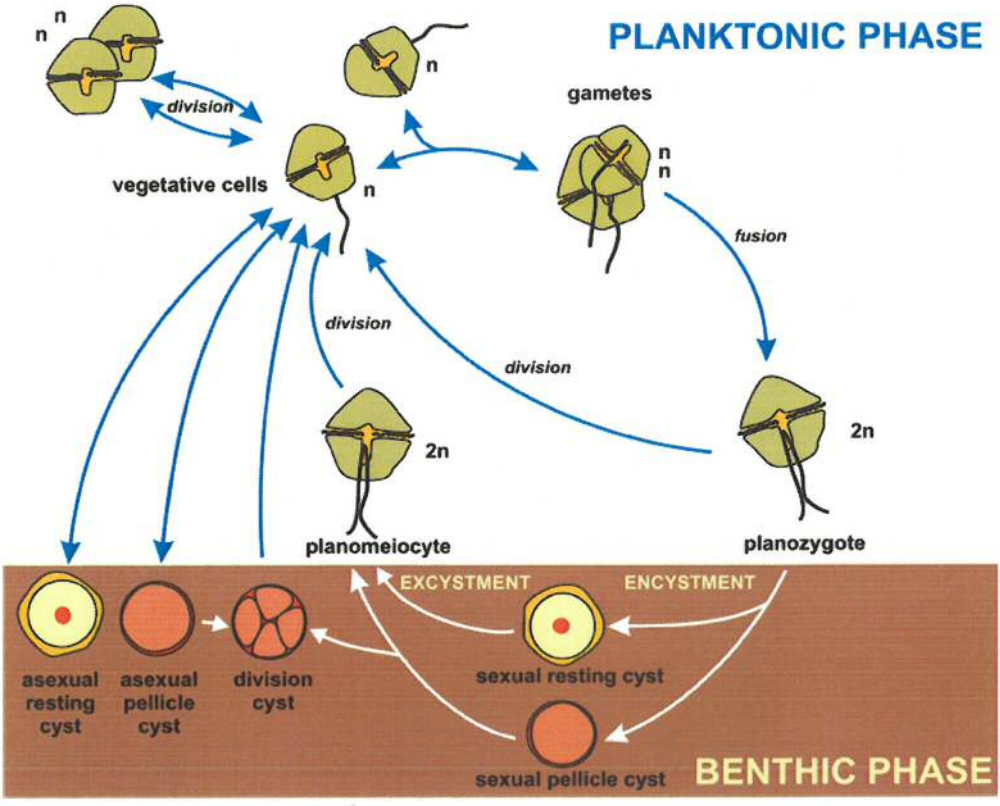


Fig. 2. The general life cycle of cyst-producing dinoflagellates (adapted from Walker, 1984) updated with the new stages as explained in the text. Life stages drawings are courtesy of R. Figueroa.

Until the first observations of the dinoflagellate life cycle, carried out by Von Stosch (e.g., Von Stosch, 1969, 1972, 1973), dinoflagellates were generally believed to reproduce only asexually. Furthermore, asexual and sexual stages of the same species were in some cases described as separate taxa. Nowadays, several dinoflagellates are known to undergo sexual reproduction, and the number of species recognized is progressively increasing. After a process still poorly understood, vegetative cells become gametes, two of which fuse during sexual reproduction. Gamete fusion may involve equal (isogamy) or unequal (anisogamy) gamete sizes and may occur between cells of the same (homothallism) or different (heterothallism) mating types. The resulting stage is the **planozygote**, a diploid cell with two pairs of flagella (one from each fused gamete) that remains motile for several hours to a few days before it becomes a nonmotile zygote. During this transformation process, known as **encystment**, the planozygote loses all of its flagella and forms a thick and resistant wall. The resulting nonmotile stage is the **resting cyst** (or hypnozygote), which sinks to the bottom sediments, thus entering the **benthic phase** (Fig. 2). After a **mandatory dormancy period** lasting days or several months, **excystment** (or germination) of the resting cyst gives rise to the **planomeiocyte.** This diploid motile cell undergoes division to produce two haploid vegetative cells, thereby reestablishing the planktonic phase (Walker, 1984). Meiosis usually occurs in the zygote, but the timing of this process is poorly understood. Nuclear cyclosis was proposed by von Stosch (1973) as a diagnostic characteristic of dinoflagellate meiosis, but it has yet to be observed in a sufficient number of species.

With the development of culturing techniques, researchers have been able to culture species and to follow their complete life cycle in the laboratory. As a result, an increasing number of scientific studies have shown that life-cycle phases of some dinoflagellates are relatively complex, as listed below. Figure 2 summarizes the general life cycle of cyst-forming dinoflagellates and the main findings reported to date:

1) During the planktonic phase, vegetative cells can form pellicle cysts (also called temporary or ecdysal cysts) (Bravo et al., 2010; Dale, 1977; Walker, 1984). This type of cysts is different from the resting cyst: it possesses a thin wall (pellicle) and is able to excyst within a short time (from hours to few days) due to the absence of a mandatory dormancy period. Pellicle cyst formation has been classically linked to stressful conditions, such as changes in temperature and light, nutrient depletion, or as a means to avoid predation or attack by viruses, bacteria, and parasites. However, evidence exists that pellicle cysts are an essential stage in the population dynamics of certain species (see review in Garcés et al., 2002). Cell division has also been observed to occur in pellicle cysts (so-called **division cysts**), giving rise to two or more vegetative cells (Figueroa and Bravo, 2005; Garcés et al., 1998; Kita et al., 1985).

2) Vegetative cells have also been observed to produce asexual resting cysts (Kremp and Parrow, 2006). These have the same morphology and the same mandatory

dormancy period of several months as the sexual resting cysts produced through planozygote formation. In addition, vegetative cells can form a temporary stage, whose origin is not well-understood, that can either give rise to motile cells in a few days or remain viable for up to two years (Rintala et al., 2007).

3) The transformation of the planozygote into a resting cyst is the most common pathway described in cyst-producing species, but it was recently reported that the planozygote stage of some species may follow multiple subsequent routes: i) division and reversion to the asexual stage (vegetative cell), ii) encystment as a sexual resting cyst, iii) encystment as a sexual pellicle cyst that can undergo division (Uchida, 1991; Figueroa et al., 2006a, 2006b).

4) Gametes can revert to the asexual stage and undergo binary fission instead of fusion (Figueroa et al., 2006a, 2006c).

Integration of the life cycle into dinoflagellate population dynamics

Population dynamics describes the changes that a population experiences in space and time over the course of its lifetime and the factors that trigger these changes. An adaptive strategy common to many dinoflagellates, including HA species, is a life cycle involving resting stages. Accordingly, phytoplankton species can be divided into two main groups based on their life cycle: **holoplanktonic** (present in the water year round) and **meroplanktonic** (with a documented resting stage that inhabits the benthos). In meroplanktonic species, the alternating planktonic and benthic phases imply different ploidy stages that occupy different niches in space and time. Different physical, chemical, and biological factors interact and regulate the distinct phases as well as their alternations, with important implications for a species' success (Steidinger and Garcés, 2006; Valero et al., 1992). Therefore, research into the population dynamics of dinoflagellate species needs to incorporate all phases of their life cycle and the environmental factors that regulate each one. This research approach can be developed along three main lines:

1) Study of the planktonic-phase dynamics of target species in diverse embayments.

2) Identification and quantification of the benthic phase, and investigation of its dynamics. In this thesis, the term benthic phase makes reference to the sexual resting cyst.

3) Assessment of the planktonic-benthic transitions of target species at selected sites, specifically, excystment and encystment processes.

The work described in this thesis consists of an analysis of the dynamics of target species within semi-enclosed systems. Across these sites, bloom dynamics vary depending on the particular topographic and hydrographic characteristics as well as on the ecological and

biological traits of the causative organisms. Nevertheless, it is possible to draw general conclusions regarding the significant processes controlling bloom dynamics across similar ecosystems (Anderson et al., 2005; GEOHAB, 2001). The target species discussed in the present thesis belong mostly to the genus *Alexandrium*, a group of cyst-forming dinoflagellates that largely rely on the excystment of resting stages for bloom initiation and recurrence. Furthermore, this genus includes 13 harmful species, 10 of which are among the most toxic (Moestrup et al., 2004). Finally, it is one of the main genera causing toxic events in the Mediterranean Sea (Vila et al., 2001). *Alexandrium* species can be studied in different habitats, where the relative importance of life cycle stages may vary due to depth, advection, and other factors.

In the following, a general overview together with a discussion of the current state of the art of the three above-mentioned lines of research is given, while pointing out gaps in our knowledge, some of which have been addressed by this doctoral work.

The planktonic phase

From the perspective of HABs, planktonic-phase dynamics has been of particular interest since toxic or noxious events are caused by the vegetative stage. In accordance, it is essential to understand why and when harmful species blooms occur, and once the bloom is initiated, the duration and intensity of the planktonic phase. In cyst-producing species, the different life-cycle stages play distinct and important roles in each phase of bloom development, with profound implications for bloom dynamics:

1) **Bloom initiation**: This first phase of the bloom requires a vegetative cell inoculum, which can originate from the excystment of resting cysts. Steidinger (1975) proposed the botanical term "**seed beds**" for resting stages acting to supply coastal blooms occurring worldwide, and pointed out the relevance of resting cysts in bloom initiation. The inoculum can derive from resting cysts germinated within the same area where the bloom develops or from advected sources originating from adjacent seed beds. The factors that trigger bloom initiation are poorly understood for most harmful dinoflagellates, mainly due to the difficulty in obtaining sufficient data on pre-bloom and initial bloom conditions.

2) **Exponential growth and maintenance**: During exponential growth, the vegetative cell responds to environmental conditions (e.g., light, photoperiod, nutrient or prey availability, temperature, salinity, and turbulence), which determine growth and the increase in population biomass (Steidinger, 1975; Steidinger and Garcés , 2006). During the maintenance phase, the population reflects the balance between vegetative growth and loss factors, which include grazing, cell death, parasitism, and dispersion, among others (Burkholder et al., 2006; Taylor, 1987). At this phase

of the bloom, any adaptive strategy to enhance maintenance of the population is critical. Vegetative cells of many dinoflagellates form pellicle cysts capable of withstanding adverse environmental conditions (Garcés et al., 1999). In addition, vertical migration is a successful strategy to reach zones richer in light or nutrients, as well as to avoid cell dispersion (Basterretxea et al., 2005; Eppley et al., 1968). In this regard, knowledge on the coupling of physical factors with biological behavior is required to understand vertical and horizontal distributions and the bloom dynamics of a species. However, these small-scale interactions are often not well quantified under field conditions, since phytoplankton distributions tend to be patchy in space and time. Therefore, studies of bloom dynamics can be greatly improved by the incorporation of instrument deployments that yield measurements at the appropriate spatio-temporal resolution (Babin et al., 2005, 2008)

3) **Termination**: Bloom termination is usually attributed to adverse conditions for vegetative growth (e.g., unfavorable temperatures or salinities, insufficient light, and nutrient depletion). Other environmental factors may include cell mortality, grazing, competition between species, dispersion, and the transition of vegetative cells to the resting stage (Anderson et al., 1983; Burkholder et al., 2006; Calbet et al., 2003). In many cases, the reasons for bloom termination have yet to be fully elucidated, probably because they involve a combination of factors (Garcés et al., 2004; Van Lenning et al., 2007).

The dynamics of the planktonic phase has been investigated throughout this thesis, including long-term temporal fluctuations of vegetative cells (**Papers 3** and **4**) and their spatio-temporal variations during bloom development (**Papers 3, 4** and **5**). In addition, short-term spatio-temporal variability of vegetative cells during bloom maintenance has been assessed using high-resolution technologies (laser in situ scattering and transmissometry, LISST) to ascertain the physical-biological coupling underlying this phase of the bloom (**Paper 1**).

The benthic phase

Brief history of dinoflagellate cyst investigations

Earlier studies on dinoflagellate cysts were conducted in the 19th century by palynologists working on microfossils in sediments. However, due to their distinct morphology with respect to planktonic cells, cysts were neither related to dinoflagellates nor identified as resting cysts. Paleontological research into "hystrichospheres," as they were called at the time, increased considerably from the 1930s based on their importance as paleoecological

indicators. However, biologists interested in the study of planktonic dinoflagellates paid scant attention to resting cysts, with only a few exceptions, such as the studies on dinoflagellate encystment by Huber and Nipkow (1922, 1923), Braarud (1945), and Nordli (1951), who suggested that hystrichospheres were dinoflagellate cysts. In addition, another group of palynologists investigating paleoclimate also found hystrichospheres but considered them as remnants of extinct organisms. Conversely, Erdtman (1949, 1950, 1954) demonstrated that they were extant by providing evidence of living representatives of hystrichospheres recovered from sediment traps. Still, the realization that hystrichospheres had a living equivalent was not broadly recognized by the palynologist community until the definitive evidence provided by Evitt (1961): the presence of an opening (archeopyle) in the cyst and common features between motile stages and fossil cysts (paratabulation). Meanwhile, Wall and Dale demonstrated that hystrichospheres collected from sediments produced motile dinoflagellate cells, providing the first evidence on excystment and the role of hystrichospheres as resting cysts of dinoflagellates (Wall, 1965; Wall and Dale, 1966). The discovery of the connection between hystrichospheres and dinoflagellate resting cysts revolutionized both palinology and biology fields. Palynologists initiated numerous investigations into fossil cysts and their corresponding living cells, resulting in an extensive taxonomic classification of cysts ("**dinocysts**"). In parallel, biological studies were (and still are) more focused on the ecological role of resting cysts as zygotes and their equivalent biological species. Perhaps not surprisingly, as a result of the two independent research lines, there are two different taxonomic denominations for dinoflagellate cysts: palynological and biological. In order to join the two and to share knowledge among cyst researchers from both fields, Evitt initiated a series of conferences ("The International Conference on Modern and Fossil Dinoflagellates"), which began in 1978 in Colorado Springs and are still held on a regular basis. In this thesis cysts are examined from a biological point of view.

Importance of the benthic phase

Nowadays, approximately 200 species of dinoflagellates are known to produce resting cysts (Head, 1996), a number that is progressively increasing but may never accurately reflect the one in nature. Resting cysts are essential stages for the ecology of dinoflagellates. The functions of this benthic stage, modified from the five functions first enounced by Wall (1971) and completed below based on a compilation from the literature, include:

1) **Genetic variability**: Resting cysts represent one of the sexual stages of the dinoflagellate life cycle that contribute to chromosomal recombination, with obvious implications for the plasticity, fitness, and success of the species (Figueroa et al., 2006c)

2) **Recurrence**: Resting cysts are a seed source in the initiation and recurrence of vegetative blooms. The hypothesis that benthic stages are involved in recurrent

HABs was introduced by Prakash (1967), with several studies later providing evidence of recurrent blooms at sites where resting cysts "overwintered" in the sediments (e.g., Anderson and Wall, 1978; Dale, 1977).

3) **Seasonality**: Braarud (1962) suggested that the seasonality observed in several phytoplankton species was related to the alternation of their life-cycle phases, and studies have shown that resting cysts are responsible for the synchronization between bloom initiation and seasonal environmental factors (Anderson and Wall, 1978; Anderson and Morel, 1979).

4) **Survival**: The benthic stage is much longer in duration than the vegetative stage and its resistance to unsuitable environment is much greater, allowing species survival when environmental conditions are unfavorable.

5) **Species dispersal**: Resting cysts may act as vectors for geographical dispersion. As passive particles, they can be transported by currents and dispersed within the sedimentary regime, extending the biogeographic range of the species through the invasion of new territories (Dale, 1983). In addition, the spreading of species via ballast water or during the transfer of shellfish stocks from one area to another is recognized to be mainly vectored through dormant stages (Hallegraeff et al., 1993).

6) **Defense**: Resting cysts have been suggested as a mechanism to avoid predation of the planktonic stage in both marine and freshwater enviroments (Hansson, 1996; Rengefors et al., 1998).

7) **Biodiversity**: Resting cysts form a "**seed bank**" that can be activated when environmental conditions are adequate, thereby guaranteeing the continuity of the species in space and time and constituting a reservoir of biodiversity (Boero et al., 1996; Margalef, 1994, 2002). In other words, resting cysts represent a part of the genetic memory of an ecosystem.

Resting cyst morphology

Dinoflagellate resting cysts are nonmotile benthic stages and possess a thick multilayered wall (Evitt et al., 1977; Von Stosch, 1973). The composition of the different wall layers has not been fully determined, and it may vary depending on the species. The walls of most resting cysts are composed of organic matter (organic cysts). Some contain cellulose, whereas in others a complex polymer similar to sporopollenin, termed dinosporin, has been identified. The presence of dinosporin is considered to be responsible for the fossilization of resting cysts (Dale, 1983; Fensome et al., 1993). In addition, the resting cysts of a number of species are mineralized with calcite (calcareous cysts). Resting-cyst walls can be smooth or present a wide variety of ornamentations, such as spines, projections, ridges, horns, or calcareous

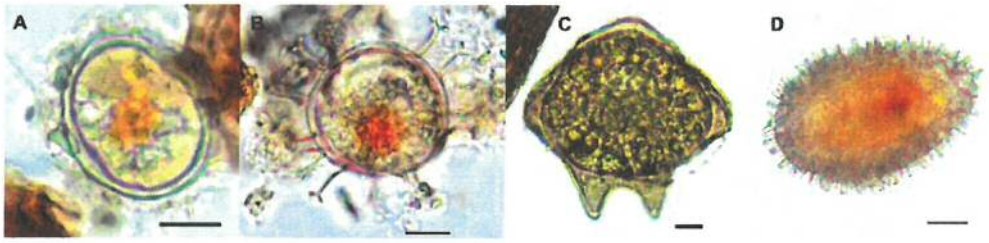


Fig. 3. Several examples of the morphological variability of resting cysts: (A) smooth organic-walled resting cyst (*Alexandrium minutum*); (B) organic-walled resting cyst with projections (*Gonyaulax sp.*); (C) unornamented organic-walled resting cyst with shape resembling the vegetative cell (*Protoperidinium leonis*); (D) calcareous resting cyst (*Scrippsiella trochoidea*). Scale bar 10 µm.

processes (Fig. 3). The shape of the resting cyst can be spherical, ellipsoidal, or ovoidal, or may resemble that of the vegetative cell. Their content consists of a nucleus, one or several yellow- to red-pigmented accumulation bodies (resulting from the accumulation of carotenoid pigments derived from plastid degradation), and a number of storage products, such as starch grains or oil droplets (Chapman et al., 1981). In contrast to vegetative cells, there is a reduction or disappearance of cytoplasmic structures, including chloroplasts and Golgi bodies (Bibby and Dodge, 1972). All these morphological characteristics, i.e., shape, size, type of ornamentation, and content, are used as taxonomic criteria for the classification of resting cysts.

A fundamental part of this thesis consisted of the taxonomic identification of dinoflagellate resting cysts from coastal ecosystems (**Papers 2, 3, 4** and **5**).

Resting cyst dynamics

The reduced metabolic activity (Bibby and Dodge 1972) of resting cysts enables them to survive for several years buried in the sediments. This survival is also favored by their characteristic thick, dense wall, which is resistant to biological (e.g., bacterial and fungal) and chemical attack (Dale, 1983). Although survival times vary within species, the half-lives of living resting cysts can be of 2–10 years (Keafer et al., 1992). More recently, Ellegaard et al. (2008) were able to germinate cysts isolated from sediments dated at 100 years old.

Resting cysts in sediments behave as passive sedimentary particles; consequently, they are mainly controlled by the sedimentary regime. Since the behavior of these benthic stages resembles that of the fine-sediment fraction (silts and clays), zones with higher proportions of fine sediments generally have higher resting cyst abundances (Dale, 1976). Thus, processes determining sediment dispersion and accumulation influence horizontal and vertical resting cyst distribution and abundance. Other processes include biotic and

abiotic factors such as germination, mortality, predation, resting cyst formation, burial, bioturbation by benthic organisms, and resuspension (Anderson et al., 1982; Giangrande et al., 2002; Persson, 2000).

Mapping of resting cyst in the sediments provides useful information on their species composition, distribution, and abundance. Most of the research in this field has focused on the species composition of resting cyst assemblages (seed bank) in the sediments. The main objective of these studies was to assess the biogeographic distribution of species occurring presently or in the past (Amorim and Dale, 2006; Bolch et al., 1990; Joyce, 2004; Nehring, 1994), or to determine their relation with both the presence of vegetative cells and the environmental conditions in the overlying water column (An et al., 1992; Godhe et al., 2003; Matsuoka et al., 2003). Surveys of resting cyst assemblages are also a useful means to reveal species rarely observed in the plankton and to assess the potential for future blooms (Bravo et al., 2006; Joyce, 2005). However, only a few field studies on resting stage dynamics in the sediments have considered the biological, physical, and other environmental mechanisms that control the spatio-temporal abundance and distribution of resting cysts. In addition, how these multiple mechanisms interact is not fully understood.

The diversity within dinoflagellate seed banks in two semi-enclosed sites of the Mediterranean Sea was evaluated and compared (**Paper 2**). In addition, the spatial distribution and temporal fluctuations of resting cysts were assessed in order to: i) explore the influence of resuspension by physical processes on resting cyst distribution, and the relationship of this distribution to resting cyst formation in the overlying water column (**Paper 3**), and ii) obtain basic information to provide a better understanding of life-cycle stage transitions (**Papers 4** and **5**).

Planktonic–benthic phase transitions

Planktonic-benthic transitions are an obvious key component in cyst-producing species. As mentioned above, many of these species exhibit recurrent and/or seasonal patterns of abundance of their motile cells in the water column. Major transition processes in the life cycle of these species are **excystment** and **encystment** (regarded in this thesis as the transition from resting cyst to vegetative cell, and from vegetative cell to resting cyst, respectively). These planktonic-benthic transitions are marked by particular characteristics and modulated by several endogenous and exogenous factors.

Excystment

Excystment of the resting cyst occurs after a **mandatory dormancy period**, which is defined as the time needed for maturation. During this period, there is an endogenous inhibition of germination even under environmentally favorable conditions. The length of the mandatory dormancy period of resting cysts is species-specific and may be affected by environmental conditions, such as temperature (Anderson, 1980), and genetic factors (e.g., inheritance, Figueroa et al., 2005). Cellular amounts of storage products have also been suggested to have an effect (Steidinger and Haddad, 1981).

Once the mandatory dormancy period is overcome, resting cysts can, if environmental conditions are favorable, excyst or, in the absence of appropriate conditions, remain quiescent (dormant). Of the environmental factors that modulate the germination of dinoflagellate resting cysts, temperature is recognized as significant (Dale, 1983; Pfiester and Anderson, 1987). Species have a temperature window within which resting cysts excyst (Anderson and Morel, 1979). Studies have shown that a major cue for excystment is a change in temperature to favorable levels, as occurs in seasonal warming or cooling in temperate waters (Anderson, 1980). Similar to temperature, there is an optimum species-specific salinity range for excystment (Band-Schmidt et al., 2003; Canon, 1993; Kim et al., 2002). Intracellular nutrient reserves also may be a factor terminating quiescence (Steidinger and Haddad, 1981), but only the effects of environmental nutrient concentrations have been investigated in laboratory experiments. According to these studies, nutrient levels have a species-specific effect on excystment. For example, germination of the resting cysts of some *Alexandrium* species is not influenced by nutrient concentrations (e.g., *A. tamarense*, Anderson, 1980; *A. minutum*, Canon, 1993), whereas other studies reported a negative effect of nutrient repletion in *A. catenella* (Figueroa et al., 2005). In contrast, nutrient depletion retards or inhibits germination in other dinoflagellate species (e.g., *Scrippsiella trochoidea*, Binder and Anderson, 1987; or *Ceratium hirundinella*, Rengefors and Anderson, 1998). According to Rengefors et al. (1996), since external nutrient levels may be a critical parameter affecting germling viability and survival, resting cysts ready to excyst may be able to utilize nutrients present in the environment. This hypothesis was supported by evidence that resting cysts of *S. trochoidea* are capable of take up inorganic phosphorous. However, to date, this capability of phosphorous uptake remains to be confirmed for other dinoflagellates and for other nutrients, such as nitrogen. Anoxia and darkness, conditions that surround resting cysts in the sediments, are known to inhibit the germination of quiescent resting cysts of most species (Anderson et al., 1987). This mode of excystment inhibition has several implications: the germination of resting cysts is restricted to the upper millimeters of the sediment surface, whereas resting cysts in deeper layers will remain unable to excyst unless external factors (e.g., bioturbation or resuspension) result in a sufficiently irradiant and oxygenated environment. The influence of different light intensities has been investigated in very few laboratory studies, but again the results showed varying effects according to species. For example, *A. minutum*

germination varied between different light intensities, with higher rates observed at light levels as low as 20 μE $m^{-2}s^{-1}$ (Canon, 1993; Blanco et al., 2009), while resting cysts of the *A. tamarense* complex germinated at similar rates independent of the tested irradiances. Finally, an endogenous annual clock modulating the excystment of resting cysts was reported for populations inhabiting deep-ocean areas, where environmental cues such as light are absent (*A. tamarense* cysts of the Gulf of Maine, Anderson and Keafer, 1987; Matrai et al., 2005).

Encystment

Gamete fusion gives rise to the planozygote, a motile cell that often resembles the vegetative cell, which is one of the reasons why in earlier studies the planozygote stage was frequently mistaken for vegetative cells. However, the planozygote can be morphologically distinguished by its two trailing flagella and the fact that it is frequently larger, darker, and swims more slowly than vegetative cells (Anderson et al., 1983; Probert, 1999). After their formation, planozygotes remain motile for a few days or several weeks, until the completion of encystment. The processes by which the planozygote becomes a resting cyst are largely unknown.

Resting-stage formation in dinoflagellates has been typically linked to suboptimal conditions for growth. Specifically, nutrient limitation has been largely used in laboratory studies to induce sexuality and resting cyst production. As a consequence, it is widely accepted that nutrient depletion is the main cue stimulating sexuality and encystment. In spite of these laboratory findings, observations of resting cyst formation in the field do not frequently support the relationship between low nutrient availability and encystment (Garcés et al., 2004; Kremp and Heiskanen, 1999; Pitcher et al., 2007; Wall, 1970). In addition, Sgrosso et al. (2001) observed resting cyst formation at high external nutrient levels in culture experiments. Therefore, some authors have argued that optimal ambient conditions are needed for cysts to undergo sexuality (Wall, 1970). Such discrepancies may result from intracellular nutrient deficiencies, since a drop in internal nutrients to threshold levels was shown to be involved in the transitioning of vegetative cells to sexual stages. Moreover, the general metabolic state (carbon metabolism) of the cells apparently also influenced the sexual response (Probert, 1999). This implies the need to assess nutrient limitation in the context of intracellular nutrient and metabolic state instead of external nutrient availability, but these parameters are difficult to measure under field conditions.

Other factors influencing encystment have been reported, such as changes in temperature or day length or the presence of bacteria, allelochemicals, or parasites (Adachi et al., 1999; Pfiester and Anderson, 1987; Sgrosso et al., 2001). In addition, cell density may be an important parameter. Wyatt and Jenkinson (1997) suggested that a requisite threshold in

cell abundance must be reached before encystment can proceed. Indeed, several studies showed that planozygotes of the species *S. trochoidea* and *Gyrodinium instriatum* did not encyst if cell density was below a certain threshold (Uchida 1991; Uchida et al., 1996). In a field study, Garcés et al. (2004) also observed the necessity of a threshold abundance before encystment occurred. Uchida (2001) reported cell contact as a potentially determining factor for the encystment of planozygotes. In fact, mating success may increase as the chances of cell encounters increase, such that the need for cell-to-cell communication cannot be ruled out (Wyatt and Jenkinson, 1997). In laboratory experiments, clumping behavior of gametes and the formation of clumps by resting cysts in culture plates are often observed (e.g., Figueroa et al., 2006b; Uchida et al., 1996). In the field, although observations are scarcer, planozygotes were seen in the water column within a frontal convergence, in which increased sexual activity was detected (Tyler et al. 1982). Hypothetical communication signals could be chemical substances such as pheromones, mechanical stimuli, or "quorum-sensing" molecules analogous to those used by bacteria to detect nearby cells (Persson et al. 2008; Uchida 2001; Wyatt and Jenkinson 1997).

In summary, excystment and encystment processes can influence population dynamics of cyst-producing species by determining the inoculum for bloom initiation and defining the size of the seed stock, respectively. In situ data on these life-cycle transitions and the endogenous and exogenous factors that may regulate them are needed to improve our understanding of the life cycle of individual species. This information will allow comparison between species and eventually generate and parameterize models of population dynamics.

In situ excystment and encystment of target species have been quantified in the present thesis, and the results integrated with those describing the dynamics of planktonic and benthic phases in order to gain insight into the population dynamics of these species (**Papers 4** and **5**).

Objectives of the thesis

OBJECTIVES OF THE THESIS

This thesis is aimed at contributing to the knowledge on dinoflagellate population dynamics by integrating the planktonic and benthic phases. The main goal was to evaluate the role of the different life-cycle stages using methodologies that involve observation and experimentation in natural field conditions. Since studies on the benthic phase are scarcer, a large part of the research developed within this thesis has been focused on this phase of the life cycle.

According to the main lines of research exposed in the General introduction, the specific objectives of this thesis were:

1) Study of the planktonic-phase dynamics of target species in diverse embayments:

   - To investigate the short-term spatial and temporal variability of the planktonic stage during bloom maintenance and the underlying physical and biological factors using LISST technology (**Paper 1**)
   - To assess long-term temporal fluctuations of the vegetative stage (**Papers 3** and **4**)
   - To characterize and quantify different life-cycle stages during bloom development: vegetative cells (**Papers 3, 4** and **5**) and planktonic planozygotes (**Paper 5**)

2) Identification and quantification of the benthic phase, and investigation of its dynamics:

   - To evaluate and compare the diversity of resting cysts in sediments from semi-enclosed embayments (**Paper 2**)
   - To determine temporal and spatial distribution of resting cysts of key species in the sediments at selected semi-enclosed sites (**Papers 3, 4** and **5**)
   - To investigate the role of the physical process (seiche-forced resuspension) and biological resting-stage production in resting cyst distribution dynamics (**Paper 3**)

3) Assessment of the planktonic-benthic transitions of target species at selected sites:

   - To quantify the flux of life-cycle stages of key species in different coastal embayments from and to the sediments (**Papers 4** and **5**), and to estimate in situ excystment (**Paper 4**) and encystment rates (**Papers 4** and **5**)
   - To investigate the factors influencing excystment and encystment, and the contribution of these transitions to bloom development (**Papers 4** and **5**)

The research developed during this thesis to address the objectives is presented as five scientific articles. Following, a General discussion of all the results and the General conclusions are given.

1

HIGH-RESOLUTION SPATIO-TEMPORAL DISTRIBUTION OF A COASTAL PHYTOPLANKTON BLOOM USING LASER IN SITU SCATTERING AND TRANSMISSOMETRY (LISST)

Harmful Algae 7, 808–816 (2008)

High-resolution spatio-temporal distribution of a coastal phytoplankton bloom using laser in situ scattering and transmissometry (LISST)

Sílvia Anglès[1], Antoni Jordi[2], Esther Garcés[1], Mercedes Masó[1], Gotzon Basterretxea[3]

[1] Institut de Ciències del Mar (CMIMA-CSIC), Pg. Marítim de la Barceloneta, 37-49, 08003 Barcelona, Spain.

[2] Marine Sciences Research Center, Stony Brook University, 101 Endeavour Hall, Stony Brook, NY 11794-5000

[3] IMEDEA (UIB-CSIC), Miquel Marqués 21, 07190 Esporles, Baleares, Spain.

Abstract

Development of optical observation technologies provides new insights into harmful algal bloom (HAB) detection and assessment of HAB species dynamics. Basing on preliminary laboratory tests, a laser in situ scattering and transmissometry instrument (LISST-100X) was used to monitor a high-biomass phytoplankton proliferation in the field. Short-term spatial and temporal changes in particle size distribution were measured during a recurrent *Alexandrium taylori* outbreak. Since the bloom was not monospecific, a size-fraction method to discriminate particular species from LISST-100X measurements was proposed. Results were validated to simultaneous microscopic counts of phytoplankton, and the significantly positive correlation obtained between the two methodologies confirmed the instrument's ability to discriminate phytoplankton at the group and species level. The LISST-100X obtains high-resolution in situ data, and is therefore a better alternative than the traditional microscope for assessing temporal and spatial evolution of HABs. Field observations showed high variability over a short time scale associated with diel vertical migration of *A. taylori* and the whole phytoplankton population (nanoplankton and microplankton). A numerical circulation model was used to investigate the influence of beach hydrodynamics in the observed horizontal variability. Simulations of the model suggested an important role of daily coastal circulation in determining the distribution of *A. taylori* in coastal environments.

Resum

El desenvolupament de tecnologies d'observació òptica proporciona nous coneixements sobre la detecció de proliferacions d'algues nocives (PAN) i l'avaluació de la dinàmica d'espècies d'algues nocives. Basant-se en proves preliminars de laboratori, es va utilitzar un instrument *Laser in situ scattering and transmissometry* (LISST-100X) al camp per quantificar una proliferació de fitoplàncton d'alta biomassa. Es van mesurar els canvis espacials i temporals a curt termini en la distribució de mida de partícules durant una proliferació recurrent d'*Alexandrium taylori*. Atès que la proliferació no va ser monoespecífica, es va proposar un mètode de fraccions per mides per discriminar espècies concretes a partir de les mesures del LISST-100X. Els resultats es varen validar amb recomptes simultanis de fitoplàncton mitjançant microscòpia, i la correlació significativament positiva obtinguda entre les dues metodologies va confirmar la capacitat de l'instrument per discriminar el fitoplàncton a nivell de grup i d'espècie. El LISST-100X obté dades in situ d'alta resolució temporal, i per tant és una alternativa millor que el microscopi tradicional a l'hora de mesurar l'evolució temporal i espacial de les proliferacions d'algues nocives. Les observacions de camp van mostrar una alta variabilitat a curta escala temporal associada amb la migració vertical d'*A. taylori* i de la població total de fitoplàncton (nanoplàncton i microplàncton). Es va utilitzar un model numèric de circulació marina per investigar la influència de la hidrodinàmica en la variabilitat horitzontal observada. Les simulacions del model suggereixen un paper important de la circulació diària en la determinació de la distribució d'*A. taylori* en la zona costanera.

Introduction

Research into the population dynamics and ecology of microalgae that produce harmful algal blooms (HABs) is an essential issue for a better understanding of such events. The first step is to characterize the HAB species involved and the accompanying phytoplankton community, along with the biological, physical and chemical processes that interact with them in their natural environment. The variable nature of HABs, which tend to be episodic and patchy, requires a high resolution measurement of all these parameters. In certain high-biomass HAB events, when discoloration is not readily observed in the early stages, adequate evaluation of the bloom requires high-resolution data. Furthermore, precise estimation of cell abundance at this stage of bloom development is essential for forecasting purposes. Monitoring programs for HABs, aimed at detecting and characterizing distributions of HAB species, involve determining the target organisms at the species level and other components of the community as taxonomic or functional groups (GEOHAB, 2001). This is traditionally accomplished by laborious microscopic examination of discrete water samples collected along the coast at intermittent intervals of time, but these direct microscope counts are inadequate for rapid evaluation of the large quantity of samples required to describe the space and time variations of bloom-forming species.

To overcome this problem, a new generation of bio-optical instruments capable of rapidly assessing different aspects of phytoplankton dynamics is emerging. Technologies such as the FlowCAM, based on the combination of flow cytometry and image analysis (Buskey and Hyatt, 2006) and the CytoBuoy, a moorable cytometer (Dubelaar and Gerritzen, 2000), have successfully confirmed their ability to detect phytoplankton at the group and species level and have provided information on algal biomass. Recently, laser in situ scattering and transmissometry (LISST-100X) became available for automated detection of suspended particle size distribution (Agrawal and Pottsmith, 2000). Gentien et al. (1995) used the same optical principles to design a particle size analyzer that demonstrated its reliability in describing the distribution of particles along vertical profiles. Although the LISST-100X was originally designed for sediment analysis, previous studies have demonstrated its utility for determining phytoplankton and bacteria distributions (Serra et al. 2001, 2002). However, its application to detection at the species level in mixed phytoplankton communities has not been tested until now.

Frequent high-biomass blooms that occur along the Mediterranean coasts provide a good opportunity to test the ability of LISST-100X for detection at the species level in phytoplankton communities. Among the high-biomass bloom-producing species in the Mediterranean Sea, the noxious *Alexandrium taylori* Balech is responsible for most of the near-beach outbreaks. Appearance of these deleterious blooms leads to loss of aesthetic value caused by water discoloration, with considerable economic impacts on the local tourist industry (Masó and Garcés, 2006). These proliferations, often exceeding 10^6 cells l^{-1}, occur as green-brown patches that are usually noticeable for two months during the

summer. The exceptional duration of these blooms is due to relatively high growth rates and low loss of cells (Basterretxea et al., 2007; Garcés et al., 1999).

The objective of the present study is to assess the utility of the LISST-100X instrument short-term spatial and temporal changes of a noxious *A. taylori* bloom on a Mediterranean beach. Since the bloom was not monospecific, we propose a new method for calibrating the instrument measurements to determine the phytoplankton concentration for each species involved in the bloom. To explore the ability of the LISST-100X to detect and count phytoplankton at the group and species level, automated field cell abundance estimations for each species obtained by the instrument are compared with simultaneous samples counted by traditional microscopy.

In addition, the influence of coastal circulation on the observed cell abundance distribution is investigated using numerical simulations of a hydrodynamic model. Several studies have demonstrated that large-scale seasonal bloom dynamics are associated with major oceanographic processes such as shelf currents and tidal fronts (e.g., (Anderson et al., 2005; McGillicuddy et al., 2005). For smaller scales, Basterretxea et al. (2005) have demonstrated by numerical simulations that wind-induced currents can accumulate phytoplankton cells in near-coastal areas, thus favoring the occurrence and persistence of blooms. However, the interconnections between small-scale coastal blooms and local circulation in areas of restricted dynamism such as harbors, bays, lagoons and enclosed beaches remain largely unknown.

Materials and Methods

LISST-100X description

A laser in situ scattering and transmissometry probe (LISST-100X Type-C, Sequoia Scientific, Inc.) was used to measure in situ the particle size distribution and concentration. Detailed information of the LISST-100X operation is given in Agrawal and Pottsmith (2000). Basically, the LISST-100X instrument obtains the particle volume concentration by size ranges using a technique based on the laser diffraction theory. A collimated laser beam (wavelength 670 nm) illuminates particles and the light scattered is sensed by a 32-ring detector. Each ring measures the scattering intensity over a range of small forward angles for 32 different size classes logarithmically spaced from 2.5 to 500 μm. At these small angles, laser diffraction is unaffected by composition of particles because light scattering is determined almost entirely by light diffracted by the particle. With the software provided by the manufacturers, the scattering intensities measured by the detector are mathematically inverted to obtain the particle volume concentration assuming that particles are spheres.

LISST-100X calibration

LISST-100X measures the particle volume concentration (PVC_i) in the range 2.5-500 μm (d_i). Considering that LISST-100X assumes that particles are spherical, the particle number concentration (PNC_i) can be calculated from

$$PNC_i = \frac{PVC_i}{\frac{4}{3}\pi\left(\frac{d_i}{2}\right)^3} \quad (1)$$

And the total particle number concentration (PNC_T) is obtained by integration over all the size classes:

$$PNC_T = \sum_{i=1}^{32} PNC_i \quad (2)$$

These calculations can be applied to estimate the concentration for one single species (cultures or monospecific natural assemblages) or for phytoplankton groups. However, proliferations are often multi-specific, as was the case in the bloom monitored in this study. For this reason, we applied a size-fraction method (SFM) in order to consider the particle number concentration for each species involved (PNC_s) in the bloom rather than the total particle number concentration. Calculations of the PNC_T were modified as follows:

$$PNC_{s,j} = \sum_{i=1}^{32} f_{ij} PNC_i \quad \text{for} \quad j = 1...N \quad (3)$$

where N is the number of species involved in the bloom and f_{ij} the size fraction of species j in the size class i, which is subject to

$$0 \leq \sum_{j=1}^{N} f_{ij} \leq 1 \quad \text{for} \quad i = 1...32 \quad (4)$$

This condition implies that f_{ij} is 1 for species j sizes ranging over a whole size class i and 0 for species j sizes ranging outside a size class i. The size fraction f_{ij} was determined by linear regression between total particle number concentration for each species (PNC_s) measured by microscopic phytoplankton analysis and particle number concentration for each size class (PNC_i) measured by the LISST-100X. The type of linear regression used in this work was a least squares fit constrained to the condition (4).

To evaluate the instrument performance, laboratory measurements of two different strains of *A. taylori* (EMBL AJ251654, ICMB219) were made with the LISST-100X. Strains were cultured in 50 ml polycarbonate flasks using F2 media (Guillard, 1973) and incubated in a growth chamber at 20ºC and 100 µmol m^2 s^1 irradiance on a 12:12 h light:dark cycle. In order to test the instrument sensitivity at different cell densities, a dilution series of the cultures (10^2 to 10^6 cells l^{-1}) was measured with the instrument's mixing chamber. After processing of the cultures, a subsample was collected and immediately fixed with Lugol's iodine solution for microscopic phytoplankton counting. The general procedure for phytoplankton enumeration involved sedimentation (24 h) of a subsample in a 10 to 50 ml settling chamber and subsequent counting of cells in an appropriate area (Andersen and Throndsen, 2003) using a Leica-Leitz DM-IL inverted microscope.

Field work

From 31 July to 2 August 2006 an in situ experiment was performed during the maintenance phase of an *A. taylori* bloom in La Fosca, a small (~300 m long) sandy beach located in the NW Mediterranean Sea (Fig. 1), where recurrent blooms of this species are observed during the summer (Garcés et al., 1999, 2005). The shorefront has a fairly uniform bathymetry sloping gently between 2 and 7 m. Typical summer meteorological conditions at the sampling site are dominated by a breeze regime, which may be disrupted by traveling frontal systems for periods of a few days (Soriano et al., 2006). The tidal regime is microtidal with a spring range of less than 0.25 m (Tsimplis et al., 1995). Calm conditions were experienced during the sampling and no significant sediment resuspension that could interfere in the particle analysis was observed.

To determine the temporal variations of the phytoplankton population, the first part of the experiment consisted in a continuous in situ record of the particle volume concentration during a diel cycle (from 14:00 GMT on 31 July until 19:00 GMT on 1 August) obtained with the LISST-100X deployed at the northern part of the beach at a depth of 0.5 m (Fig. 1). Simultaneous measurements of temperature and salinity were determined with a SBE 19plus CTD probe deployed at the same place. Water samples for microscopic phytoplankton analysis and chlorophyll *a* (Chl *a*) quantification were collected every hour during daytime. In order to estimate the spatial distribution of the phytoplankton, the second part of the experiment consisted in along- and cross-shore transects with a total number of 23 sampling points carried out from 12:00 to 13:20 GMT on 2 August (Fig. 1). Water surface measurements of the particle volume concentration were obtained in situ with the LISST-100X during 30 s at a depth of 0.5 m (the same like for the moored case). In order to remove short time scale variability, the particle volume concentration was time-averaged at each sampling point.

Subsamples (150 ml) of the water collected from La Fosca beach were immediately preserved with Lugol's iodine solution in the field. Posterior microscopic phytoplankton analysis in the laboratory involved the general procedure described above. For the identification of *Alexandrium* species, fixed specimens were stained with Calcofluor White M2R (Fritz and Triemer, 1985) and examined in an epifluorescence microscope under UV excitation (Axioplan, filter set Zeiss 487902, 1000x magnification). Tabular formula and morphological features of the thecal plates were studied following the criteria of Balech (1995). Subsamples (60 ml) for the quantification of total Chl *a* were filtered on 25 mm Whatman GF/F glass fiber filters and frozen. In the laboratory, filters were extracted in 8 ml 90% acetone and concentrations of Chl *a* were measured with a Turner Designs fluorometer.

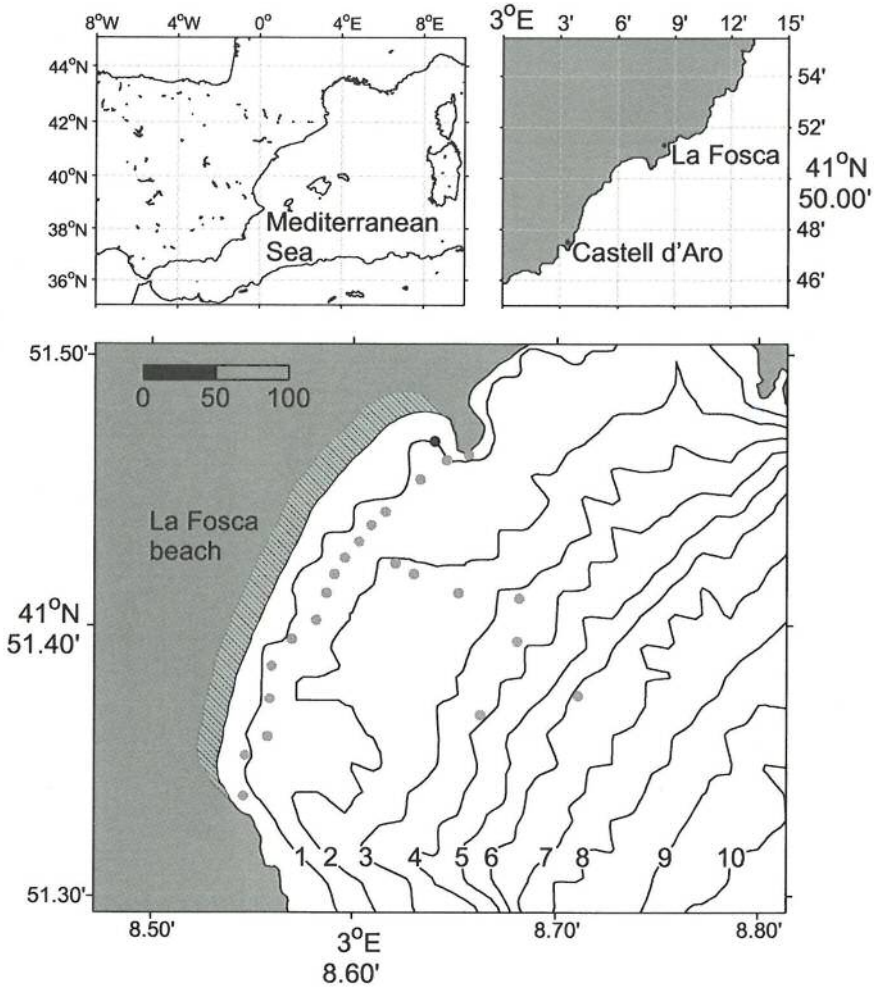


Fig. 1. Map of the study area displaying the coastal bathymetry and the location of the station for the diel cycle (black dot) and for the spatial distribution (gray dots).

Meteorological data of air temperature (accuracy ± 0.1°C), wind velocity (accuracy ± 0.1 m s^{-1}), wind direction (accuracy ± 1°) and radiation (accuracy ± 1 W m^{-2}) were provided from a nearby permanent station located in Castell d'Aro (Fig. 1) by the Catalan Meteorological Service (MeteoCat).

Hydrodynamic model

A numerical circulation model (FUNDY) was implemented for La Fosca beach. The FUNDY is a linear, shallow-water, sigma-coordinate, three-dimensional finite element model with spherical-polar extensions formulated in the frequency domain (Greenberg et al., 1998). This model has previously been used, among other applications, to study the influence of coastal circulation in *A. taylori* bloom dynamics in a pocket beach of the Mediterranean Sea (Basterretxea et al., 2005). The model solves the shallow water equations in linear

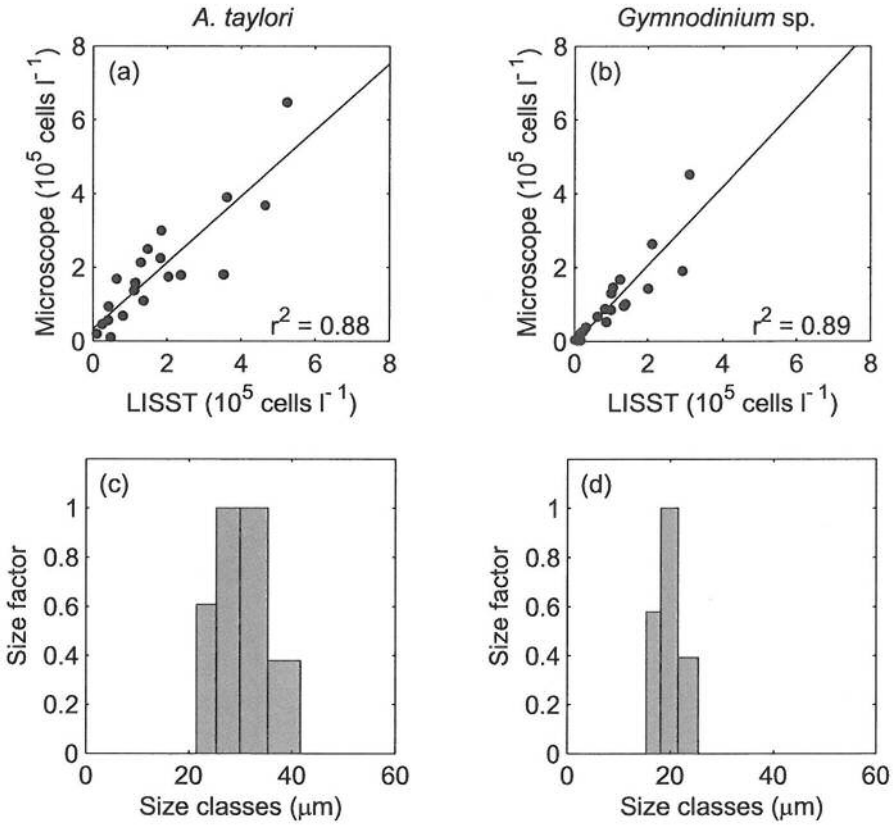


Fig. 2. Linear regression between counts of (a) *Alexandrium taylori* and (b) *Gymnodinium* sp. cells made using an inverted microscope and automated counts made using the LISST-100X. Correlation coefficient is indicated in the lower right corner. Size factor distribution of (c) *A. taylori* and (d) *Gymnodinium* sp. cells made using the LISST-100X and applying the SFM.

form with the Boussinesq and hydrostatic approximations. The vertical eddy viscosity is parameterized according to the Mellor and Yamada level 2.5 turbulence closure scheme, which has successfully been used to simulate the temporal variability of mixing on the continental shelf and the coastal sea (e.g., Wijesekera et al., 2003, Jordi et al., 2008). The computational domain extends from the beach to the inner shelf. Ocean boundaries lie far from the beach and hence are unlikely to influence the circulation there. Bathymetric data in the area were obtained with a shipmounted Biosonics DE-4000 echosounder equipped with a 200 kHz transducer. The mesh contained 6627 elements and 3504 nodes in the horizontal. Under each horizontal node, 10 one-dimensional linear elements were connected following a σ coordinate system.

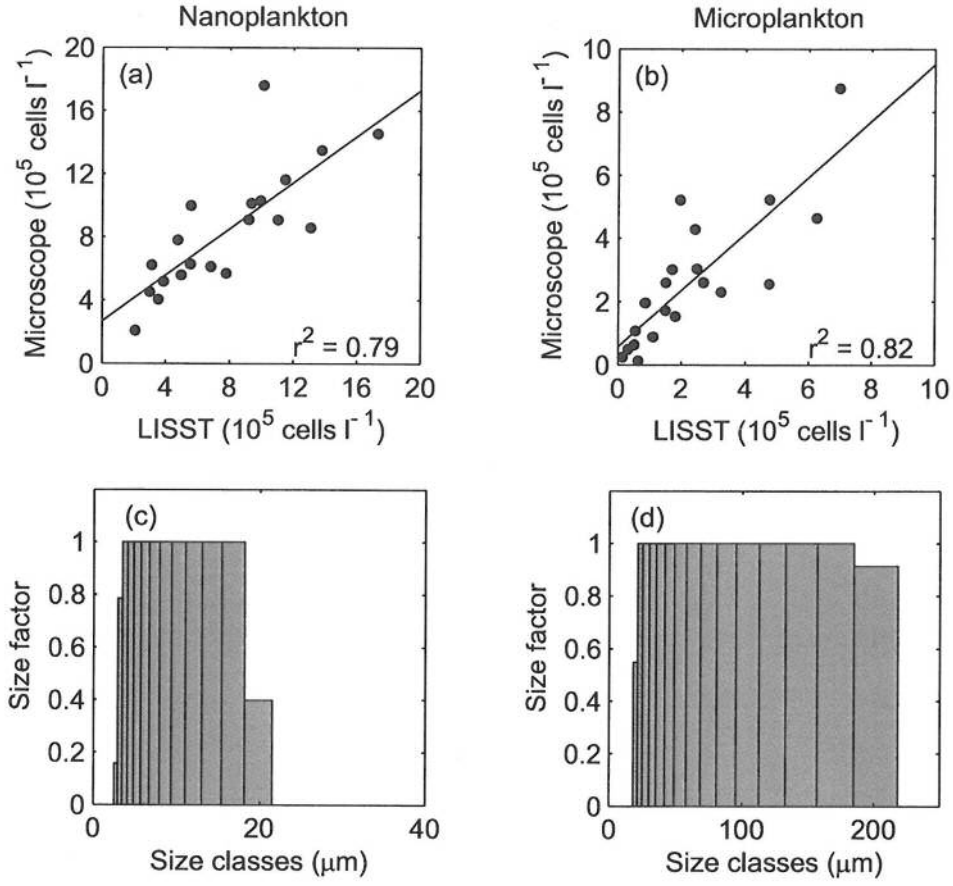


Fig. 3. Linear regression between counts of (a) nanoplankton and (b) microplankton cells made using an inverted microscope and automated counts made using the LISST-100X. Correlation coefficient is indicated in the lower right corner. Size factor distribution of (c) nanoplankton and (d) microplankton cells made using the LISST-100X and applying the SFM.

Results

LISST calibration

In order to determine the potential of the LISST-100X for the in situ detection of A. *taylori* and co-blooming species, we first performed laboratory tests in which measurements of cultured strains of *A. taylori* using LISST-100X and microscopy cell counts were compared. The high correlation obtained between the two techniques ($r^2 = 0.95$) provided background for the in situ application of laser transmissometry in a natural environment.

Microscopic analysis of the water samples collected at La Fosca beach revealed that *A. taylori* was the most abundant species. The other co-blooming species was *Gymnodinium*

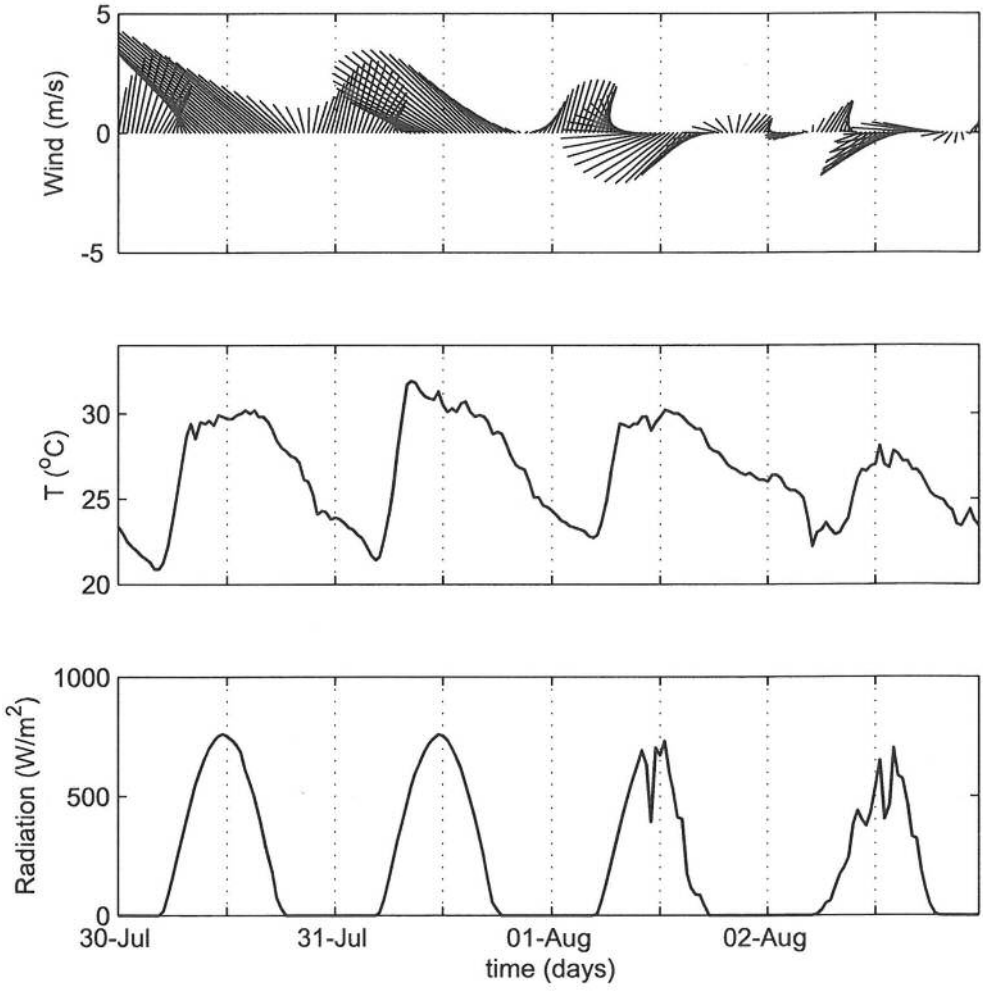


Fig. 4. Meteorological records from Castell d'Aro station: low-passed wind, air temperature and solar radiation. Wind vectors have been rotated 45° clockwise for convenience.

sp., a common accompanying species during *A. taylori* outbreaks. Therefore, we first focused our analysis on these two species and their particle number concentrations were estimated using the SFM following equation (3) and condition (4). Comparisons between microscopic cell counts and LISST-100X particle number concentrations of *A. taylori* (Fig. 2a) and *Gymnodinium* sp. (Fig. 2b) showed good agreement, with similar correlations of 0.88 and 0.89, respectively. Detected size fractions for both species varied from 23 to 45 µm for *A. taylori* and from 17 to 28 µm for *Gymnodinium* sp., which were also in accordance with the cell sizes observed under the microscope (Figs. 2c and 2d). Other phytoplankton species such as *Ostreopsis* sp, *Prorocentrum rathymum, P. micans,* and *Scrippsiella* spp. were detected, but in very low concentrations. Thus, in order to characterize the entire

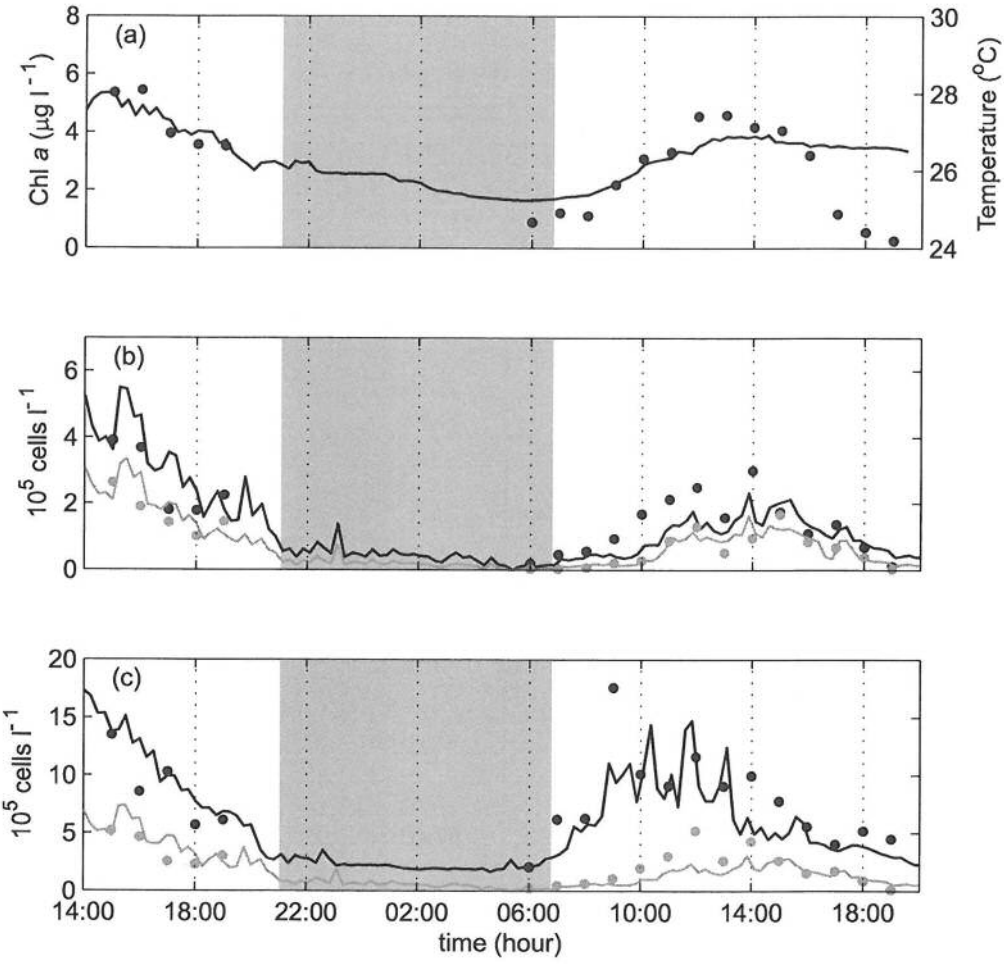


Fig. 5. Diel cycle from 31 July until 1 August for (a) chlorophyll a concentration (dots) and sea temperature (line), (b) *Alexandrium taylori* (black) and *Gymnodinium* sp. (gray) counts made using an inverted microscope (dots) and automated counts made using the LISST-100X (line), and (c) microplankton (black) and nanoplankton (gray) counts made using an inverted microscope (dots) and automated counts made using the LISST-100X (line).

phytoplankton population, all microscopic cell counts were grouped into nanoplankton (2-20 µm) and microplankton (20-200 µm), and LISST-100X concentrations were estimated for these groups. Correlations obtained by comparisons between the two methods were also significant (Fig. 3). Furthermore, LISST-100X size fractions for both groups fitted into the corresponding microscopy size estimations. It is important to note that occurrence of mesoplankton (>200 µm) was not detected by either the LISST-100X or the microscope, which confirms that planktonic macrograzers are scarce in this coastal environment (Calbet et al., 2003).

Temporal variations

Meteorological conditions experienced in the area during the previous day and the days of the experiment are shown in Figure 4. A breeze regime, resulting from the contrasting thermal response of land and sea, was the dominant pattern during the first two days

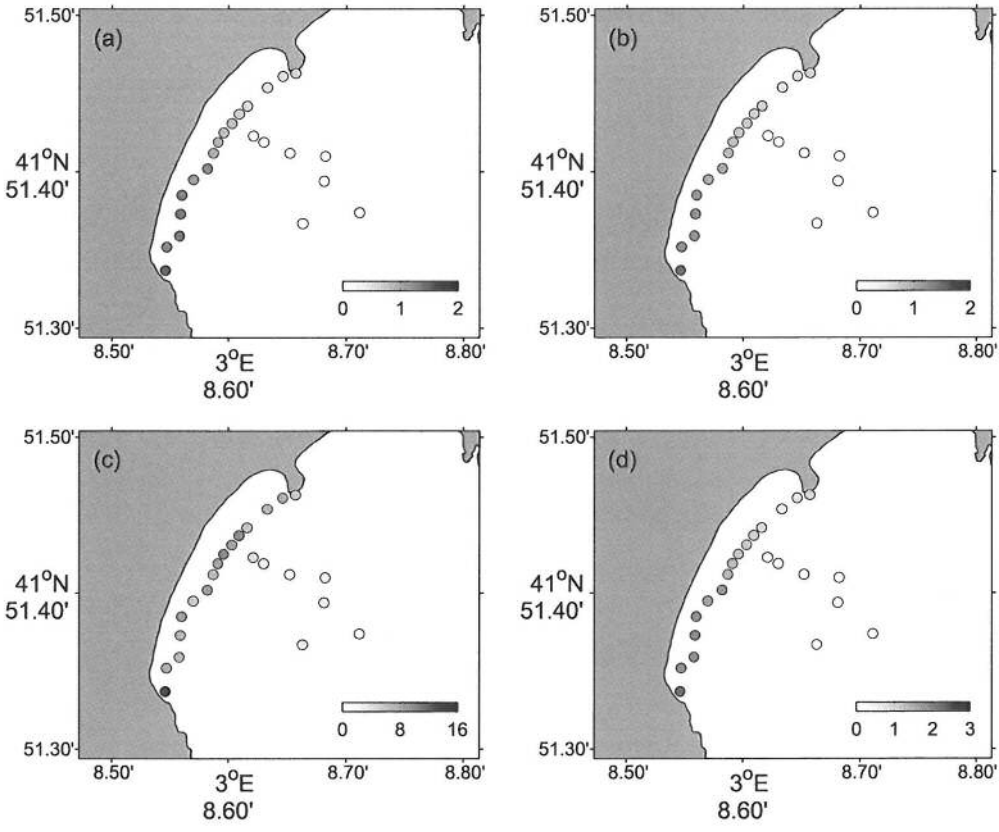


Fig 6. Spatial distribution on 2 August of (a) *Alexandrium taylori*, (b) *Gymnodinium* sp., (c) nanoplankton and (d) microplankton cell counts made using the LISST-100X. Units are 10^5 cells l^{-1}.

(30 and 31 July). A sea breeze blew from the southeast (onshore) during the day and there was a weaker land breeze circulation at night. Solar radiation and diurnal temperatures were lower during the experiment (1 and 2 August) than on previous days, which could have been responsible for the weak northeasterly flow that developed at midday on these two days.

Water temperature (Fig. 5a) reached maximum values of 28°C on 31 July. On 1 August temperature values were lower, and always below 27°C. No significant salinity variations were observed during the experiment (38.1 - 38.4). Chl *a* concentrations ranged from 5.5 μg l^{-1} at midday on 31 July to 0.25 at night on 1 August (Fig. 5a) and water discoloration was clearly perceivable along the shoreline in the northern part of the beach. Generally, Chl *a* showed good agreement with phytoplankton cell abundances measured by the two methodologies, with a better correlation coefficient for the LISST-100X ($r^2 = 0.91$) than for the microscope ($r^2 = 0.81$).

Figure 5b shows the diel variation of the particles as depicted from in situ LISST-100X measurements of this part of the beach. Surface cell concentrations of *A. taylori* reached values of 10^5 cells l^{-1} from 14:00 to 20:00 GMT, with a substantial cell decrease at night from 21:00 to 08:00 GMT. Maximum cell densities were attained on 31 July, whereas cell concentrations were lower on 1 August. Cell abundances showed a diel cycle typical of vertically migrating organisms, even though a stock of 10^4 cells l^{-1} remained in surface waters at all times. *Gymnodinium* sp. followed a similar pattern to *A. taylori*, with lower concentrations in both methodologies (microscope counts and LISST-100X estimations). Nanoplankton and microplankton also followed this daily pattern (Fig. 5c).

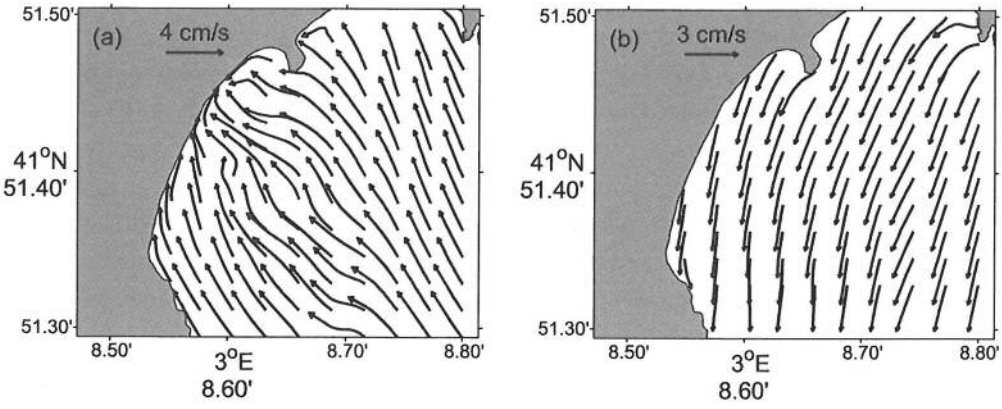


Fig 7. Wind-induced surface circulation patterns in La Fosca corresponding to the (a) averaged breeze regime at midday for 30-31 July, and (b) the averaged northeasterly wind at midday for 1-2 August.

Spatial distributions

Cell distributions along and across the beach measured in situ with the LISST-100X on 2 August under northeasterly winds are displayed in Figure 6. Inshore stations revealed a north-south gradient with *A. taylori* cell densities increasing to the south, whereas abundances rapidly decreased in the offshore direction. A similar spatial pattern was observed in *Gymnodinium* sp. and microplankton although abundances were higher in the latter. Nanoplankton cells showed a slightly different pattern, with enhancement in the nearshore but a less clear alongshore distribution and abundances reaching 10^4 cells l^{-1} offshore.

The numerical simulation of the FUNDY model was forced by the observed meteorological conditions (wind and heat flux) from 30 July to 2 August. Modeled surface averaged circulation patterns at midday for the first two days (breeze regime) and for the last two days (northeasterly winds) are displayed in Figure 7. Prior to compare coastal currents and phytoplankton distribution, it is convenient to check if the measurement of phytoplankton can be considered representative and therefore gives an overall picture of the horizontal distribution. If the time of measurement would be larger than the time taken by the current to move from the farthest station to the closest station, then the spatial distribution presented would be not representative. The distance between the farthest station and the closest was less than 300 m and the coastal currents are of the order of 3 cm s^{-1} (for northeasterly winds), therefore the fluid would take more than 165 min to move from the first to the last station. This time doubles the time in the measurement of the spatial distribution (about 80 min) and therefore we can consider our measurements as representative.

The response of surface currents to breeze regime generates a northwesterly flow (4-5 cm s^{-1}) and a progressive northward deflection of the currents near La Fosca beach (Fig. 7a). These conditions favor particle accumulation in the vicinity of the northern edge of the beach, where currents are weakened by the presence of a headland (note that this was the location of the LISST-100X during the first part of the experiment). Conversely, northeasterly winds produce currents flowing cyclonically along the coast (Fig. 7b). This flow tends to advect surface particles released near the beach southward, promoting cell accumulation at the southern boundary of La Fosca beach. Differences in *A. taylori*, *Gymnodinium* sp., nanoplankton and microplankton cell abundance between the first part (31 July with breeze regime) and the second part (2 August with northeasterly winds) of the experiment could be at least partially explained by the above mentioned patterns of surface flow.

Discussion

Advances in optical technologies provide new perspectives for the analysis of HAB-producing species and their dynamics. Herein we present a case study in which laser diffractometry has been successfully employed for rapid in situ assessment of a bloom-producing community in a coastal marine environment. The significantly positive correlation obtained between microscopy cell counts and LISST-100X measurements for the different species and phytoplankton groups demonstrates the viability of rapid cell abundance assessment using laser scattering transmissometry in both laboratory and field conditions. These results are in agreement with the values obtained by Serra et al. (2001) for purple sulphur bacteria, although better correlations were obtained in our case, which can be attributed to the use of the SFM. The fact that better correlations are attained for comparisons at the species level than for phytoplankton groups suggests that the SFM is suitable and improves LISST-100X estimations at the species level. The disagreements between the two techniques can be attributed to the intrinsic error they involve. Inverted microscope counting sensitivity is 20-500 cells l^{-1}, depending on the sample volume and on the number of fields counted (Andersen and Throndsen, 2003), and an important limitation of the LISST-100X is that particles outside the range of measurement are included in the nearest size, producing an overestimation of the smallest and largest particle sizes (Agrawal and Pottsmith, 2000). In our study, while particles larger than 200 µm were not detected, a low abundance of particles smaller than 2 µm was observed. Although the concentration was not significant and the SFM might have almost discriminated them, it is a possible source of error that could explain the lowest correlation obtained for nanoplankton.

Another interesting feature which should be considered when the LISST-100X is used in field studies is the assumption of a spherical shape when the measurements are converted into particle size distributions. In non-spherical particles, LISST-100X detects an altered diffraction pattern since the method provides the concentration of equivalent spheres. This may result in a deviated estimation of the particle number concentration, depending on the particle aspect ratio (lower over higher diameter) (Pedocchi and Garcia, 2006). Considering that phytoplankton displays a wide range of cell shapes, this should be taken into account in measuring natural phytoplankton assemblages. In our study, the two main species found in the field (*A. taylori* and *Gymnodinium* sp.) were nearly spherical cells, and their size ranges were thus clearly distinguishable. Another source of potential counting error is size overlapping of species. Whereas in monospecific blooms cell densities can be easily obtained from the particle number concentration for the size range of the species measured by the LISST-100X, in multispecific blooms discriminating phytoplankton species may be not as simple. As shown in this study, this can be solved by an appropriate calibration such as the SFM. Hence, as also recommended by Serra et al. (2001), the previous analysis of a sample under the microscope is required in order to identify all the species involved and to evaluate possible sources of errors. Afterwards, the LISST-100X can be deployed to acquire field measurements and subsequently apply the proper data

calibration to differentiate species.

The main advantage of LISST-100X over traditional microscope counting is the high-resolution data of phytoplankton that can be attained. Counting under the microscope is a time-consuming technique and the number of samples that can be handled is therefore lower. Other instruments (CytoBuoy, FlowCAM) have the ability to detect phytoplankton at the species level, but they need to be complemented by continuous in situ deployments of other sensors capable of highly resolved sampling on the HAB scales (Babin et al., 2005). While high-resolution time series of physical parameters such as temperature, salinity, turbulence and irradiance can be accomplished by a wide range of devices, high-resolution data of phytoplankton can only be acquired by means of bio-optical devices based mainly on fluorescence (Doubell et al., 2006) or apparent and inherent optical properties (Robbins et al., 2006). All these bio-optical instruments reach high-resolved sampling that can be easily related to phytoplankton and other water components. Here we suggest that the LISST-100X is a valuable tool for rapidly assessing HAB species in field environments and providing high-resolution data at an appropriate scale autonomously.

Phytoplankton variability observed over a short time scale indicates the profound importance of microscale and small-scale processes in the ecology of communities of marine microorganisms. In our first field experiment, surface densities of *A. taylori* showed a diurnal vertical migration pattern. The increase in cells in the water column in the morning through midday, with concentrations peaking in the afternoon, was followed by lower levels at night. Since no bottom densities were measured during the experiment, this diurnal pattern could be the result of an accumulation of cells on the surface during the day and dispersion at night caused by land-breeze. Nevertheless, vertical migration for the dinoflagellate has been previously described (Garcés et al., 1999), and therefore this biological process may influence its horizontal distribution over a time scale of hours. Moreover, the vertical migration was observed for *Gymnodinium* sp., nanoplankton and microplankton. This vertical migration of the whole phytoplankton community has also been hypothesized for bacteria in an *A. taylori* bloom, where strong daily changes in bacterial abundance occurred in the presence of large dinoflagellate populations performing daily vertical migrations (Gasol et al., 2005). In particular, *A. taylori* and *Gymnodinium* sp. populations seem to have a very similar behavior in vertical migration.

In the second part of the experiment, our results suggest that the observed horizontal distribution is linked to the coastal circulation. A typical summer sea breeze provokes the accumulation of the organisms at the northern part of the beach where discoloration is most often observed, and changes in the prevailing wind regime modify the horizontal distribution in agreement with the north-south distribution and accumulation in the southern edge of the beach measured in the second part of the experiment. However, this north-south gradient in the nearshore appears slightly modified by small-scale variability, which is more significant for the nanoplankton distribution. This could be explained by

the presence of thin layers for different phytoplankton populations. Unfortunately, we are unable to resolve the presence of thin layers with our sampling strategy. A detailed study of the role played by thin layers in phytoplankton distribution should be addressed. The possible inaccuracy of the LISST-100X for measuring particles near the smallest size range of the instrument detection could also contribute to the higher small-scale variability for nanoplankton. Furthermore, it is known that wind may prevent bloom maintenance by dispersing organisms offshore and/or through turbulent mixing (Yamamoto et al., 2002). However, we observed that wind-induced currents during the day accumulated the organism near the beach. The potential dispersion effect of currents at night, induced by land breeze and directed offshore, was limited by the vertical migration of *A. taylori,* with settling during the night mitigating cell dispersal (Basterretxea et al., 2005). Moreover, during the experiment, wind intensity was weak and consequently turbulence mixing did not overcome the thresholds for the appearance of negative effects on the bloom maintenance.

Conclusions

The LISST-100X provides rapid and consistent particle counts, and is therefore a valuable tool for assessing HAB species dynamics in field environments. The high-resolution in situ data that can be attained by this instrument allows the temporal and spatial evolution of HABs to be described. During the phytoplankton bloom investigated, temporal variability was due to diel vertical migration of the phytoplankton population, whereas the horizontal distribution seemed to be linked to the coastal circulation resulting from the prevailing wind regime.

Acknowledgements

This research was funded by the EU Projects SEED (GOCE-CT-2005-003875) and STRATEGY (EVK3-CT-2001-00046), and an MEC project (CTM2005-23781-E/MAR). The work of A. Jordi and E. Garcés was supported by a Postdoctoral Grant and a Ramon y Cajal contract, respectively, both from the Spanish Ministry of Education and Science. We thank the various people who helped in fieldwork (O. and R. Brascó, B. Casas and H. Illoul) and in the lab (A. Reñé), and the people who provided the cultures (L. Cros and N. Sampedro). Meteorological data were kindly provided by the Catalan Meteorological Service (MeteoCat).

References

Agrawal, Y.C., Pottsmith, H.C., 2000. Instruments for particle size and settling velocity observations in sediment transport. Marine Geology 168, 89-114.

Andersen, P., Throndsen, J., 2003. Estimating cell numbers. In: Hallegraeff, G.M., Anderson, D.M., Cembella, D.A. (Eds.), Manual on harmful marine microalgae. UNESCO Publishing, Paris, pp. 99-129.

Anderson, D.M., Keafer, B.A., Geyer, W.R., Signell, R.P., Loder, T.C., 2005. Toxic *Alexandrium* blooms in the western Gulf of Maine: The plume advection hypothesis revisited. Limnology and Oceanography 50, 328-345.

Babin, M., Cullen, J.J., Roesler, C.S., Donaghay, P.L., Doucette, G.J., Kahru, M., Lewis, M.R., Scholin, C.A., Sieracki, M.E., Sosik, H.M., 2005. New approaches and technologies for observing Harmful Algal Blooms. Oceanography 18, 213-227.

Balech, E., 1995. The genus *Alexandrium* Halim. Sherkin Island Marine Station, Sherkin Island, Co. Cork, Ireland.

Basterretxea, G., Garcés, E., Jordi, A., Masó, M., Tintoré, J., 2005. Breeze conditions as a favoring mechanism of *Alexandrium taylori* blooms at a Mediterranean beach. Estuarine Coastal and Shelf Science 62, 1-12.

Basterretxea, G., Garcés, E., Jordi, A., Anglès, S., Masó, M., 2007. Modulation of nearshore harmful algal blooms by in situ growth rate and water renewal. Marine Ecology Progress Series 352, 53-65.

Buskey, E.J., Hyatt, C.J., 2006. Use of the FlowCAM for semi-automated recognition and, enumeration of red tide cells (*Karenia brevis*) in natural plankton samples. Harmful Algae 5, 685-692.

Calbet, A., Vaque, D., Felipe, J., Vila, M., Sala, M.M., Alcaraz, M., Estrada, M., 2003. Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate *Alexandrium minutum*. Marine Ecology Progress Series 259, 303-309.

Doubell, M.J., Seuront, L., Seymour, J.R., Patten, N.L., Mitchell, J.G., 2006. High-resolution fluorometer for mapping microscale phytoplankton distributions. Applied and Environmental Microbiology 72, 4475-4478.

Dubelaar, G.B.J., Gerritzen, P.L., 2000. CytoBuoy: a step forward towards using flow cytometry in operational oceanography. Scientia Marina 64, 255-265.

Fritz, L., Triemer, R.E., 1985. A rapid simple technique utilizing calcofluor white M2R for the visualization of dinoflagellate thecal plates. Journal of Phycology 21, 662-664.

Garcés, E., Masó, M., Camp, J., 1999. A recurrent and localized dinoflagellate bloom in Mediterranean beach. Journal of Plankton Research 21, 2373-2391.

Garcés, E., Vila, M., Masó, M., Sampedro, N., Giacobbe, M.G., Penna, A., 2005. Taxon-specific analysis of growth and mortality rates of harmful dinoflagellates during bloom conditions. Marine Ecology Progress Series 301, 67-79.

Gasol, J.M., Garcés, E., Vila, M., 2005. Strong small-scale temporal bacterial changes associated with the migrations of bloom-forming dinoflagellates. Harmful Algae 4, 771-781.

GEOHAB, 2001. Global Ecology and Oceanography of Harmful Algal Blooms, Science Plan. In: Glibert, P., Pitcher, G. (Eds.). Scientific Committee on Oceanic Research and Intergovernmental Oceanographic Commission, Baltimore and Paris, p. 87.

Greenberg, D.A., Werner, F.E., Lynch, D.R., 1998. A diagnostic finite-element ocean circulation model in spherical-polar coordinates. Journal of Atmospheric and Oceanic Technology 15, 942-958.

Guillard, R.R.L., 1973. Methods for microflagellates and nanoplankton. In: Stein, J.R. (Ed.), Handbook of Phycological Methods. Culture methods and growth measurements. Cambridge University Press.

Masó, M., Garcés, E., 2006. Harmful microalgae blooms (HAB); problematic and conditions that induce them. Marine Pollution Bulletin 53, 620-630.

McGillicuddy, D.J., Anderson, D.M., Lynch, D.R., Townsend, D.W., 2005. Mechanisms regulating large-scale seasonal fluctuations in *Alexandrium fundyense* populations in the Gulf of Maine: Results from a physical-biological model. Deep-Sea Research Part II-Topical Studies in Oceanography 52, 2698-2714.

Pedocchi, F., Garcia, M.H., 2006. Evaluation of the LISST-ST instrument for suspended particle size distribution and settling velocity measurements. Continental Shelf Research 26, 943-958.

Robbins, I.C., Kirkpatrick, G.J., Blackwell, S.M., Hillier, J., Knight, C.A., Moline, M.A., 2006. Improved monitoring of HABs using autonomous underwater vehicles (AUV). Harmful Algae 5, 749-761.

Serra, T., Colomer, J., Cristina, X.P., Vila, X., Arellano, J.B., Casamitjana, X., 2001. Evaluation of laser in situ scattering instrument for measuring concentration of phytoplankton, purple sulfur bacteria, and suspended inorganic sediments in lakes. Journal of Environmental Engineering 127, 1023-1030.

Serra, T., Casamitjana, X., Colomer, J., Granata, T.C., 2002. Observations of the particle size distribution and concentration in a coastal system using an in situ laser analyzer. Marine Technology Society Journal 36, 59-69.

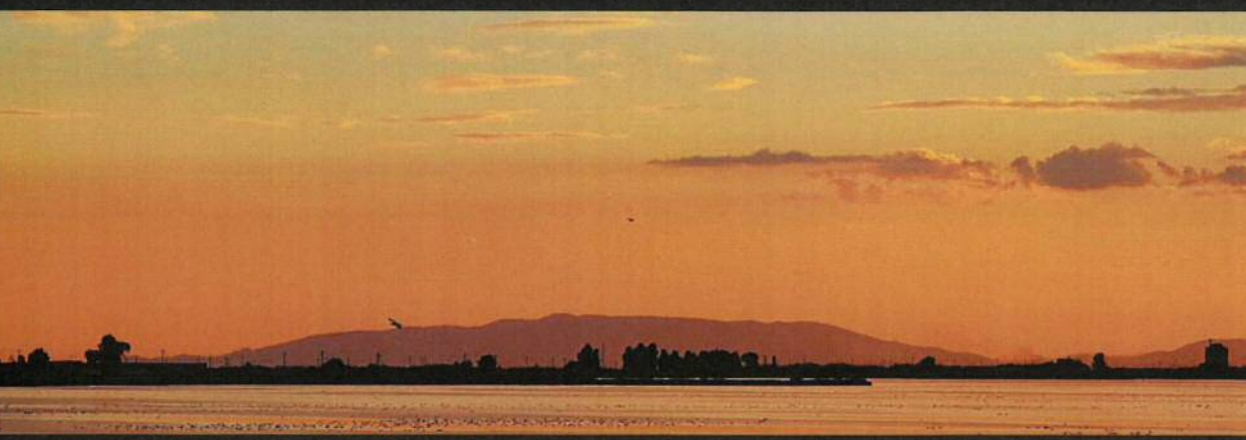
Soriano, C., Fernandez, A., Martin-Vide, J., 2006. Objective synoptic classification combined with high resolution meteorological models for wind mesoscale studies. Meteorology and Atmospheric Physics 91, 165-181.

Tsimplis, M.N., Proctor, R., Flather, R.A., 1995. A 2-dimensional tidal model for the Mediterranean Sea. Journal of Geophysical Research-Oceans 100, 16223-16239.

Yamamoto, T., Hashimoto, T., Tarutani, K., Kotani, Y., 2002. Effects of winds, tides and river water runoff on the formation and disappearance of the *Alexandrium tamarense* bloom in Hiroshima Bay, Japan. Harmful Algae 1, 301-312.

2

Dinoflagellate cysts in recent sediments from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact



Deep-Sea Research II 57, 256–267 (2010)

Dinoflagellate cysts in recent sediments from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact

Cecilia Teodora Satta[1], Silvia Anglès[2], Esther Garcés[2], Antonella Luglié[1], Bachisio Mario Padedda[1], Nicola Sechi[1]

[1] Dipartimento di Scienze Botaniche, Ecologiche e Geologiche, Via Piandanna, 4, University of Sassari, 07100 Sassari, Italy.

[2]Institut de Ciències del Mar—CSIC, Passeig Marítim de la Barceloneta, 37-49, 08003 Barcelona, Spain.

Abtract

Studies were conducted on dinoflagellate cyst assemblages from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact, Arenys de Mar harbor and the Gulf of Olbia. Sediment cores were taken from seven stations (December 2006 and August 2007) in Arenys and from eight (October 2006) and ten (May 2007) stations in Olbia. Of the 42 morphotypes found in the sediments collected at the two sites, 27 were identified at the species level, representing 10 genera. The most common cysts were those of *Scrippsiella trochoidea*, *Scrippsiella* sp. 2, Gymnodiniales type 1, and *Scrippsiella precaria*. A number of the morphotypes had not been previously described in the literature. Total cyst abundances varied substantially between the two surveys, with an increased total density in Arenys and a decrease in Olbia. However, at the latter site, a higher abundance of cysts was recorded at more confined sampling stations. Calcareous Peridiniales, belonging to the genus *Scrippsiella*, dominated the cyst assemblages of both sites, while at some stations higher numbers of Gymnodiniales (Olbia) and Gonyaulacales (Arenys) were determined. Cysts of the toxic species *Alexandrium minutum* and *A. catenella/tamarense* were also detected. *A. minutum* was present at both sites whereas *A. catenella/tamarense* was found only in Olbia. *Peridinium quinquecorne* was recovered in the sediments of both sites. In Olbia, cysts of this species were present at high densities and were detected even in deep sediments. Species such as *Pentapharsodinium* cf *tyrrhenicum*, *Scrippsiella crystallina*, *S. lachrymosa*, *S. precaria*, *S. trochoidea*, *Protoperidinium avellanum*, *P. claudicans*, *P. compressum*, *P. conicum*, *P.* cf *minutum*, *P. oblongum*, *P. pentagonum*, *P. subinerme*, and *Zygabikodinium lenticulatum* were not detected as motile stages in the study areas. The results of this study, the first on dinoflagellate cyst assemblages at these two sites, further our knowledge of cyst diversity and confirm the importance of embayments and hydrographically confined areas as reservoirs for planktonic dinoflagellates.

Resum

Es va realitzar un estudi sobre el repertori de cists de dinoflagel·lades a dues àrees semitancades de la Mediterrània occidental subjectes a un alt impacte humà, el port d'Arenys de Mar i el Golf d'Òlbia. Es van prendre testimonis de sediment de set estacions (desembre de 2006 i agost de 2007) a Arenys i de vuit (octubre de 2006), i deu (maig de 2007) estacions a Òlbia. Dels 42 morfotipus trobats en els sediments recollits en els dos llocs, 27 es van identificar a nivell d'espècie, representant 10 gèneres.Els cists més freqüents van ser els de *Scrippsiella trochoidea*, *Scrippsiella* sp. 2, Gymnodiniales tipus 1, i *Scrippsiella precaria*. Alguns dels morfotipus no havien estat descrits prèviament a la literatura. L'abundància total de cists va variar substancialment entre els dos mostreigs, amb un augment de la densitat total a Arenys i una disminució a Òlbia. No obstant, en aquest últim lloc, es va registrar una major abundància de cists a les estacions més confinades. Dins del grup de les Peridinials calcàries, el gènere *Scrippsiella* va dominar el repertori de cists a ambdós llocs, mentre que a certes estacions es van determinar major nombre de Gymnodinials (Òlbia) i Gonyaulacals (Arenys). També es van detectat cists de les espècies tòxiques *Alexandrium minutum* i *A. catenella/tamarense*. L'*A. minutum* va estar present als dos llocs, mentre que l'*A. catenella/tamarense* només es va trobar a Òlbia. El *Peridinium quinquecorne* es va observar en els sediments d'ambdós llocs. A Òlbia, els cists d'aquesta espècie van estar presents en altes densitats i es van detectar fins i tot en capes de sediment profundes. Espècies com *Pentapharsodinium* cf *tyrrhenicum*, *Scrippsiella crystallina*, *S. lachrymosa*, *S. precaria*, *S. trochoidea*, *Protoperidinium avellanum*, *P. claudicans*, P. *compressum*, *P. conicum*, *P.* cf *minutum*, *P. oblongum*, *P. pentagonum*, *P. subinerme*, i *Zygabikodinium lenticulatum* no s'havien detectat com a estadi planctònic a les àrees d'estudi. Els resultats d'aquest estudi, el primer sobre el repertori de cists de dinoflagel·lades a aquests dos llocs, amplia el nostre coneixement de la diversitat de cists i confirma la importància de badies i àrees hidrodinàmicament confinades com a reservoris de dinoflagel·lades planctòniques.

Introduction

The life cycle of many dinoflagellates is composed of an asexual vegetative phase, with reproduction by binary fission, and a sexual phase, involving reproduction by gamete fusion. However, sexual reproduction is well-documented for less than 50 of the approximately 2000 dinoflagellate species known (Pfiester and Anderson, 1987). Sexuality is primarily linked to the influence of environmental factors, such as variations in nutrients, temperature, and salinity (Anderson, 1998; Ellegaard et al., 1998; Sgrosso et al., 2001), and it is regulated by endogenous and exogenous conditions (Adachi et al., 1999; Kremp and Heiskanen, 1999; Uchida, 2001). Sexual reproduction yields a motile cell, the zygote, which can either return to the vegetative stage or become a hypnozygote, or resting cyst, which is unable to swim and sinks towards the bottom sediments (Figueroa and Bravo, 2005; Figueroa et al., 2006, 2007). Furthermore, the vegetative cells of some dinoflagellates can form sexual and asexual pellicle cysts during their life cycle (Bravo et al., 2010; Figueroa and Bravo, 2005; Garcés et al., 2002).

Recently, as a result of detailed and extensive dinoflagellate cyst studies, the number of species known to be cyst producers has increased, from sixty in the early 1980s (Dale, 1983) to 80 (Sonneman and Hill, 1997) and to more than 200 (Head, 1996) in the mid-1990s. This number is expected to increase even further, as many previously described cyst morphotypes have yet to be related to the vegetative phase of the species and new cyst morphotypes continue to be discovered. The taxonomic identification of cysts is not simple, and several steps, such as isolation, germination, and culture preparation, are often required to achieve classification. New molecular methodologies are being developed that could help in identifying new morphotypes (Erdner et al., 2010; Penna et al., 2010). Similarly, taxonomic identification of the vegetative phase of natural plankton can be very complicated and often necessitates the use of a range of different methods, including light, electron, and epifluorescence microscopy, specific staining methods for morphological assessment, and molecular probes or genetic approaches. In certain genera (e.g., *Scrippsiella*), however, morphological differences between species are more obvious at the cyst level than at the vegetative stage (Lewis, 1991).

One approach to obtain useful information on plankton diversity is through combined studies on planktonic and benthic cellular stages (Boero et al., 1996; Rubino et al., 2000, 2002). In fact, dinoflagellates may remain in the vegetative stage for as long as several months, or for as short as days; they may be present year round, seasonally, or sporadically. Moreover, species can have a multi-annual cycle, alternating between abundance and rarity (Moscatello et al., 2004), such that it is not always possible to accurately determine the species record at the time of sampling (Dale, 1983; Nehring, 1997). Thus, a complete description of a phytoplanktonic community in a particular area requires intensive studies, including the collection of horizontal and vertical samples, carried out within very short intervals of time and with multiple sampling stations. However, the resources available

for such efforts, i.e., an adequate budget, sufficient research time and personnel, are often inadequate to achieve these goals. An alternative approach is provided by the study of benthic stages, as they yield integrated data on the planktonic assemblage over time and space, at least with respect to cyst-producing species. Moreover, cysts represent the potential diversity of a planktonic population and also its expressed diversity, in the form of vegetative phases inhabiting a particular site (Belmonte et al., 1995; Margalef, 2002). Indeed, in some bloom-forming species, resting cysts are the key to bloom initiation as well as a tool for species maintenance when conditions become unfavorable (Anderson and Wall, 1978; Estrada et al., 2010). The efficacy of studying benthic stages was shown in a study by Bravo et al. (2006) on the resting cysts of the *Alexandrium* genus in several semi-enclosed environments (harbors, beaches, and embayments) in the Western Mediterranean. The authors reported data on viable cysts from recent sediments of five species previously not recorded by plankton monitoring. This was also the case for *A. andersonii* Balech in the Gulf of Naples (Zingone et al., 2006).

Coastal confined areas, such as harbors, protected beaches and embayments, are characterized by their low hydrodynamic forcings and, in some cases, by high nutrient availability, conditions that favor dinoflagellate blooms (Garcés et al., 1999). In such areas, and especially those with aquaculture activities, the risk of introducing non-indigenous species can be high (Hégaret et al., 2008). In the Mediterranean region, the number of sites with these characteristics has been increasing since the 1970s because of intense human exploitation of coastal marine zones (e.g., for recreational use) (Garcés et al., 2000). Furthermore, semi-enclosed areas, where sedimentation prevails over particle transport, can act as "sediment traps" and thus as potential reservoirs of benthic stages due to the fact that the hydrodynamic behavior of cysts is similar to that of fine silt particles (Dale, 1976; Anglès et al., 2010).

Our ability to forecast algal blooms can be improved by comparing regions that have been subject to similar events. This is the case for Arenys de Mar harbor (Catalan coast, Spain) and the Gulf of Olbia (northwest Sardinia, Italy, Tyrrhenian Sea), both of which have been sites affected by dinoflagellate blooms, mainly of the genus *Alexandrium*. Bloom events have been well-documented in both areas since the 1990s (*A. minutum* in Arenys, Garcés et al., 2004; Vila et al., 2005; and both *A. minutum* and *A. catenella* in Olbia, Lugliè et al., 2003, 2004). This study provides the first detailed analysis of species composition, abundance, and distribution of dinoflagellate cysts in these two anthropogenically modified confined areas. The results increase our knowledge of plankton diversity in the Western Mediterranean Sea.

Methods

Sampling areas

Arenys de Mar harbor is located on the coast of Catalonia, Spain (Fig. 1). It is an artificial harbor with 0.4 km² of total area and comprises a commercial fishing port, shipyards, and a marina. The harbor is one of the sampling sites in the regional toxic phytoplankton monitoring program (Vila et al., 2001). In this study, surveys were carried out at seven stations within the harbor that were chosen on the basis of data obtained in previous surveys (Anglès et al., 2010).

The Gulf of Olbia is situated on the eastern coast of Sardinia, Italy (Fig. 1). Morphologically, it is a typical ria, with a total area of 6.5 km², and receives flow from the Padrongianus River

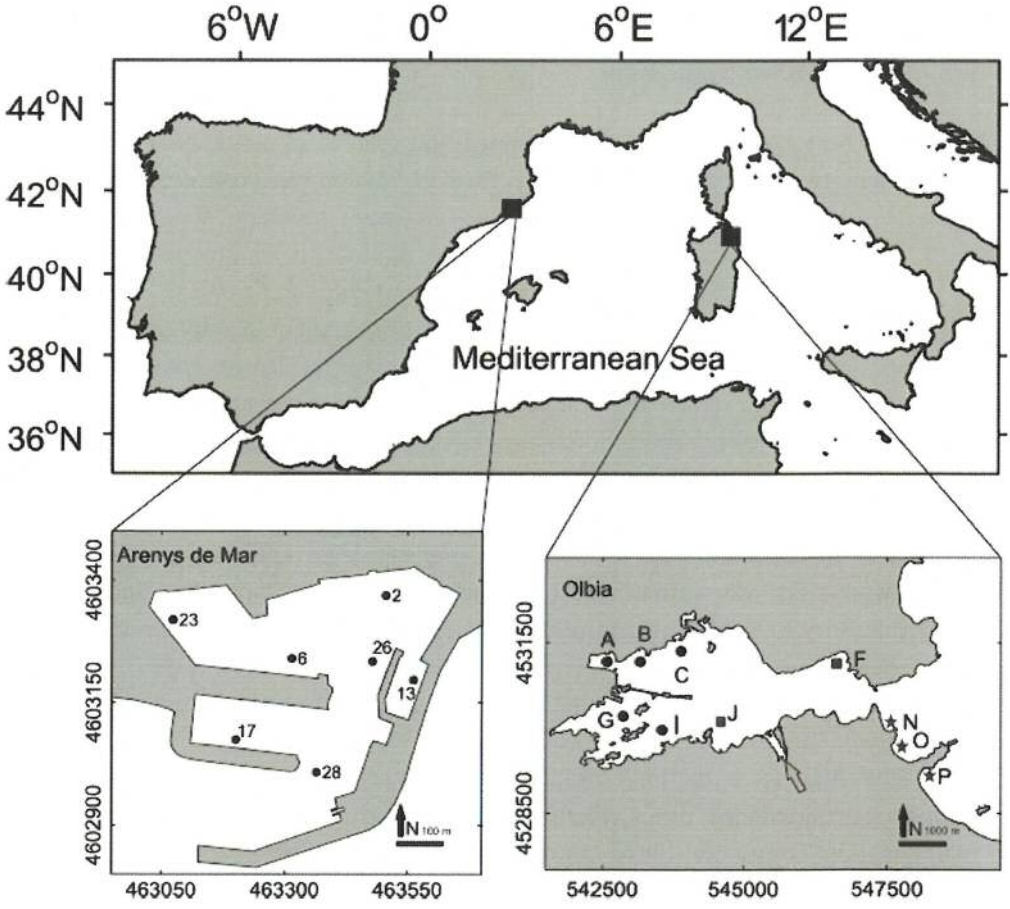


Fig. 1. Maps of the two studied areas, showing sampling stations and kilometric coordinates at Arenys de Mar harbor and Olbia Gulf (in the latter, the Padrongianus river discharge is indicated with a gray arrow, and stations as: inner •, intermediate ▪, and outer ★).

in its southern part. The town of Olbia, located in the inner part of the Gulf, is the site of one of the most important passenger harbors in the Mediterranean (about 5 million passengers per year; www.olbiagolfoaranci.it) in addition to a commercial and an industrial harbor. It is also the largest mussel- and clam-farming area in Sardinia (5000 t yr^{-1}). Since the early 1990s, the Gulf of Olbia has been monitored with respect to phytoplankton abundance and composition, and environmental variables. In the present study, dinoflagellate cysts were investigated at 8–10 stations: A, B, C, G, I, J, F, N, O, and P. These sites represent different bathymetric and hydrographic conditions and were chosen on the basis of preliminary information on granulometry and cyst presence/absence (Satta et al., 2008). Accordingly, the stations were split into three groups: A, B, C, G, and I, with depths of 1–2 m, low hydrodynamism, and relatively more confined; J and F, with depths of 5–7 m and an intermediate degree of confinement; and N, O, and P, with a depth of 4–6 m and situated outside the ria, 200 m away from the shore.

Sample collection and processing

Sediment cores were taken by a scuba diver using cylindrical plastic corers (20 cm long with a diameter of 5 cm). Three replicates for each station were collected only in Olbia. Two surveys were conducted both in Arenys (December 2006 and August 2007) and in Olbia (October 2006 and May 2007). In the latter, the first survey covered eight stations (A, B, C, G, I, J, F, N), and the second two additional stations (O and P). Samples were stored in the dark at 4°C (Olbia) and 10°C (Arenys) until analysis. A total of 54 (Olbia) and 14 (Arenys) samples were analyzed. All samples were left undisturbed for 1–4 days and were processed within 1 month. Water above the sediment was carefully removed, and the top undisturbed 1 cm of sediment from each core was sectioned. Subsamples (2–3 cm^3) of this portion were suspended in filtered seawater (FSW) and sonicated for 2 min using a Bandelin Sonoplus (Olbia) and Dynatech Sonic Dismembrator (Arenys). The sonicated suspension was sieved through 10- and 100-µm mesh sieves. The slurry remaining on the 10-µm mesh sieve was washed with FSW and collected in a 50-ml tube. Subsamples (5 ml) of the slurry were processed for cyst concentration and separation from the sediment using the sodium polytungstate density gradient method of Bolch (1997), adding the SPT solution at the second centrifugation as proposed by Amorim et al. (2001) and Bravo et al. (2006). The resulting sample was rinsed in a 10-µm sieve and collected with 5–15 ml of FSW. Aliquots of the final sample were counted in sedimentation chambers with an inverted microscope (Leica DMIRB for Arenys and Axiovert 100 for Olbia) at 200× magnification. Empty cysts were not considered. Densities were expressed in cysts cm^{-3} of wet sediment (ws). Selected cysts were photographed and then isolated with a glass Pasteur micropipette and transferred into IWAKI tissue-culture multi-well plates. These were filled with f/2 or f/16 medium prepared with FSW from the two sites. These multi-well plates were subsequently maintained at 18–20 ± 1°C in a 12:12 h light:dark cycle.

Fluorescence tubes provided illumination at a photon irradiance of 100 µmol photons m^{-2} s^{-1}. Plates were controlled every 2–4 days for germination and species identification. Cyst identification and the terminology used to describe the dinoflagellate cysts were based on the work by Wall and Dale (1968), Dale (1983), and Matsuoka and Fukuyo (2000, 2003). Thecal plates of germinated cells of armored dinoflagellates were analyzed by staining with Calcofluor White and examined under epifluorescence. For scanning electron microscopy (SEM) observations, 5 ml of the cultures obtained by the isolation and germination of cysts were fixed for 2 h in 4% OsO_4 (Sigma-Aldrich, St. Louis, MO, USA). These cells were washed with distilled water, filtered onto a Nuclepore filter (8- and 0.8-mm pore size; Whatman, Maidstone, UK), and dehydrated in increasing ethanol concentrations (25%, 50%, 75%, 95%, and 100%) for 10 min at each concentration. After three 3-min rinses in 100% ethanol, the filters were critical-point dried in liquid CO_2 using a BAL-TEC CPD 030 critical-point drying apparatus. Filters were subsequently glued to SEM stubs with colloidal silver, sputter-coated with gold palladium, and examined with a Hitachi S-3500N (Nissei Sangyo Co. Ltd., Tokyo, Japan) scanning electron microscope operating at 5 kV.

To support the assessment of the presence of *Alexandrium catenella/tamarense* cysts, samples collected in the first survey in Olbia were stained according to the primuline method (Yamaguchi et al., 1995). Subsamples (5 ml) of the slurries collected in the 50-ml tubes after sieving (for all stations) were fixed with 1% glutaraldehyde for 30 min in 15-ml polycarbonate centrifuge tubes. After fixation, the suspension of fixed cysts was centrifuged (700 × g for 15 min) and the supernatant discarded. Methanol (10 ml) was added to the pellets and the tubes were placed in the refrigerator. After 2 days, the samples were centrifuged and the methanol replaced by distilled water. One ml of a stock solution of 2.0 mg primuline ml^{-1} was added to the tubes, which were then allowed to stand for an hour. After staining, the samples were centrifuged to remove the primuline. The remaining pellet was resuspended in distilled water, washed again by centrifugation, and then resuspended in 5 ml of distilled water. Aliquots of this suspension were observed under an epifluorescence inverted microscope (Axiovert 10, equipped with a Zeiss Attoarc 2 100W lamp) using blue light excitation with a 450–490 nm pass filter.

Statistical analysis

Differences among the sites over time were tested using one-way pair-wise analysis of similarity (ANOSIM) (Clarke and Warwick, 2001). Tests based on a Bray-Curtis rank similarity matrix (constructed using the single morphotype abundances found in all samples for Arenys and considering the average values of the three replicates for Olbia) were calculated using log (x+1) transformed data. This transformation was used to normalize the asymmetric distribution of the data and to reduce the effects of outliers. A two dimensional non-metric multidimensional scaling (nMDS) ordination was carried out. This representation derived

from the Bray-Curtis similarity measure. Similarity percentage analysis (SIMPER test) was used to determine the percentage contribution of each morphotype to the average dissimilarity among samples in each site and between sites.

Group	Biological species name	Arenys	Olbia	Figures	Germination	
					Arenys	Olbia
Calcareous Peridiniales	*Calciodinellum* cf *operosum* Deflandre	+	+	Plate I a	No	No
	Pentapharsodinium cf *tyrrhenicum* type 1 (Balech) Montresor, Zingone et Marino	+	+	Plate I b	No	No
	Pentapharsodinium cf *tyrrhenicum* type 2 °	+	+	Plate I c	No	No
	Scrippsiella crystallina Lewis		+	Plate I d		No
	Scrippsiella lachrymosa Lewis	+	+	Plate I e-g	No	No
	Scrippsiella precaria Montresor et Zingone	+	+	Plate I h	Yes	No
	Scrippsiella ramonii Montresor	+		Plate I i	No	
	*Scrippsiella trochoidea** (Stein) Loeblich III	+	+	Plate I l-o	Yes	Yes
	Scrippsiella sp 1 °	+		Plate I p-q	Yes	
	Scrippsiella sp 2 °	+	+	Plate II a-b	Yes	No
	Scrippsiella sp 3		+	Plate II c		No
	Scrippsiella sp 4		+	Plate II d-g		Yes
	Calcareous cyst 1 °	+	+	Plate II h	No	No
Organic (Non-calcareous) Peridiniales	*Protoperidinium avellana* (Meunier) Balech		+	Plate II i-m		Yes
	Protoperidinium claudicans (Paulsen) Balech	+	+	Plate II n	No	Yes
	Protoperidinium compressum (Abé) Balech	+	+	Plate III a	No	No
	Protoperidinium conicum (Gran) Balech	+	+	Plate III b	No	No
	Protoperidinium leonis (Pavillard) Balech	+	+	Plate III c	No	Yes
	Protoperidinium cf *minutum* (Kofoid) Loeblich		+	Plate III d		Yes
	Protoperidinium oblongum (Aurivillius) Parke et Dodge	+	+	Plate III e	No	Yes
	Protoperidinium pentagonum (Gran) Balech		+	Plate III f		No
	Protoperidinium subinerme (Paulsen) Loeblich III		+	Plate III g-h		Yes
	Zygabikodinium lenticulatum (Paulsen) Loeblich et Loeblich III		+	Plate III i-l		Yes
Gonyaulacales	*Alexandrium catenella/tamarense* complex		+	Plate IV a		No
	Alexandrium minutum Halim	+	+	Plate IV b-c	Yes	No
	Gonyaulax cf *membranacea* (RossigNol) Ellegaard, Daugbjerg, Rochon, Lewis et Harding	+		Plate IV d	No	
	Gonyaulax cf *scrippsae* Kofoid	+	+	Plate IV e	No	No
	Gonyaulax cf *spinifera* (Claparéde et Lachmann) Diesing	+	+	Plate IV f	No	No
	Gonyaulax verior Sournia	+		Plate IV g	Yes	
	Lingulodinium polyedrum (Stein) Dodge	+		Plate IV h	No	

Group	Biological species name	Arenys	Olbia	Figures	Germination	
					Arenys	Olbia
GymNodiniales	*Gymnodinium impudicum* Fraga et Bravo		+	Plate IV i-l		Yes
	Gyrodinium instriatum Freudenthal et Lee	+	+	Plate IV m	No	Yes
	Polykrikos complex	+	+	Plate IV n	No	Yes
	GymNodiniales type 1 °		+	Plate V a- d		Yes
	GymNodiniales type 2 °		+	Plate V e		Yes
	GymNodiniales type 3 °		+	Plate V f		Yes
	GymNodiniales type 4 °	+		Plate V g- i	Yes	
Unidentified morphotypes	Cyst type A °	+	+	Plate V l	No	No
	Cyst type B (Round brown)	+	+	Plate V m-n	No	No
	Cyst type C		+	Plate V o	No	
	Cyst type D °		+	Plate V p	No	
	Cyst type E		+	Plate V q	No	

Table 1. Biological names of the dinoflagellate cyst types identified from the two studied areas and numbers of all figures. + = occurrence of cyst; ° = first morphotype description.

Results

Morphotype composition

The sediments of the two studied areas yielded 42 morphotypes, representing 10 genera, of which 27 were identified at species level and five at the genus and group level. A list of the biological species names is provided in Table 1. Cyst types that could not be identified are referred to as types A–E. Since many of the cyst morphotypes recorded have been well-documented in the literature, here, we give a detailed morphological description only of those that have not been previously described. However, descriptions of some morphotypes already reported in the literature are included, due to the interest of the species to which they belong.

Pentapharsodinium cf *tyrrhenicum* type 2 (Plate I, Fig. c)

This cyst has a characteristic brown calcareous layer that completely surrounds the inner body. The latter is oval-shaped and its contents are granular with a red accumulation body. The size of the cysts ranges from 32 to 55 µm in length and from 30 to 42 µm in width (45–62 µm long and 40–51 µm wide, considering the calcareous layer; n=2).

Scrippsiella sp.1 (Plate I, Figs. p–q)

The cyst (33–46 µm long, 29–35 µm wide; n=5) has a granular content and a red accumulation body. The cyst is smooth, with a pale brown calcareous layer that covers half of the cyst. Germination of the cyst produced a cell belonging to the genus *Scrippsiella*. The clonal culture obtained yielded cysts with the same morphology. This morphotype is widely distributed in Arenys.

Scrippsiella sp.2 (Plate II, Figs. a, b)

This morphotype is a small, circular cyst (18–25 µm in diameter; n=5). The cyst wall is smooth and sometimes surrounded by mucus. The cellular content is granular and includes a yellow accumulation body. Germination of the cyst produced a cell belonging to the genus *Scrippsiella*. The morphotype is widespread in Arenys, but not often observed in Olbia.

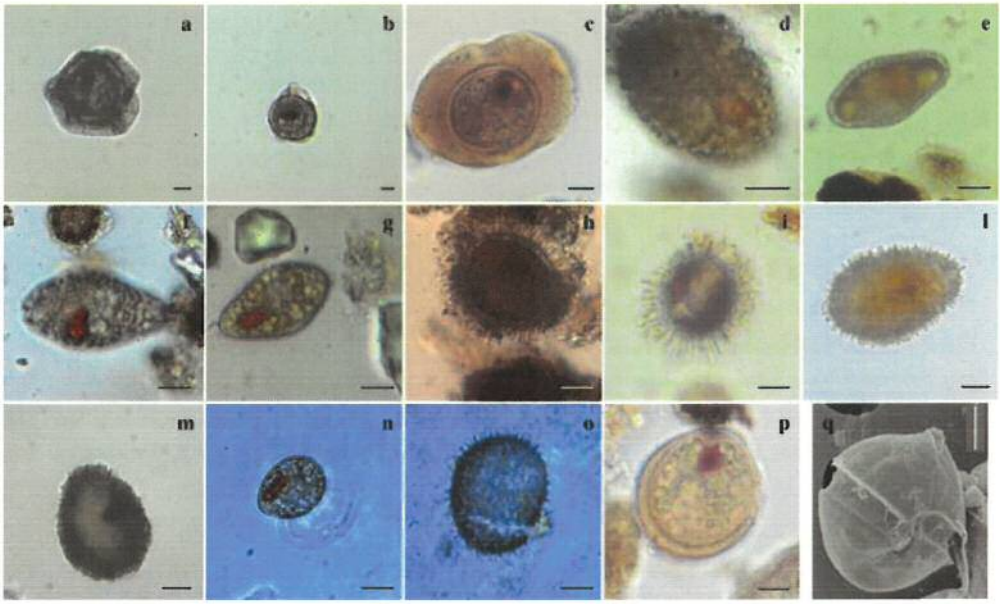


Plate I. Dinoflagellate cysts isolated from recent sediments of the Western Mediterranean Sea. Figures a–q: Calcareous Peridiniales (a) *Calciodinellum* cf *operosum*. (b) *Pentapharsodinium* cf *tyrrhenicum* type 1. (c) *Pentapharsodinium* cf *tyrrhenicum* type 2. (d) *Scrippsiella crystallina*. (e–g) *Scrippsiella lachrymosa*: (e) cyst from Arenys sediments, (f) cyst from Olbia sediments, (g) cyst without calcareous crystals. (h) *Scrippsiella precaria*. (i) *Scrippsiella ramonii*. (l–o) *Scrippsiella trochoidea*: (l) cyst from Arenys sediments, (m) cyst from Olbia sediments, (n) cyst produced in culture, without calcareous crystals, (o) empty cyst. (p, q) *Scrippsiella* sp1: (p) viable cyst, (q) SEM, ventral view of motile cell. All scale bars 10 µm.

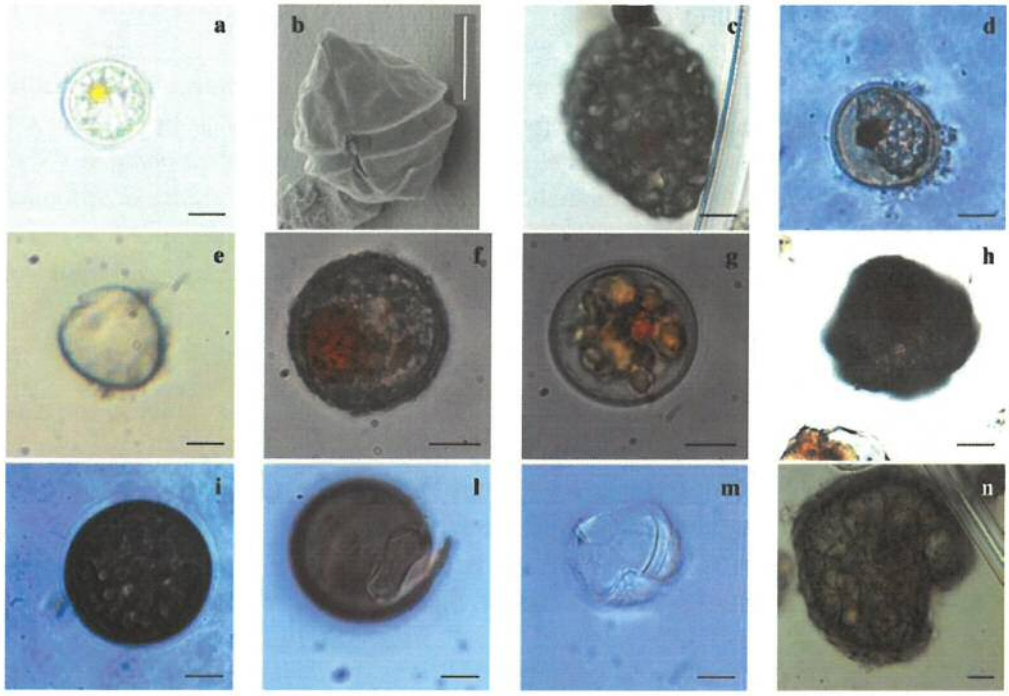


Plate II. Dinoflagellate cysts isolated from recent sediments of the Western Mediterranean Sea. Figures a–h: Calcareous Peridiniales (a, b) *Scrippsiella* sp2: (a) viable cyst, (b) SEM, ventral view of motile cell. (c) *Scrippsiella* sp3. (d–g) *Scrippsiella* sp4: (d) wild viable cyst, (e) empty cyst, (f) cyst obtained in culture, with calcareous crystals, (g) the same cyst after HCl treatment. (h) Calcareous cyst 1. Figures i–n: Organic (non-calcareous) Peridiniales (i–m) *Protoperidinium avellanum*: (i) viable cyst, (l) empty cyst with the archeopyle, (m) theca of the motile cell. (n) *Protoperidinium claudicans*. All scale bars 10 µm.

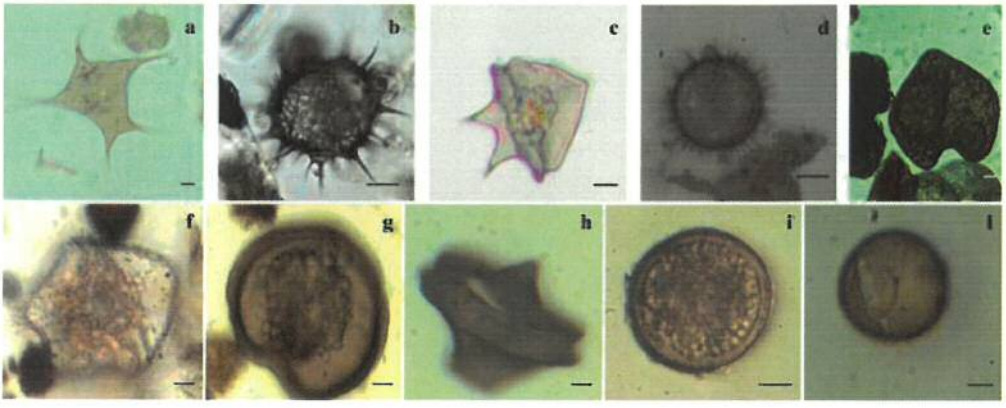


Plate III. Dinoflagellate cysts isolated from recent sediments of the Western Mediterranean Sea. Figures a–l: Organic (non-calcareous) Peridiniales (a) *Protoperidinium compressum*. (b) *Protoperidinium conicum*. (c) *Protoperidinium leonis*. (d) *Protoperidinium* cf *minutum*. (e) *Protoperidinium oblongum*. (f) *Protoperidinium pentagonum*. (g, h) *Protoperidinium subinerme*: (g) viable cyst, apical view, (h) empty cyst, lateral view. (i, l) *Zygabikodinium lenticulatum*: (i) viable cyst, (l) empty cyst. All scale bars 10 µm.

Scrippsiella sp.3 (Plate II, Fig. c)

The cyst is ovoid (length 38–50 µm, width 28–35 µm; n=3), dark brown in color, with a red accumulation body. Calcareous crystals surround the cyst wall. The germination experiment was negative. Montresor et al. (1994) described this morphotype in a study of the Gulf of Naples. The cyst's general characteristics are similar to those of *Scrippsiella crystallina*, but germination experiments conducted by the authors produced vegetative cells with a body shape and plate pattern common to both *S. trochoidea* and *S. crystallina*.

Scrippsiella sp.4 (Plate II, Figs. d–g)

The cyst is round to oval (25–30 µm in diameter; n=3), with clear contents and a red accumulation body. The cyst wall is smooth and thick. The archeopyle is present and remains attached to the cyst body, although the type is difficult to distinguish. The germinated cell is small and *Scrippsiella*-like. Cysts produced in the unique culture that was established exhibited small calcareous crystals, a characteristic not observed in wild-isolated cysts. The cultured cysts resembled those of *S. rotunda* Lewis. This is a common

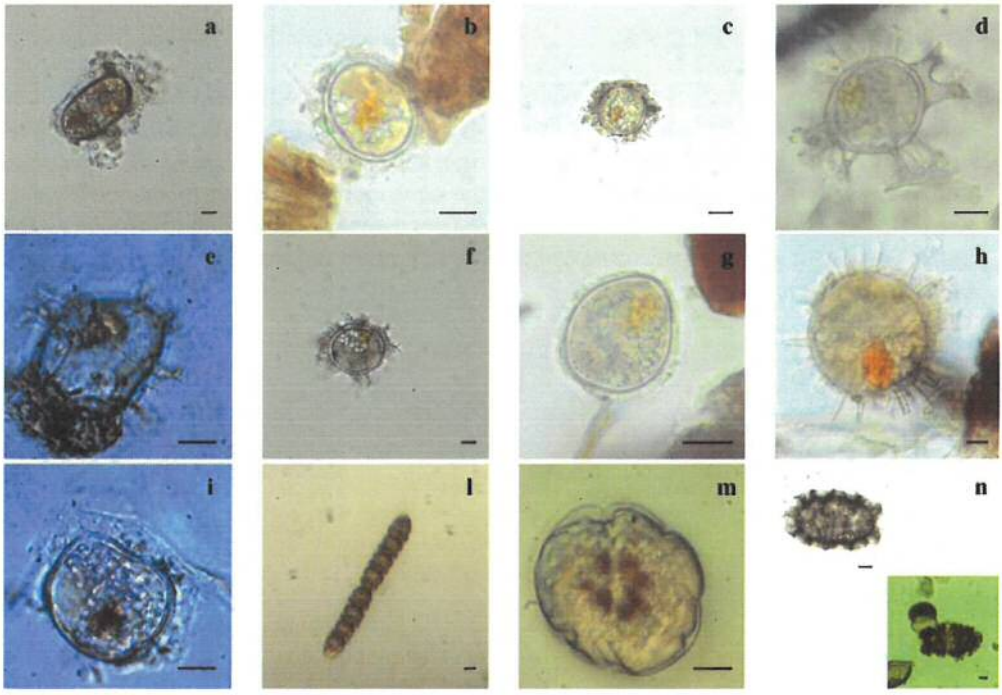


Plate IV. Dinoflagellate cysts isolated from recent sediments of the Western Mediterranean Sea. Figures a–h: Gonyaulacales (a) *Alexandrium catenella/tamarense*. (b, c) *Alexandrium minutum*: (b) cyst from Arenys sediments, (c) cyst from Olbia sediments. (d) *Gonyaulax* cf *membranacea*. (e) *Gonyaulax* cf *scrippsae*. (f) *Gonyaulax* cf *spinifera*. (g) *Gonyaulax verior*. (h) *Lingulodinium polyedrum*. Figures i–n: Gymnodiniales (i, l) *Gymnodinium impudicum*: (i) viable cyst, (l) chain of motile cells. (m) *Gyrodinium instriatum*. (n) *Polykrikos* complex. All scale bars 10 µm.

and widespread cyst type in Olbia. A similar taxon has been reported from the Russian East Coast (Orlova et al., 2004).

Calcareous cyst 1 (Plate II, Fig. h)

This morphotype was seldom present in Olbia sediments. The cyst is characterized by an evident excavated girdle, 40 µm long and 40 µm wide (n=2), and is dark brown in color with a red accumulation body. Small calcareous crystals cover the surface.

Alexandrium catenella/tamarense complex (Plate IV, Fig. a)

A. catenella/tamarense cysts are cylindrical (48–50 µm long, 22–30 µm wide; n=3) with rounded ends and an orange accumulation body. Morphological observations were in accordance with the description by Fukuyo (1985). *A. catenella/tamarense* cysts were observed only in Olbia sediments. In samples from the first survey, *A. catenella/tamarense* cysts were detected using the primuline method, whereas detection by the sodium polytungstate density gradient method was negative. In the second survey, the presence of cysts was confirmed using the latter method. In both surveys, *A. catenella/*

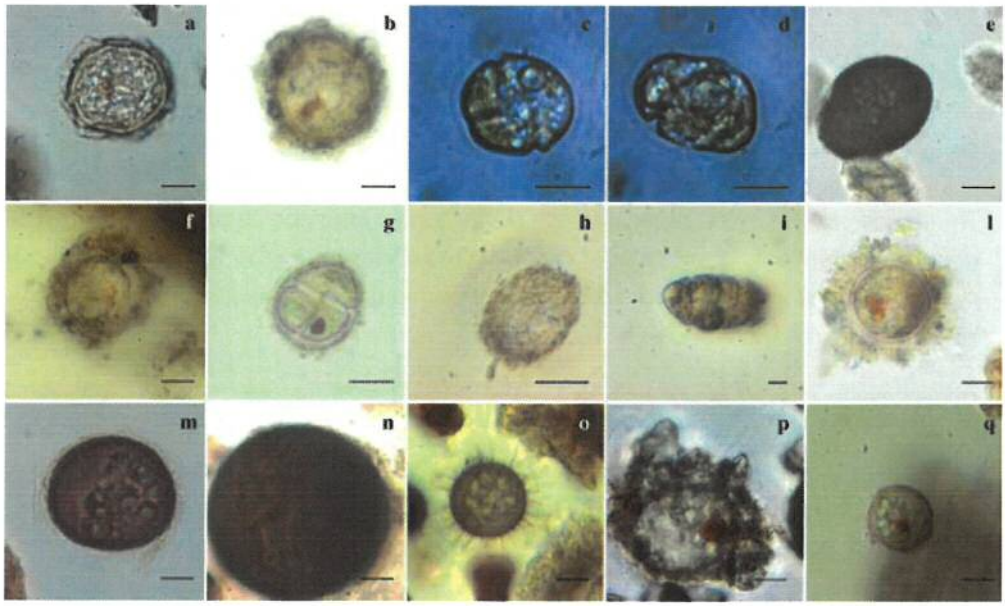


Plate V, Dinoflagellate cysts isolated from recent sediments of the Western Mediterranean Sea. Figures a–i: Gymnodiniales (a–d) Gymnodiniales type 1: (a, b) different viable cysts of the same morphotype, (c) ventral view of motile cell, (d) lateral view of motile cell. (e) Gymnodiniales type 2. (f) Gymnodiniales type 3. (g–i) Gymnodiniales type 4: (g) viable cyst, (h) empty cyst, (i) motile cell. Figures l–q: Unidentified morphotypes (l) Cyst type A. (m, n) Cyst type B, (m) cyst from Arenys sediments, (n) cyst from Olbia sediments. (o) Cyst type C. (p) Cyst type D. (q) Cyst type E. All scale bars 10 µm.

tamarense cysts were restricted to only one of the external stations (N and P, respectively). As reported by Fukuyo (1985), the cysts of *A. catenella* (Whedon et Kofoid) Balech and *A. tamarense* (Lebour) Balech are very similar and hard to distinguish without germination and observation of the vegetative cells. Since germination experiments were unsuccessful and both species were present in Olbia plankton, we report them as *A. catenella/tamarense* complex.

Alexandrium minutum (Plate IV, Figs. b–c)

A. minutum cysts were observed at both sites, but only those from Arenys germinated (germination success: 50%). *A. minutum* cysts are round to oval, compressed in lateral view (20–25 µm long, 20–23 µm wide; n=3), with a granular content and a yellowish accumulation body. Morphological observations were in accordance with the description by Bolch et al. (1991) and Bravo et al. (2006).

Gymnodiniales type 1 (Plate V, Figs. a–d)

The cyst is round to oval (25–30 µm in diameter; n=22). The wall is clear, thick, and sometimes covered with detrital particles, probably indicative of a mucilage layer. The cyst has a pale granular content with a red/orange accumulation body. Germination of this cyst produced a small gymnodinioid cell. This morphotype is widely distributed in the Olbia sediments.

Gymnodiniales type 2 (Plate V, Fig. e)

This darkly colored cyst is ovoidal to ellipsoidal, 38 µm long and 28 µm wide (n=2), with a granular content only on one side of the cyst. A red accumulation body is present. Only one cyst germinated, producing a short-lived gymnodinioid cell. A more-detailed identification was therefore not possible.

Gymnodiniales type 3 (Plate V, Fig. f)

This unique cyst is oval in shape, 20 µm long and 18 µm wide (n=1), and surrounded by a conspicuous layer of mucilage and detrital particles. The wall is thin, and the pale content is not well-defined but large pale grains are evident. The accumulation body is yellowish-pale orange. Germination produced a small gymnodinioid cell that died a few days later.

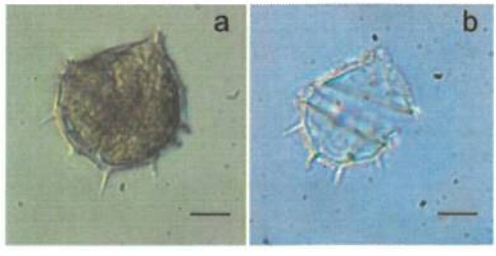


Fig. 2. *Peridinium quinquecorne*, (a) viable "stage," (b) empty theca. Scale bars 10 µm.

Gymnodiniales type 4 (Plate V, Figs. g–i)

This cyst is characterized by its transparent content, which reveals a gymnodinioid cell with a ruby-red accumulation body (20–38 µm long, 18–30 µm wide; n=3). The wall is thin and surrounded by hair-like structures. The two cysts that were isolated germinated and divided once, but cultures could not be established. The vegetative cell has a gymnodinioid shape, but taxonomic identification was not possible.

Cyst type A (Plate V, Fig. l)

The cyst of this morphotype is circular (15–25 µm in diameter; n=2), with a granular content and a yellowish-orange accumulation body. The cyst wall is covered by acicular processes 5–7 µm long.

Cyst type B (Plate V, Figs. m–n)

This group comprises "round brown cysts." These morphotypes are brown in color, rounded in shape, with a granular content and lacking a colored accumulation body. The size varied substantially. Dinoflagellates belonging to the genus *Protoperidinium* or to the Diplopsalis group often produce these kinds of cysts. In the absence of information on archeoplyle or germinated cells, it was impossible to identify them.

Cyst type C (Plate V, Fig. o)

This is a small rounded cyst (22 µm in diameter; n=1) with a green-gray granular content and lacking a colored accumulation body. Several capitate processes surrounded the cysts (8 µm long). This morphotype is widely distributed in Olbia. A similar taxon has been reported on the East Coast of Russia (Orlova et al., 2004).

Cyst type D (Plate V, Fig. p)

The cyst has an oval shape and is 32–40 µm long and 28–30 µm wide (n=2), with a thin wall covered by a layer of detrital particles due to the conspicuous presence of mucilage. The cyst content appears granular in the apical part and a red accumulation body is present.

Cyst type E (Plate V, Fig. q)

The cyst is round and small (17.5–18 µm in diameter; n=2), with a granular content and a red accumulation body. The wall is smooth and thick.

The case of Peridinium quinquecorne

The shape of this "stage" resembles that of the vegetative cell (Fig. 2), with a sharp epitheca and five processes in the rounded hypotheca. The length ranges from 25 to 35 µm, and the width from 20 to 35 µm (n=9). Processes are 5–8 µm long. The content is granular, with an expanded and orange accumulation body. All the isolated "stages" germinated in one or two days, losing the theca but producing only one cell, which did not divide. This species has not been reported to be a cyst-producer and its life cycle has not yet been described. For these reasons, we could not affirm whether this morphotype is a "resting cell" or a resting or temporary cyst; consequently, we excluded it from both the list of morphotypes and data elaborations. *P. quinquecorne* was found in Arenys and Olbia sediments. In Olbia, it reached high densities (max. of 296 cysts cm^{-3}) even in the deeper layers (6–7 cm).

Abundance and assemblage composition

Total cyst densities in Arenys varied from 480 cysts cm^{-3} ws to 10679 cysts cm^{-3} ws. The range in Olbia was 20–5484 cysts cm^{-3} ws. At all stations, abundance increased between the first and second survey in Arenys but decreased in Olbia, except at station N. Maximum

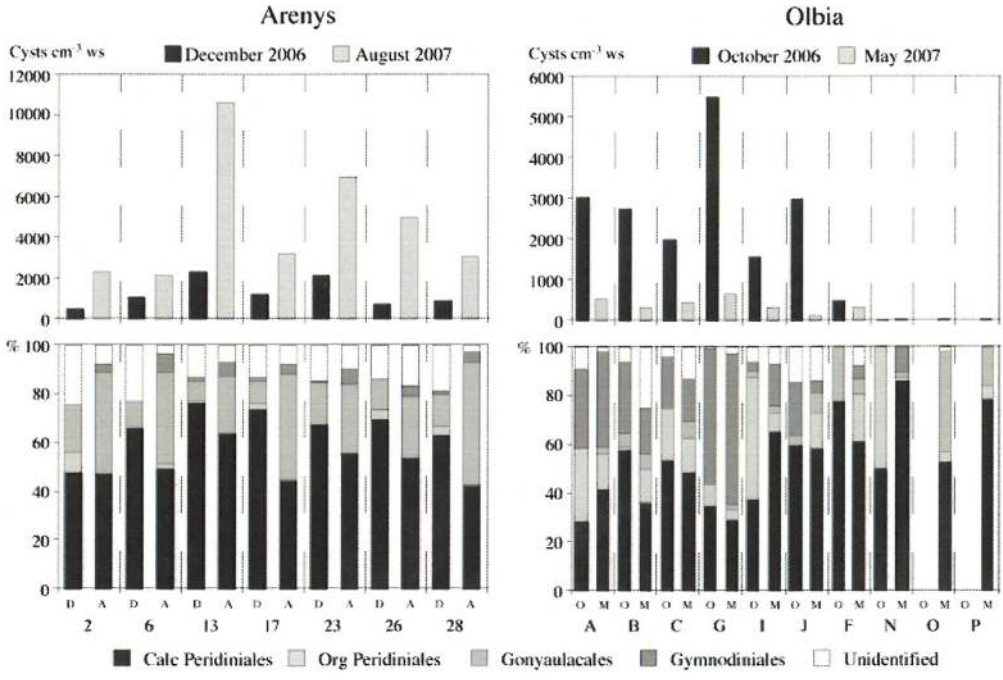


Fig. 3. Total cyst densities (top panel) and class composition (bottom panel) in Arenys de Mar harbor (left) and Olbia Gulf (right).

cyst abundance occurred at station 13 in Arenys, both in December and in August, and in Olbia at the most confined station (G in both surveys) (Fig. 3, top panel). Relative class percentages (Fig. 3, bottom panel) indicated that calcareous Peridiniales was the most numerous class at both sites during the two surveys. In Olbia, the classes showed different distribution patterns, with a higher percentage of calcareous Peridiniales in the outer (N, O and P) and intermediate (J and F) stations while Gymnodiniales prevailed in the inner stations (especially G). Apart from these general trends, there was notable heterogeneity in the percentages of the class compositions at each station between the two surveys. Class assemblage compositions in Arenys de Mar were more similar among stations. In addition to the dominance of calcareous Peridiniales, the relative importance of Gonyaulacales and Gymnodiniales increased in Arenys in the second survey, contemporaneous with the decrease of calcareous and organic Peridiniales.

Average similarity (%)			
ARENYS	72.80%	OLBIA	43.99%
December	75.43%	October	40.13%
August	82.57%	May	45.52%
Contributory species			
Arenys		**Olbia**	
Scrippsiella trochoidea	16.16%	*Scrippsiella trochoidea*	26.10%
Gonyaulax verior	13.35%	*Scrippsiella* sp.4	13.47%
Scrippsiella sp.2	13.32%	Others Calc Per*	11.72%
Alexandrium minutum	11.29%	*Gymnodinium impudicum*	11.63%
Scrippsiella precaria	11.20%	*Protoperidinium avellana*	8.40%
December		**October**	
Scrippsiella trochoidea	17.82%	*Scrippsiella trochoidea*	26.10%
Gonyaulax verior	13.46%	*Scrippsiella* sp.4	13.47%
Scrippsiella sp.2	13.38%	Others Calc Per*	11.72%
Scrippsiella precaria	12.29%	*Gymnodinium impudicum*	11.63%
Alexandrium minutum	11.86%	*Protoperidinium avellana*	8.40%
August		**May**	
Gonyaulax verior	15.56%	*Scrippsiella trochoidea*	23.47%
Scrippsiella trochoidea	14.84%	*Scrippsiella lachrymosa*	12.89%
Scrippsiella sp.2	13.23%	*Gonyaulax spinifera*	7.32%
Alexandrium minutum	11.48%	Others Calc Per*	7.29%
Gyrodinium instriatum	10.56%	*Zygabikodinium lenticulatum*	6.48%

Table 2. Important morphotypes identified by SIMPER (similarity percentages procedure) analysis: average similarity and percent contribution to each site and considering the single surveys. *= sum of *Calciodinellum* cf *operosum* and *Pentapharsodinium* cf *tyrrhenicum* type 1 and 2.

The SIMPER test confirmed a greater similarity among samples from Arenys (average similarity: 72.80%) than among those from Olbia (43.99%) (Table 2). As the species with the highest contribution, *Scrippsiella trochoidea* was important at both sites, whereas there were substantial differences in the other characterizing species. In the analysis of the surveys, the SIMPER test revealed a higher similarity in Arenys (average: 75.43% in December and 82.57% in August) than in Olbia (average respectively 40.13% and 45.52% in October and May). As expected, the nMDS ordination (Fig. 4) showed a strong clustering of samples taken in Arenys stations in both surveys, whereas samples taken in Olbia seemed more dissimilar. The Olbia stations A, B, C, G, and I, located in the inner part of the ria (Fig. 1), form a main group in contrast to the outer stations (N, O, P), which are distinct with respect to the stations in the main group and among each other. One-way ANOSIM showed significant differences between sites (Table 3). The SIMPER test revealed that *Gonyaulax verior*, *Scrippsiella* sp.2, *Alexandrium minutum*, *Scrippsiella* sp.1, and cyst type B were the morphotypes that most contributed to differences between sites.

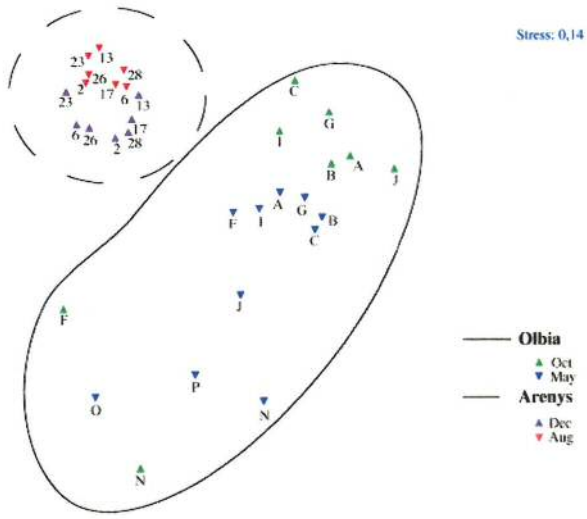


Fig. 4. Results of nMDS ordination plots derived from the Bray-Curtis similarity measure of morphotype abundances.

ANOSIM		SIMPER		
R	p	Average dissimilarity (%)	Most discriminating species	Contribution (%)
0.609	0.1%	68.42	*Gonyalulax verior*	10.47
			Scrippsiella sp.2	8.40
			Alexandrium minutum	6.83
			Scrippsiella sp.1	6.50
			Cyst type B	6.11
			Scrippsiella precaria	6.10
			Scrippsiella sp.4	5.04

Table 3. Results of one-way ANOSIM (R value and significant level) and SIMPER analyses of the morphotype abundances at the two sites.

Discussion

The composition of dinoflagellate resting cyst assemblages in recent sediments from the Mediterranean Sea has been examined in only a few studies (e. g. Della Tommasa et al., 2000; Giannakourou et al., 2005; Montresor et al., 1998; Rubino et al., 2000, 2002). Instead, most studies have focused on species-specific investigations, the description of new morphotypes (Bravo et al., 2006; Garcés et al., 2004; Genovesi et al., 2007; Giacobbe and Yang, 1999; Montresor and Zingone, 1988; Montresor et al., 1993; Montresor, 1995), or have assessed particular groups (e.g., Meier and Willems, 2003; Montresor et al., 1994, for calcareous cysts). To our knowledge, ours is the first study that examines recent dinoflagellate resting cyst assemblages in semi-enclosed (confined) areas of the Western Mediterranean. The study sites are representative of the numerous man-made and over-exploited coastal zones in the Mediterranean area (Garcés et al., 2000). However, each of these sites has its own unique characteristics and, consequently, specific hydrological behaviors that may affect cyst deposition (Dale, 1976). Arenys is a small harbor where ship traffic is linked to recreational and fishing activities. It is an artificial harbor with straight sidewalls and shallow and regular bathymetry that protects the area from the sea and from general winds and is subject to restricted hydrodynamic forces (Anglès et al., 2010). Conversely, Olbia is a larger area that receives river discharge (Padrongianus River) and is influenced to a greater degree by physical forces, mostly on the side open to the Gulf. It is also a highly impacted site, given the presence of the most important passenger harbor and mussel-farming activities in Sardinia. Both these activities strongly affect the harbor's sediments due to the high turbulence caused by heavy ship traffic, the continuous movement of mussel long-lines, and the deposition of waste from the shellfish (i.e. metabolic products and shells). Despite their different dimensions and general features, the two harbors were studied in parallel because both are affected by recurrent *Alexandrium* blooms, as revealed during multi-annual monitoring activities: *A. minutum* in Arenys (Garcés et al., 2004; Vila et al., 2005), and both *A. minutum* and *A. catenella* in Olbia (Lugliè et al., 2003, 2004). Nevertheless, the differences between the two sites could partly explain the lower spatial variability of cyst abundance in Arenys than in Olbia. Calcareous Peridiniales dominated the cyst assemblages in both areas, similar to the findings of other studies done in the Mediterranean (Gulf of Naples; Montresor et al., 1994, 1998). In addition, cosmopolitan dinoflagellates belonging to the genera *Scrippsiella*, *Protoperidinium*, *Gonyaulax*, and *Polykrikos* were identified in Arenys and in Olbia (Bolch and Hallegraeff, 1990; Montresor et al., 1998; Morquecho and Lechuga-Devéze, 2003; Persson et al., 2000; Rubino et al., 2000, 2002; Orlova et al., 2004). Conspicuous differences between the two assemblages were, however, revealed by nMDS ordination analysis (Fig. 4), which also showed that the Arenys stations formed a cluster that did not group with the inner stations of Olbia. This finding suggests that Olbia's inner area does not behave like an artificial harbor with respect to the spatial variability of morphotype abundance. The SIMPER test confirmed *S. trochoidea* as the species with the highest contribution to the similarity percentages to both sites, consistent with its world-wide distribution (Ellegaard et al., 1994; Giannakourou

et al., 2005; Godhe et al., 2000; Montresor et al., 1998; Morquecho and Lechuga-Devéze, 2003; Nehring, 1994; Orlova et al., 2004; Wang et al., 2004), but the same test underlined the dissimilarity between the two sites regarding the other characterizing morphotypes. Arenys had a higher number of Gonyaulacales, especially in the second survey, *G. verior* being the species contributing the most to the dissimilarity between the two sites.

The genus *Scrippsiella* accounted for several common and site-specific species (common species: *S. lachrymosa*, *S. precaria*, *S. trochoidea*, *Scrippsiella* sp.2; Arenys specific-species: *Scrippsiella* sp.1; Olbia specific-species: *Scrippsiella* sp.4). This finding is in agreement with data already reported for other Mediterranean sites, which identified *Scrippsiella* species as co-associated elements of planktonic assemblages during *A. minutum* blooms (Vila et al., 2005).

Several species not previously identified in plankton samples (Sannio et al., 1996, 1997; Vila et al., 2001; Vila and Masó, 2005) were detected in the sediments analyzed in this study. These species, including *Pentapharsodinium* cf *tyrrhenicum*, *Scrippsiella crystallina*, *S. lachrymosa*, *S. precaria*, *S. trochoidea*, *Protoperidinium avellanum*, *P. claudicans*, *P. compressum*, *P. conicum*, *P.* cf *minutum*, *P. oblongum*, *P. pentagonum*, *P. subinerme*, *Zygabikodinium lenticulatum*, and *Gyrodinium instriatum*, are frequently reported as groups of species at the corresponding genus level. For example, different species belonging to the genus *Scrippsiella* are often denoted as a complex in plankton surveys (Satta, 2006; Sampedro, pers. com.). These small thecate dinoflagellates are difficult to distinguish at the species level in plankton samples without an exhaustive analysis of thecal plates (Nehring, 1994, 1997; Orlova et al., 2004). However, even an exhaustive analysis of thecal plates is not sufficient to identify some species. For example, *Scrippsiella crystallina*, *S. lachrymosa* and *S. trochoidea* share exactly the same cell shape and plate pattern (Lewis, 1991). Here, as in the case of other species of the genus *Scrippsiella* (e.g. *S. rotunda, S. operosa, S. infula*), the difference between the species is only based on their characteristic resting cysts, and is supported by molecular data (D'Onofrio et al., 1999; Montresor et al., 2003). Moreover, many species belonging to this genus were originally described on the basis of cultures obtained from cysts (Lewis, 1991; Montresor and Zingone, 1988; Montresor, 1995).

Other difficulties in identifying motile stages are encountered regarding various unarmored dinoflagellates in plankton samples (Orlova et al., 2004). In this case, the problem is due to the fact that the fixative used destroys the morphological features. These species can be identified either as live cells or by electron microscopy after osmium fixation. The rarity of a species in terms of its abundance, distribution, and duration of presence in the water column must also be considered. In this case the sediment acts as a "recorder" of species' presence.

The case of *P. quinquecorne* is very interesting. There is no evidence that this species is a cyst-producer, even though it was already reported as a germinating cell following the

incubation of untreated sediments collected in Naantali harbor in the Baltic Sea (Pertola et al., 2006). Our finding agrees with the probable benthic habitat of *P. quinquecorne* (Okolodkov et al., 2007) at least as far as the surface sediment observation is concerned, but records of specimens apparently viable in deeper sediments (6–7 cm) suggest the need for further study of the life cycle of this species. This would be of particular relevance if its ability to cause high biomass blooms is confirmed in Mediterranean waters.

Two toxic species belonging to the genus *Alexandrium* were found as resting cysts. *A. minutum* was identified in both Arenys and Olbia samples whereas *A. catenella/tamarense* was present only in Olbia. These data confirm observations already reported for Arenys (Anglès et al. 2010; Bravo et al., 2006; Garcés et al., 2004) but provide the first report of these species as resting cysts in Olbia. *A. catenella/tamarense* cysts were only detected in the outer stations. In this external area, *A. catenella* vegetative cells were found in samples obtained during a cruise in 2002 (Vila and Lugliè, 2004). This concurrence, and the fact that *A. tamarense* is only sporadically observed in plankton samples at very low densities, supports the hypothesis that the *Alexandrium* resting cysts observed probably belong to *A. catenella*.

Conclusions

Our results reveal the richness of the dinoflagellate resting cysts of the two areas and provide useful information on the presence of dinoflagellate species, increasing our knowledge on the biodiversity of the study sites. This work confirms the importance of semi-enclosed or confined areas as reservoirs for planktonic dinoflagellates. Moreover, our study led to the description of 10 new morphotypes, thereby contributing to our knowledge of recent dinoflagellate cysts and providing the basis for further studies to match these cysts with their vegetative stage and thereby indicate their species designation.

Acknowledgements

The authors wish to thank K. Van Lenning, X. Novell, and the personnel at the Creu Roja of Arenys de Mar for their help with the fieldwork; I. Bravo, M. Montresor, and J. Lewis for their kind and useful suggestions; and J.M. Fortuño for his technical help during SEM analyses. This study was financed by the EU-funded Research Project SEED (GOCE-CT-2005-003875). E. Garcés' work is supported by a Ramon y Cajal contract from the Spanish Ministry of Science and Innovation.

References

Adachi, M., Kanno, T., Matsubara, T., Nishijima T., Itakura, S., Yamaguchi, M., 1999. Promotion of cyst formation in the toxic dinoflagellate *Alexandrium* (Dinophyceae) by natural bacterial assemblages from Hiroshima Bay, Japan. Marine Ecology Progress Series 191, 175-185.

Amorim, A., Dale, B., Godinho, R., Brotas, V., 2001. *Gymnodinium catenatum*-like cysts (Dinophyceae) in recent sediments from the coast of Portugal. Phycologia 40 (6), 572-582.

Anderson, D.M., 1998. Physiology and Bloom Dynamics of Toxic *Alexandrium* species, with Emphasis on Life Cycle Transitions. In: Anderson, D.M., Cembella, A.D., Hallegraeff, G.M., (Eds.), Physiological Ecology of Harmful Algal Blooms. NATO ASI Series, Springer-Verlag, Berlin, pp. 29-48.

Anderson, D.M., Wall, D., 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. Journal of Phycology 14, 224-234Anglès, S., Garcés, E., Jordi, A., Basterretxea, G., Palanques, A., 2009. *Alexandrium minutum* resting cyst distribution dynamics in a confined site. Deep Sea Research Part II-Topical Studies in Oceanography 57, 210-221.

Belmonte, G., Castello, P., Piccinni, M.R., Quarta, S., Rubino, F., Boero, F., Geraci, S., 1995. Resting stages in marine sediments off the Italian coasts. In: Eleftheriou, A., Ansell, A.D., Smith, C.J. (Eds.), Biology and Ecology of Shallow Coastal Waters. Olsen and Olsen, Fredensborg, pp. 53-58.

Boero, F.G., Belmonte, G., Fanelli, S., Piratino, S., Rubino, F., 1996. The continuity of living matter and the discontinuities of its constituents: do plankton and benthos really exist? Trends in Ecology and Evolution 11, 177-180.

Bolch, C.J.S., 1997. The use of sodium polytungstate for the separation and concentration of living dinoflagellate cysts from marine sediments. Phycologia 36 (6), 472–478.

Bolch, C.J.S., Hallegraeff, G.M., 1990. Dinoflagellate cysts in recent marine sediments from Tasmania, Australia. Botanica Marina 33 (2), 173-192.

Bolch, C.J.S., Blackburn, S.I., Cannon, J.A., Hallegraeff, G.M., 1991. The resting cyst of the red-tide dinoflagellate *Alexandrium minutum* (Dinophyceae). Phycologia 30 (2), 215-219.

Bravo, I., Garcés, E., Diogeène, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including first description of cysts of *A. kutnerae* and *A. peruvianum*. European Journal of Phycology 41 (3), 293–302.

Bravo, I., Isabel Figueroa, R., Garcés, E., Fraga, S., Massanet, A., 2010. The intricacies of dinoflagellate pellicle cysts: The example of *Alexandrium minutum* cysts from a bloom-recurrent area (Bay of Baiona, NW Spain). Deep Sea Research Part II: Topical Studies in Oceanography 57, 166-174.

Clarke, K.R., Warwick, R.M., 1994. Change in marine communities: an approach to statistical analysis and interpretation. Natural Environmental Research Council, Plymouth Marine Laboratory, Plymouth, UK, pp. 1-172.

Dale, B., 1976. Cyst formation, sedimentation, and preservation: factors affecting dinoflagellate assemblages in recent sediments from Trondheimsfjord, Norway. Review in Palaeobotany and Palynology 22, 39-60.

Dale, B., 1983. Dinoflagellate resting cysts: 'benthic plankton'. In: Fryxell, G.A. (Ed.), Survival Strategies of the Algae. Cambridge University Press, Cambridge, pp. 69-136.

Della Tommasa, L., Belmonte, G., Palanques, A., Puig, P., Boero, F., 2000. Resting stages in a submarine canyon: a component of shallow-deep sea coupling? Hydrobiologia 440 (1-3), 249-260.

D'Onofrio, G., Marino, D., Bianco, L., Busico, E., Montresor, M., 1999. Toward an assessment on the taxonomy of dinoflagellates that produce calcareous cysts (Calciodinelloidae, Dinophyceae): a morphological and molecular approach. Journal of Phycology 35 (5), 1063-1078.

Ellegaard, M., Christensen, N.F., Moestrup, Ø., 1994. Dinoflagellate cysts from recent Danish marine sediments. European Journal of Phycology 29, 183-194.

Ellegaard, M., Kulis, D.M., Anderson, D.M., 1998. Cysts of Danish *Gymnodinium nolleri* Ellegaard et Moestrup sp. ined. (Dinophyceae): studies on encystment, excystment and toxicity. Journal of Plankton Research 20 (9), 1743-1755.

Erdner, D.L., L., Percy, Lewis, J., Anderson, D.M., 2009. A quantitative real-time PCR assay for the identification and enumeration of *Alexandrium* cysts in marine sediments. Deep Sea Research Part II- Topical Studies in Oceanography 57, 279-287.

Estrada, M., Solé, J., Anglès, S., Garcés, E. 2009. The role of resting cysts in *Alexandrium minutum* population dynamics. Deep Sea Research Part II- Topical Studies in Oceanography 57, 308-321.

Figueroa, R.I., Bravo, I., 2005. A study of the sexual reproduction and determination of mating type of *Gymnodinium nolleri* (Dinophyceae) in culture. Journal of Phycology 41 (1), 74-83.

Figueroa, R.I., Bravo, I., Garcés E., 2006. Multiple routes of sexuality in *Alexandrium taylori* (Dinophyceae) in culture. Journal of Phycology 42 (5), 1028-1039.

Figueroa, R.I., Garcés E., Bravo, I., 2007. Comparative study of the life cycles of *Alexandrium tamutum* and *Alexandrium minutum* (Gonyaulacales, Dinophyceae) in culture. Journal of Phycology 43 (5), 1039-1053.

Fukuyo, Y., 1985. Morphology of *Protogonyaulax tamarensis* (Lebour) Taylor and *Protogonyaulax catenella* (Whedon and Kofoid) Taylor from Japanese coastal waters. Bulletin of Marine Science 37, 529-537.

Garcés E., Masó, M., Camp, J., 1999. A recurrent and localized dinoflagellate bloom in a Mediterranean beach. Journal of Plankton Research 21 (12), 2373-2391.

Garcés, E., Masó, M., Camp J., 2002. Role of temporary cysts in the population dynamics of *Alexandrium taylori* (Dinophyceae). Journal of Plankton Research 24 (7), 681-686.

Garcés, E., Masó, M., Vila, M., Camp, J., 2000. HABs events in the Mediterranean Sea: are they increasing? A case study the last decade in the NW Mediterranean and the genus *Alexandrium*. Harmful Algae News 20, 1-11.

Garcés, E., Bravo, I., Vila, M., Figueroa, R.I., Masó, M., Sampedro, N., 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbor (NW Mediterranean). Journal of Plankton Research 26 (6), 637–45.

Genovesi, B., Mouillot, D., Vaquer, A., Laabir, M., Pastoureaud, A., 2007. Towards an optimal sampling strategy for *Alexandrium catenella* (Dinophyceae) benthic resting cysts. Harmful Algae 6 (6), 837-848.

Giacobbe, M.G., Yang, X., 1999. The life history of *Alexandrium taylori* (Dinophyceae). Journal of Phycology 35 (2), 331-338.

Giannakourou, A., Orlova, T.Y., Assimakopoulou, G., Pagou, K., 2005. Dinoflagellate cysts in recent marine sediments from Thermaikos Gulf, Greece: effects of resuspension events on vertical cyst distribution. Continental Shelf Research 25 (19-20), 2585-2596.

Godhe, A., Karunasagar, I., Karunasagar, I., Karlson B., 2000. Dinoflagellate cysts in recent marine sediments from SW India. Botanica Marina 43 (1), 39–48.

Head, M.J., 1996. Modern dinoflagellate cysts and their biological affinities. In: Jansonius, J., McGregor, D.C., (Eds.), Palynology: Principles and Applications. The American Association of Stratigraphic Palynologists, Dallas, pp. 1197–1248.

Hégaret, H., Shumway, S.E., Wikfors, G.H., Pate, S., Burkholder, J.M., 2008. Potential transport of harmful algae via relocation of bivalve molluscs. Marine Ecology Progress Series 361, 169-179.

Kremp, A., Heiskanen, A.S., 1999. Sexuality and cyst formation of the spring-bloom dinoflagellate *Scrippsiella hangoei* in the coastal northern Baltic Sea. Marine Biology 134 (4), 771-777.

Lewis, J., 1991. Cyst-theca relationships in *Scrippsiella* (Dinophyceae) and related orthoperidinoid genera. Botanica Marina 34 (2), 91-106.

Lugliè, A., Giacobbe, M.G., Sannio, A., Fiocca, F., Sechi, N., 2003. First record of *Alexandrium catenella* (Whedon & Kofoid) Balech (Dinophyta), a potential producer of paralytic shellfish poisoining in Italian waters (Sardinia, Tyrrhenian Sea). Bocconea 16 (2), 1045-1051.

Lugliè, A., Giacobbe, M.G., Fiocca, F., Sannio, A., Sechi, N., 2004. The geographical distribution of *Alexandrium catenella* is extending to Italy! First evidences from the Tyrrhenian Sea. In: Steidinger, K.A., Landsberg, J.H., Tomas, C.R., Vargo, G.A., (Eds.), Harmful Algae 2002. Florida Fish and Wildlife Conservation Commission, Florida Institute of Oceanography, and Intergovernmental Oceanographic Commission of UNESCO, St. Petersburg, Florida, USA, pp. 329-331.

Margalef, R., 2002. Diversidad y biodiversidad. In: Pineda, F.D. (Ed.), La diversidad biológica de España. Prentice-Hall, Madrid, pp. 3-5.

Meier, K.J.S., Willems, H., 2003. Calcareous dinoflagellate cysts in surface sediments from the Mediterranean Sea: distribution patterns and influence of main environmental gradients. Marine Micropaleontology 48 (3-4), 321-354.

Matsuoka, K., Fukuyo, Y., 2000. Technical guide for modern dinoflagellate cyst study. WESTPAC-HAB/WESTPAC/IOC.

Matsuoka, K., Fukuyo, Y., 2003. Taxonomy of cysts. In: Hallegraeff, G.M., Anderson, D.M., Cembella, A.D. (Eds.), Manual on Harmful Marine Microalgae. UNESCO, Paris, pp. 563–592.

Montresor, M., 1995. The life history of *Alexandrium pseudogonyaulax* (Gonyaulacales, Dinophyceae). Phycologia 34 (6), 444-448.

Montresor, M., Zingone, A., 1988. *Scrippsiella precaria* sp. nov. (Dinophyceae), a marine dinoflagellate from the Gulf of Naples. Phycologia 27 (3): 387-394.

Montresor, M., Zingone, A., Marino D., 1993. The calcareous resting cyst of *Pentapharsodinium tyrrhenicum* comb. nov. (Dinophyceae). Journal of Phycology 29, 223-230.

Montresor, M., Zingone, A., Sarno, D., 1998. Dinoflagellate cyst production at a coastal Mediterranean site. Journal of Plankton Research 20 (12), 2291-2312.

Montresor, M., Montesarchio, E., Marino, D., Zingone, A., 1994. Calcareous dinoflagellate cysts in marine sediments of the Gulf of Naples (Mediterranean Sea). Review of Palaeobotany and Palynology 84, 45-56.

Montresor, M., Sgrosso, S., Procaccini, G., Kooistra, W.H.C.F., 2003. Intraspecific diversity in *Scrippsiella trochoidea* (Dinophyceae): evidence for cryptic species. Phycologia 42 (1), 56-70.

Morquecho, L., Lechuga-Devéze, C.H., 2003. Dinoflagellate cysts in recent sediments from Bahía Concepción, Gulf of California. Botanica Marina 46, 132-141.

Moscatello, S., Rubino, F., Saracino, O.D., Fanelli, G., Belmonte, G., Boero F., 2004. Plankton biodiversity around the Salento Peninsula (South East Italy): an integrated water/sediment approach. Scientia Marina 68 (Suppl.1), 85-102.

Nehring, S., 1994. *Scrippsiella* spp. resting cysts from the German Bight (North Sea): a tool for more complete check-lists of dinoflagellates. Netherlands Journal of Sea Research 33 (1), 57-63.

Nehring, S., 1997. Dinoflagellate resting cysts from recent German coastal sediment. Botanica Marina 40 (4), 307-324.

Okolodkov, Y.B., Campos-Bautista,G., Gárate-Lizárraga, I., González-González, J.A.G., Hoppenrath, M., Arenas, V., 2007. Seasonal changes of benthic and epiphytic dinoflagellates in the Veracruz reef zone, Gulf of Mexico. Aquatic Microbial Ecology 47(3), 223–237.

Orlova, T.Y., Morozova, T.V., Gribble, K.E., Kulis, D.M., Anderson, D.M., 2004. Dinoflagellate cysts in recent marine sediments from the east coast of Russia. Botanica Marina 47, 184-201.

Persson, A., Godhe, A., Karlson, B., 2000. Dinoflagellate cysts in recent sediments from the West Coast of Sweden. Botanica Marina 43, 69-79.

Penna, A., Battocchi, C., Garcés, E., Anglès, S., Cucchiari, E., Totti, C., Kremp, A., Satta, C., Giacobbe, M. G., Bravo, I., Bastianini, M., 2009. Detection of microalgal resting cysts in European coastal sediments using a PCR-based assay. Deep Sea Research Part II- Topical Studies in Oceanography 57, 288-300.

Pertola, S., Faust, M.A., Kuosa, H., 2006. Survey on germination and species composition of dinoflagellates from ballast tanks and recent sediments in ports on the South Coast of Finland, North-Eastern Baltic Sea. Marine Pollution Bulletin 52, 900-911.

Pfiester, L.A., Anderson, D.M., 1987. Dinoflagellate reproduction. In: Taylor, F.J.R. (Ed.), The biology of Dinoflagellates. Blackwell Scientific Publications, London, pp. 611-648.

Rubino, F., Belmonte, G., Miglietta, A.M., Geraci, S., Boero, F., 2000. Resting stages of plankton in recent North Adriatic sediments. Marine Ecology 21, 263-284.

Rubino, F., Moscatello, S., Saracino, O.D., Fanelli, G., Belmonte, G., Boero, F., 2002. Plankton-derived resting stages in marine coastal sediments along the Salento Peninsula (Apulia, South-Eastern Italy). Marine Ecology 23 (suppl. I), 329-339.

Sannio, A., Lugliè, A., Sechi, N., 1996. The phytoplankton of the internal Gulf of Olbia (North-East Sardinia) between July 1992 and July 1993. Giornale Botanico Italiano 130, 1037-1050.

Sannio, A., Sechi, N., Lugliè, A., 1997. Potentially toxic dinoflagellates in Sardinia. Plant Biosystems 131 (1), 73-78.

Satta, C.T., 2006. Presenza di HABs (Harmful Algal Blooms) in aree marine costiere della Sardegna: studio delle specie coinvolte, dei cicli vitali e delle condizioni ambientali favorenti il loro sviluppo. Ph. D. Thesis, Dipartimento di Botanica ed Ecologia vegetale, University of Sassari, Sardinia, Italy, unpublished.

Satta, C.T., De Falco, G., Lugliè, A., Padedda, B.M., Pascucci, V., Sechi, N., 2008. Cisti di dinoflagellati in sedimenti a diversa granulometria nel Golfo di Olbia. Proceedings of the Italian Association for Oceanology and Limnology, Pallanza, pp. 441-448.

Sgrosso, S., Esposito, F., Montresor, M., 2001. Temperature and daylength regulate encystment on calcareous cyst-forming dinoflagellates. Marine Ecology Progress Series 211, 77-87.

Sonneman, J.A., Hill, D.R.A., 1997. A taxonomic survey of cyst-producing dinoflagellates from recent sediments of Victorian coastal waters, Australia. Botanica Marina 40 (3), 149-177.

Uchida, T., 2001. The role of cell contact in the life cycle of some dinoflagellate species. Journal of Plankton Research 23 (8), 889-891.

Vila, M., Lugliè, A., 2004. Harmful dinoflagellates distribution in Mediterranean confined waters. Bloom features. In: Masó, M. (Ed.), Proceedings of the International Workshop on "Management of Recreational Waters in Relationship with Harmful Microalgae Blooms (HAB) in the Mediterranean Sea", 25-26 October 2004, Calvià (Mallorca), pp. 10-16.

Vila, M., Masó, M., 2005. Phytoplankton functional groups and harmful algal species in anthropogenically impacted waters of the NW Mediterranean Sea. Scientia Marina 69 (1), 31-45.

Vila, M., Camp, J., Garcés, E., Masó, M., Delgado, M., 2001. High resolution spatio-temporal detection of HABs in confined waters of the NW Mediterranean. Journal of Plankton Research 23 (5), 497-514.

Vila, M., Giacobbe, M.G., Masó, M., Gangemi, E., Penna, A., Sampedro, N., Azzaro, F., Camp, J., Galluzzi, L., 2005. A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas. Harmful Algae 4, 673-695.

Wall, D., Dale, B., 1968. Modern dinoflagellate cysts and evolution of Peridiniales. Micropaleontology 14, 265-304.

Wang, Z., Matsuoka, K., Qi, Y., Chen, J., 2004. Dinoflagellate cysts in recent sediments from Chinese coastal waters. Marine Ecology 25 (4), 289-311.

Yamaguchi, M., Itakura, S., Imai, I., Ishida, Y., 1995. A rapid and precise technique for enumeration of resting cysts of *Alexandrium* spp. (Dinophyceae) in natural sediments. Phycologia 34 (3), 207-214.

Zingone, A., Siano, R., D'Alelio, D., Sarno, D., 2006. Potentially toxic and harmful microalgae from coastal waters of the Campania region (Tyrrhenian Sea, Mediterranean Sea). Harmful Algae 5 (3), 321-337.

3

Alexandrium minutum resting cyst distribution dynamics in a confined site

Deep-Sea Research II 57, 210–221 (2010)

ALEXANDRIUM MINUTUM RESTING CYST DISTRIBUTION DYNAMICS IN A CONFINED SITE

Sílvia Anglès[1], Antoni Jordi[2,3], Esther Garcés[1], Gotzon Basterretxea[3], Albert Palanques[1]

[1] Institut de Ciències del Mar (CSIC), Pg. Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

[2] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794, USA

[3] IMEDEA (UIB-CSIC), Miquel Marqués 21, 07190 Esporles, Spain

ABSTRACT

The life cycle of the toxic dinoflagellate *Alexandrium minutum* consists of an asexual stage, characterized by motile vegetative cells, and a sexual stage, a resting cyst that once formed remains dormant in the sediment. Insight into the factors that determine the distribution and abundance of resting cysts is essential to understand the dynamics of the vegetative phase. In investigations carried out between January 2005 and January 2008 in Arenys de Mar harbor (northwestern Mediterranean Sea), the spatial and temporal distribution patterns of *A. minutum* resting cysts and of the sediments were studied during different bloom stages of the vegetative population. Maximum cyst abundance was recorded mainly in the innermost part of the harbor while the lowest abundance always occurred near the harbor entrance, consistent with the distribution of silt-clay sediment fractions. The tendency of cysts in sediments to increase after bloom periods was clearly associated with new cyst formation, while cyst abundance decreased during non-bloom periods. Exceptions to this trend were observed in stations dominated by the deposition of coarse sediments. High correlation between the presence of cysts and clays during non-bloom periods indicates that cysts behave as passive sediment particles and are influenced by the same hydrodynamic processes as clays. In Arenys de Mar, the main physical forcing affecting sediment resuspension is the seiche, which was studied using *in situ* measurements and numerical models to interpret the observed distribution patterns. During non-bloom periods, cyst losses were smaller when the seiche was more active and at the station where the seiche-induced current was larger. Thus, seiche-forced resuspension appears to reduce cyst losses by reallocating cysts back to the sediment surface such that their burial in the sediment is avoided. The observed vertical profiles of the cysts were consistent with this process.

Resum

El cicle de vida de la dinoflagel·lada tòxica *Alexandrium minutum* es compon d'una fase asexual, caracteritzada per cèl·lules vegetatives mòbils, i una fase sexual, el cist de resistència, que una vegada format roman latent en el sediment. El coneixement dels factors que determinen la distribució i abundància de cists de resistència és fonamental per comprendre la dinàmica de la fase vegetativa. Es van estudiar els patrons de distribució espacial i temporal dels cists de resistència d'*A. minutum* i dels sediments durant diferents etapes de proliferació de la població vegetativa entre gener de 2005 i gener de 2008 al port d'Arenys de Mar (nord-oest del Mar Mediterrani). Les majors abundàncies de cists es van quantificar principalment en la part més interna del port, mentre que la menor abundància sempre es va produir prop de l'entrada del port, en concordança amb la distribució de les fraccions de sediments de llims i argiles. L' abundància dels cists en els sediments va augmentar després dels períodes de proliferació amb la formació de cists nous, mentre que l'abundància de cists va disminuir durant els períodes d'absència de proliferació vegetativa. Les excepcions a aquesta tendència es van trobar a les estacions dominades per deposicions de sediments gruixuts. L'alta correlació entre la presència de cists i argiles durant els períodes d'absència de proliferació indica que els cists es comporten com partícules passives de sediment i que estan influenciats pels mateixos processos hidrodinàmics que les argiles. A Arenys de Mar, el principal forçament físic que afecta a la resuspensió de sediments és la seixa, que es va estudiar mitjançant mesures in situ i models numèrics per tal d'interpretar els patrons de distribució observats. Durant els períodes d'absència de proliferació, les pèrdues de cists van ser menors quan la seixa va ser més activa i a l'estació on les corrents induïdes per la seixa van ser majors. Per tant, la resuspensió forçada per la seixa sembla reduir les pèrdues de cists mitjançant la recol·locació dels cists de nou a la superfície del sediment evitant el seu soterrament en el sediment. Els perfils verticals d'abundància de cists observats van ser coherents amb aquest procés.

Introduction

A heteromorphic life cycle alternating between motile and resting stages is a common feature of many bloom-forming phytoplankton species (e.g., dinoflagellates, cyanobacteria, raphidophyceans). Shifts between these different life stages occur in response to endogenous and/or environmental factors and determine species survival, persistence, and spreading (Steidinger and Garcés, 2006). Since planktonic and benthic phases occupy distinct ecological niches, the factors controlling cell populations during each of these phases are different. For these reasons, it is essential to interpret phytoplankton proliferations by considering not only the motile vegetative phase of the species but also its complete life cycle as well as the factors influencing each stage.

In the case of dinoflagellates, the sexual phase of the life cycle includes a benthic resting cyst that results from a planozygote formed by the fusion of gametes. Cyst formation plays numerous roles in the ecology of dinoflagellates, including beneficial effects on species dispersal, genetic recombination, seeding for bloom initiation, and survival during unfavorable conditions (Dale, 1983). A thick protective wall improves the survival of resting cysts and facilitates their sinking to the sea bottom (Montresor et al., 1998). Indeed, cysts can rest in the sediments for decades, thus providing a reservoir of potential diversity (Belmonte et al., 1997).

Information about the fluctuations of resting cysts and the physical factors that determine their distribution is fundamental to understand and predict the onset and fate of phytoplankton blooms. Analyses of the temporal abundance of resting cysts are essential for the development of conceptual models of bloom dynamics, as underlined by the fact that cyst abundance in the sediment is considered to reflect the potential for subsequent blooms (Anderson et al., 2005; Villanoy et al., 2006). Additionally, surveys of sedimentary cyst assemblages may reveal the existence of species rarely observed in the plankton (Joyce, 2005; Satta et al., 2010), thereby providing an early indication of the presence of species of interest.

Among the factors known to influence the resting phase, bottom-boundary processes, and, more generally, the forces controlling sediment dynamics are highly relevant (Wang et al., 2004). Resting cysts tend to gather in zones where fine sediments accumulate, suggesting that dormant stages behave as passive particles in sediment dynamics (Dale, 1976). Dispersion and accumulation regulate the horizontal distribution of cysts, whereas burial, bioturbation by benthic organisms, and resuspension contribute to their vertical redistribution (Anderson et al., 1982; Giangrande et al., 2002). In addition to these processes, biotic factors, such as germination, natural mortality, degradation, and grazing, also determine cyst abundances in the sediment (Persson, 2000).

Reports of dinoflagellate outbreaks following periods of enhanced vertical mixing (e.g., Usup and Azanza, 1998) suggest a close linkage between resuspension and the early stages of bloom development. Anoxic conditions and the absence of light in the sediment repress cyst germination in most dinoflagellate species (Anderson et al., 1987), whereas wind forcing, strong tidal currents, and episodes of stormy weather, i.e., processes that cause resuspension, are likely triggers of excystment (Kirn et al., 2005; Kremp, 2001; Nehring, 1996). While in the open sea cyst abundance and species dominance patterns are the result of a complex combination of production, lateral transport, resuspension, and accumulation processes (Richter et al., 2007), in confined areas, where dispersion is restricted, the links between cysts and vegetative populations or particular environmental conditions are more straightforward (Garcés et al., 2004). Furthermore, confined areas play an important role as one-stop accumulation sites for cyst-forming species, allowing them to expand geographically.

As a result of their particular morphology (straight sidewalls, shallow and regular bathymetry, elongated configurations, etc.), harbors and other semi-enclosed systems are prone to natural oscillatory motions that occur within a time frame of one to several minutes (seiching). Although a certain degree of oscillatory movement is a feature of all enclosed basins, some harbors are subject to considerable seiches (e.g., Gomis et al., 1993). Resonance at these sites generates strong currents that resuspend sediments and other particulate material from the seafloor. Two major initiation forces are generally responsible for harbor resonance excitation: strong wind pulses directed to the closed end of the harbor and long-wave energy-loading from the sea and atmosphere (Lee and Park, 1998). While seiching is known to be relevant for the biology of limnic ecosystems (Ostrovsky et al., 1996), in the ocean it is most significant in confined areas, where tidal currents are low (microtidal regimes), and thus is likely to contribute to the triggering of near-shore algal blooms.

In this study, we determined the spatial and temporal distribution patterns of *Alexandrium minutum* Halim resting cysts and the sediment characteristics in Arenys de Mar harbor. *A. minutum* is a bloom-forming species able to trigger outbreaks of paralytic shellfish poisoning (PSP). It is widely distributed in the Mediterranean Sea (Vila et al., 2005 and references therein), including Arenys de Mar harbor, where recurrent blooms of *A. minutum* occur. In a previous study, Jordi et al. (2008) demonstrated, based on observations obtained by acoustic Doppler current profiler (ADCP) and numerical simulations, that seiching was the main physical process controlling the resuspension of harbor sediments. The present study combines *in situ* measurements with numerical modeling to explore the potential effects of seiche-forced resuspension on cyst distribution to provide evidence that the seiche regulates the distribution dynamics of the dormant phase of *A. minutum*. Moreover, the conclusions are in accordance with the results from an analysis of the vertical distribution of *A. minutum* resting cysts in the sediments.

Methods

Study area

The study site was the harbor of Arenys de Mar, located on the coast of Catalonia (Spain, northwestern Mediterranean Sea, Fig. 1). The harbor is an artificial seaport measuring 0.4 km² and made up of shipyards, fishing docks, and a marina. The average depth is 4 m, ranging from 1 m in the most confined parts to 6 m at the entrance. The harbor receives both semi-continuous freshwater inflows along its walls and occasional discharges from land drainage following episodes of heavy rain. The tidal regime is microtidal, reaching a height of up to 0.25 m in spring-tide (Tsimplis et al., 1995). During the period of study, no dredging activities were carried out in the harbor. Water disturbance caused by ship traffic is very low and such activity consists mainly of leisure and small fishing boats.

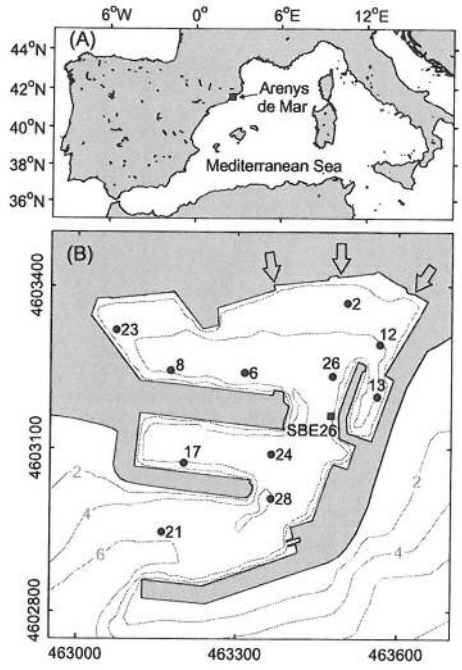


Fig. 1. (A) Location of Arenys de Mar. Latitude/Longitude coordinates in degrees. (B) Arenys de Mar harbor and the locations of the deployed instrument (square) and sampling stations (dots with station number). Arrows indicate drainage system inputs. Gray lines are isobaths (m). Coordinates are UTM (m).

Sampling strategy

Between the years 2005 and 2008, surveys were carried out in Arenys de Mar harbor in order to assess the spatial and temporal distribution patterns of *A. minutum* resting cysts and of the sediments. Surveys were conducted at different bloom conditions of the recurrent outbreaks of *A. minutum* vegetative cells that occurred in the harbor during that time. Data were obtained from 7–11 stations (Fig. 1) during five sediment surveys carried out before (C2 and C4, Fig. 2) and after (C1, C3, and C5, Fig. 2) the blooms. The exact dates of the surveys were informed by the numbers of *A. minutum* vegetative cells, obtained from the regional toxic phytoplankton monitoring program of Catalonia (station 23, Fig. 1). In addition, the abundance and spatial distribution of *A. minutum* vegetative cells during bloom development were determined by a survey performed at 14 stations on March 2006 (V, Fig. 2). Finally, to ascertain the vertical distribution of *A. minutum* resting cysts in sediments, a survey was carried out at three stations (23, 13, and 28, Fig. 1) prior to bloom development (C6, Fig. 2).

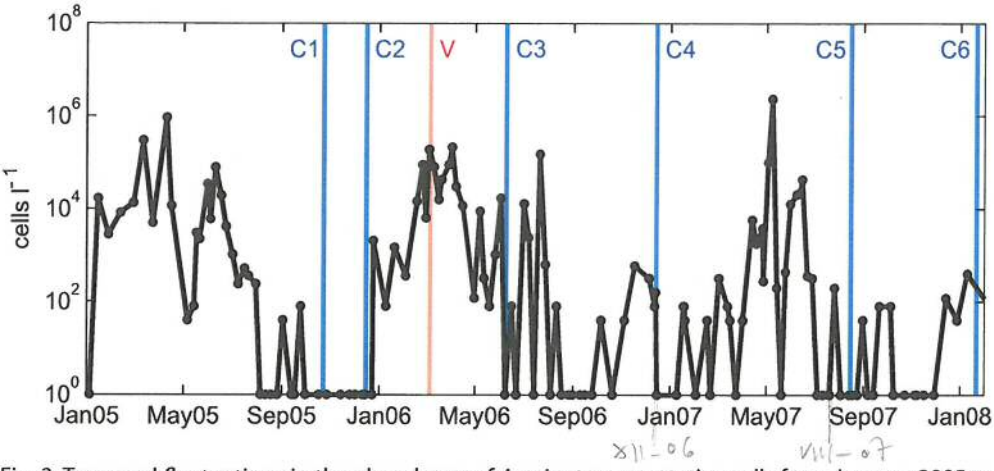


Fig. 2. Temporal fluctuations in the abundance of *A. minutum* vegetative cells from January 2005 to January 2008 (dots). Bars indicate surveys of resting cysts and sediments (C1–C5), vertical distribution of cysts (C6), and vegetative cells distribution during bloom development (V).

Sample collection, processing, and analysis

Phytoplankton samples to be evaluated in the monitoring program and the spatial survey were collected at the water surface at the same time of day (12:00 GMT) and fixed immediately with Lugol's solution. Vegetative cells were quantified by sedimentation of 50-ml subsamples in settling chambers for 24 h followed by counting of an appropriate area, depending on the density (Andersen and Throndsen, 2003) under an inverted microscope (Leica DMIRB bright-field and epifluorescence microscope). *A. minutum* cells were identified by staining with Calcofluor-White solution (Fritz and Triemer, 1985).

For sediment surveys, two samples per station were collected by a scuba diver, who inserted plastic cylindrical corers (20 cm long × 5 cm base diameter) into the sediments. For resting cyst analysis, sediment cores were stored in the dark at 10°C and left to settle for at least 24 h. All of the cores from each survey were processed within one month. The water above the core was carefully removed and the sediment cut into 1-cm slices. A subsample of the top 0–1 cm (temporal and spatial distributions) and one from each 1-cm slice of the 0–5 cm layer (vertical distribution) were sonicated, sieved to retain the 10- to 100-µm fraction, and processed by the sodium polytungstate density gradient method of Bolch (1997), as modified by Amorim et al. (2001) and Bravo et al. (2006). The resulting sample was rinsed in a 10-µm sieve and collected with 10 ml of filtered seawater. *A. minutum* resting cysts were counted in 2-ml Utermöhl sedimentation chambers under an inverted microscope (see above specifications). Empty cysts were not considered, and abundance was expressed as cysts ml^{-1} of wet sediment (ws). The cysts were almost hemispherical in shape when viewed directly and kidney shaped in lateral view, in accordance with the descriptions of Garcés et al. (2004) and Bravo et al. (2006). Further morphological details of *A. minutum* cysts identified in the harbor sediments can be found in Satta et al. (this issue).

Sediment characteristics were analyzed by measuring the grain size of the <50-μm fraction using a Sedigraph 5000D and of the >50-μm fraction using a settling tube, according to the method of Giró and Maldonado (1985). Granulometic distributions of both fractions were combined and total grain-size distribution, and textural statistical parameters for each sediment sample were calculated. Carbon was measured in duplicate using a Leco CN 2000 analyzer. Two subsamples were used to determine the total carbon percentage after combustion at 1050 °C. Another two subsamples were digested with HCl in a LECO CC 100 digester; the resulting CO_2 was measured using the same LECO CN 2000 analyzer and assigned to inorganic carbon content. The difference between the two values is the percentage of organic carbon (OC)Spatial objective analysis

Spatial maps of vegetative cells, resting cysts, and granulometry were interpolated using spatial objective analysis for each survey and an independent data set (Pedder, 1993). Since the presence of nearby sampling points separated by piers may have biased the analysis, the position of the coastline was taken into account in the interpolation in order to constrain the statistical error by computing the covariance of the marine domain only. This prevented propagation of information across the coast.

Seiche characterization

To determine the characteristics of the seiche in the Arenys de Mar harbor, an SBE26 sea-level recorder was deployed at the same location for two periods (September 22, 2005—July 26, 2006, and January 19—May 23, 2007), as shown in Figure 1. The original pressure–time series, recorded every 30 s, was adjusted to sea level with atmospheric pressure, measured at a nearby station (scale factor 1 cm mb^{-1}). Point measurements were compared and extended to the entire harbor domain using a three-dimensional finite-element model (FUNDY) based on the linear shallow-water equations. The algorithm uses conventional hydrostatic and Boussinesq approximations and Mellor-Yamada level 2.5 eddy viscosity closure. Details of the model are given in Lynch and Werner (1987) and Lynch et al. (1992). The computational domain extended from the harbor to the inner shelf. The mesh contained 4249 elements and 2428 nodes in the horizontal direction, with 11 one-dimensional linear elements connected under each horizontal node following a sigma coordinate system. The model was forced by a barotropic wave of 1-cm amplitude at the model boundaries, which lay far from the harbor and hence were unlikely to influence its response. Solutions were obtained in the frequency domain. Different simulations with wave periods ranging from 1 min to 5 h were performed. The relative amplification of waves arriving from the shelf inside the harbor was evaluated by dividing the amplitude of the sea-level response measured at the location of the sea-level recorder by the amplitude of the wave at the ocean boundaries.

Potential influence of the seiche and analysis of the vertical distribution of A. minutum cysts

To study the potential influence of the seiche on the vertical distribution of *A. minutum* resting cysts in Arenys de Mar harbor, a coupled hydrodynamic sediment-cyst one-dimensional vertical model was used to investigate the effect of resuspension events on the sediments and on resting cysts. The model was described in detail in Jordi et al. (2008) and included the simulation of two sediment classes, sands and fine sediments (the sum of silts and clays). Here, we introduced a new sediment class comprising sediments with a diameter of 20 μm and a density of 1250 kg m^{-3} (Anderson et al., 1985), thus representing *A. minutum* resting cysts. Biological processes, such as germination, mortality, and predation, were not simulated and resting cysts were treated as passive particles.

In addition, the vertical profiles of resting cysts were examined at three stations, each of which reflected different seiche effects. If we assume that seiche-induced mixing can be described as a diffusive process, then the vertical profiles obtained can be compared to the theoretical distribution as stated by the advection-diffusion equation (Guinasso and Schink, 1975):

$$\frac{\partial \rho C}{\partial t} = \frac{\partial}{\partial z}\left[D_b \frac{\partial \rho C}{\partial z} \right] - S \frac{\partial \rho C}{\partial z} \qquad (1)$$

where C is the cyst concentration (cyst cm^{-3} ws), ρ the sediment density (g dry sediment cm^{-3} ws), t the time (s), z the depth (cm), D_b the sediment mixing rate ($cm^2\ s^{-1}$), and S the sedimentation rate (cm s^{-1}).

In non-bloom periods, i.e., in the absence of cyst deposition, a steady state can be assumed for cysts in the sediment. If we also assume that the seiche-forced resuspension causes constant mixing in the sediment mixed layer (or in the depth scale of interest, 5 cm), the steady state solution of Eq. (1) becomes:

$$C = C_0 \exp\left(\frac{S}{D_b} z \right) \qquad (2)$$

where C_0 is the cyst concentration at the sediment surface.

However, after the bloom cysts are deposited in the sediment, upsetting the steady state condition assumed in Eq. (2). If the arrival of a cyst at the sediment surface is considered as a pulse input caused by the bloom, then the solution of Eq. (1) is (Crank, 1975):

$$C = C_0 \exp\left(-\frac{z^2}{4 D_b t} \right) \qquad (3)$$

assuming that sedimentation was negligible (S=0) over the time interval (t) of interest. Therefore, differences in the slope of the exponential decrease in the number of cysts [the term between parentheses in Eq. (3)] can be explained by the sediment mixing rate (D_b).

Results

Temporal abundances and spatial distribution of A. minutum vegetative cells

From January 2005 to January 2008, *A. minutum* blooms (up to 2 × 10^6 cells l^{-1}) were recurrently observed at Arenys de Mar harbor, generally over a period spanning late winter to early summer (Fig. 2). The spatial distribution of *A. minutum* vegetative cells during the maintenance phase of the 2006 bloom (V) is shown in Figure 3. Higher cell abundances were recorded at stations located in the innermost part of the harbor (1 × 10^5cells l^{-1} at station 23), whereas the lowest abundances were measured at stations on the eastern side and towards the harbor entrance.

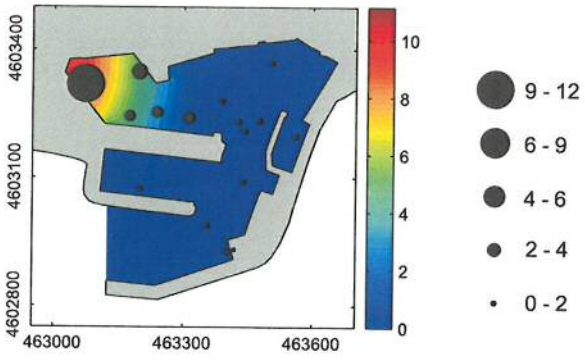


Fig. 3. Abundance of *A. minutum* vegetative cells (10^4 cells l^{-1}) in Arenys de Mar harbor during the maintenance bloom phase (2006). Data are presented as dots, with size related to cell abundance, and in colored maps from the interpolation of data by spatial objective analysis. Coordinates are UTM (m).

Temporal and spatial distribution patterns of A. minutum resting cysts

Abundances of *A. minutum* resting cysts during the period investigated ranged from 0 cysts ml^{-1} ws (C1, station 28) to 1010 cysts ml^{-1} ws (C5, station 23) (Fig. 4). Maximum values were always measured at the innermost part of the harbor (station 23). In some cases (C3 and C5), cysts also accumulated in the confined eastern basin (station 13). Cyst abundance was always lowest near the harbor entrance (stations 21 and 28). While the spatial distribution pattern of *A. minutum* resting cysts was fairly uniform throughout the different surveys,

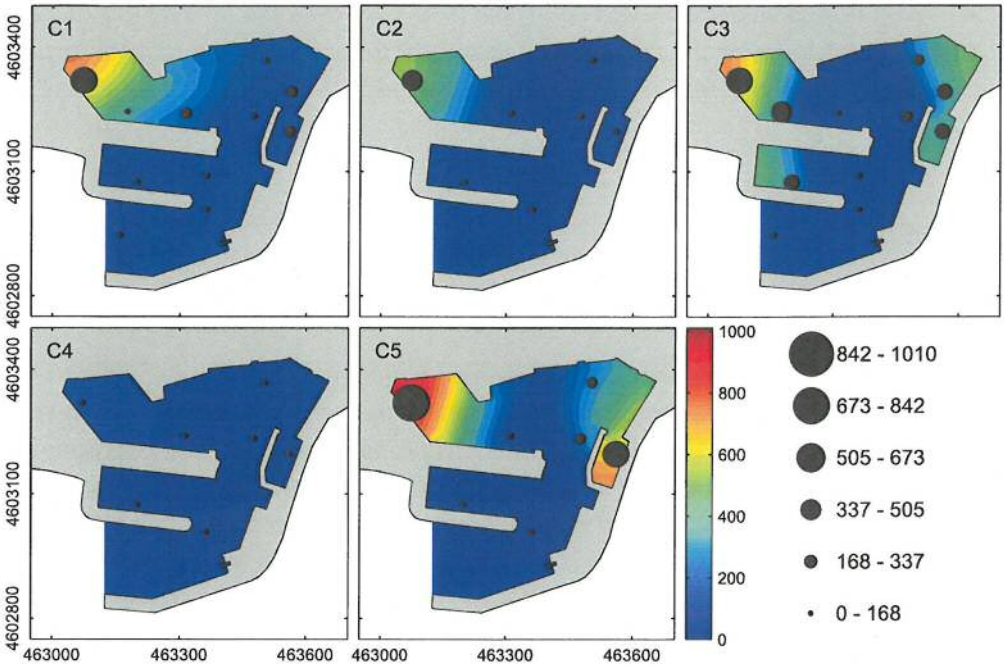


Fig. 4. Distribution and abundance of *A. minutum* resting cysts (cysts ml^{-1} ws) in Arenys de Mar harbor during surveys performed from 2005 to 2007. Data are presented as in Fig. 3. Coordinates are UTM (m).

there were large temporal variations in their abundance. A significant decreasing tendency in cyst abundance paralleled the absence of *A. minutum* blooms in the water column, while higher values were noted after the bloom, as a result of cyst production.

To evaluate gains and losses of resting cysts between bloom and non-bloom periods, the difference in total resting cyst abundances per station, as measured during two consecutive surveys, was calculated. The net balance for each period was expressed as the percent increase (positive numbers) or decrease (negative numbers) in resting cyst abundance during a given survey compared to the previous one (Table 1). The results showed a general trend in the variations of sedimented resting cysts in the harbor, with increases in cyst abundance after bloom periods and significant decreases after non-bloom periods. Exceptions occurred at some stations: an increase in resting cysts rather than a decrease was recorded at stations 17, 26, and 28 for the non-bloom period C1–C2, and a decrease in resting cysts was observed at station 6 during the post-bloom period C2–C3. However, since the resting cyst abundance measured at these stations is very low, these increases are not very meaningful. In that case, if these exceptions are excluded, cyst losses at the stations were more homogeneous throughout non-bloom periods than cyst gains occurring after blooms. The net balance of the post-bloom periods differed, with a higher increase during the C4–C5 period, whereas the extent of the decrease was greater for the non-bloom period of C3–C4.

Station	C1-C2 (non-bloom)	C2-C3 (bloom)	C3-C4 (non-bloom)	C4-C5 (bloom)
2	-56	348	-89	743
6	-58	-15	-9	41
13	-52	293	-84	1115
17	901	561	-94	347
23	-26	39	-80	525
26	982	86	-88	739
28	24	454	-59	99

Table 1. Net balance of gains and losses of resting cysts between non-bloom and bloom periods, expressed as the percent increase (positive numbers) or decrease (negative numbers) in resting cyst abundances during a given survey compared to the previous one. Only the seven stations common to all the resting cyst surveys are considered.

Temporal and spatial sediment patterns

The seafloor at Arenys de Mar is composed of fine-grained sediments with an average particle size of 14.6±12 μm (fine silt). Figures 5–7 show the spatial and temporal changes

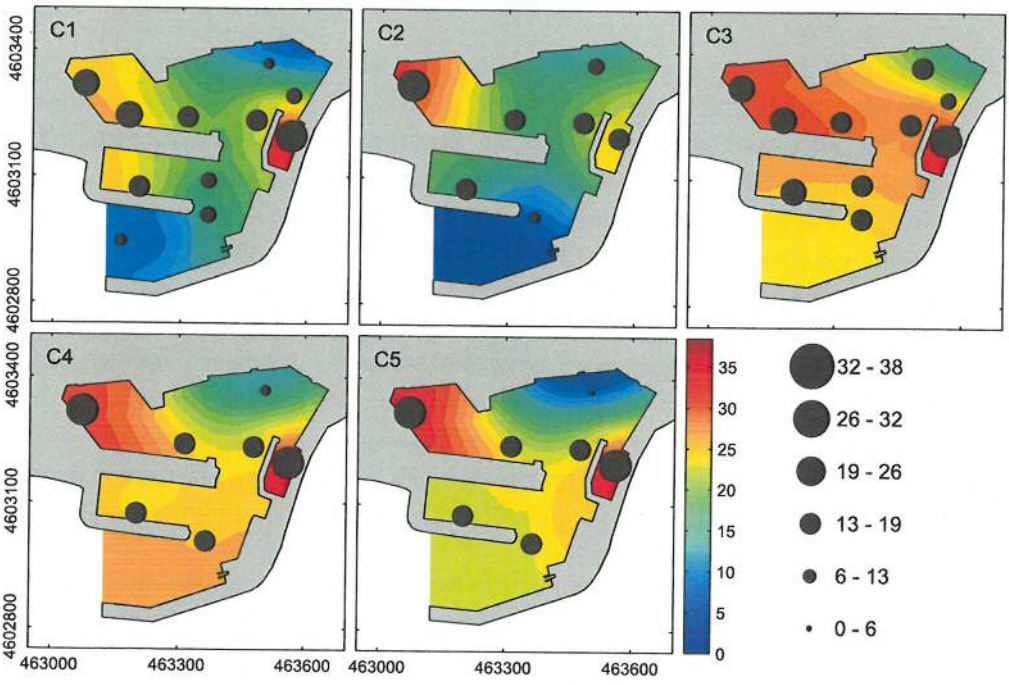


Fig. 5. Distribution of the clay fraction (%) in Arenys de Mar harbor during surveys performed from 2005 to 2007. Data are presented as in Fig. 3. Coordinates are UTM (m).

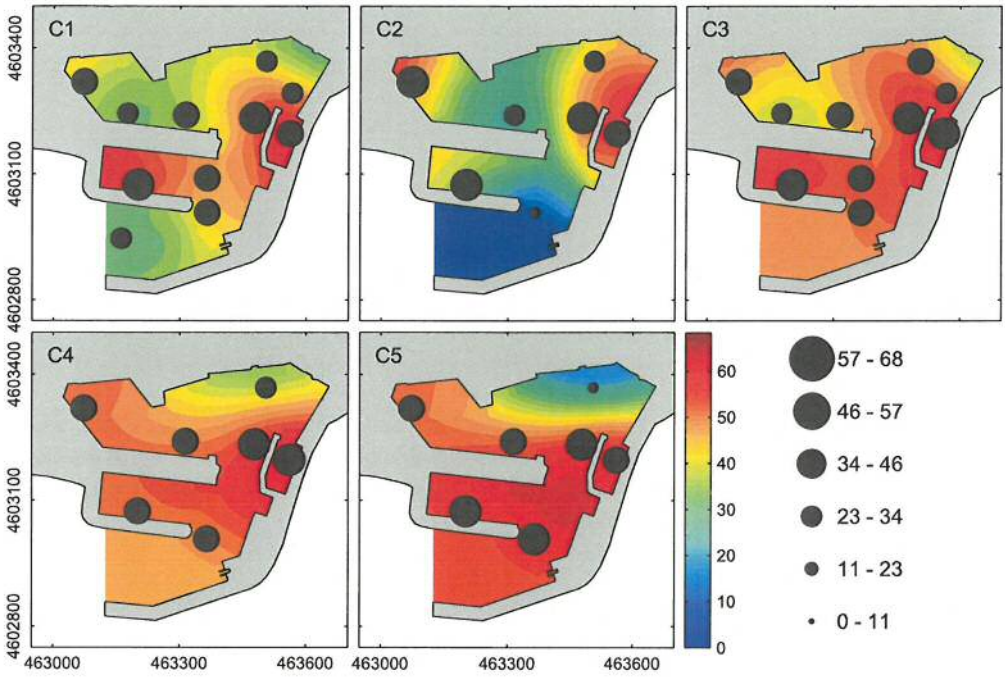


Fig. 6. Distribution of the silt fraction (%) in Arenys de Mar harbor during surveys performed from 2005 to 2007. Data are presented as in Fig. 3. Coordinates are UTM (m).

in the harbor's sediment fractions of clays, silts, and sands. Fine-sediment fractions (clay and silt) were the most abundant (average of 23% and 50%, respectively). A maximum of fine-sediment fractions frequently occurred in the inner basins of the harbor (stations 13, 17, and 23). Sands were less abundant than fine fractions, although there was noticeable variability, probably related to sediment inputs from external sources. As shown in Figure 7, these inputs affected the harbor entrance (C1 and C2), the northeast corner (station 2) where the drainage system ends (C1, C4, and C5), and the northern side of the main dock (station 6) where construction materials had on occasion been deposited (C1 and C2). The gravel-containing fraction accounted for only 0–3% of the sediments, with the highest values recorded at the harbor entrance (stations 21 and 28) and the northern side of the main dock (station 6). Therefore, these areas are prone to coarse sediment deposition. The OC content of the sediment ranged from 0.9% to 3%. OC content was higher in the inner basins of the harbor and had the same distribution pattern as fine-sediment fractions.

Correlations between resting cyst abundances and sediment fraction percentages showed that the highest correlation was between cyst concentrations and the percentage of clay (R^2=0.43), considering all data sets. A higher correlation coefficient (R^2=0.89) was obtained when this correlation was evaluated by considering only the data from pre-bloom surveys (C2 and C4).

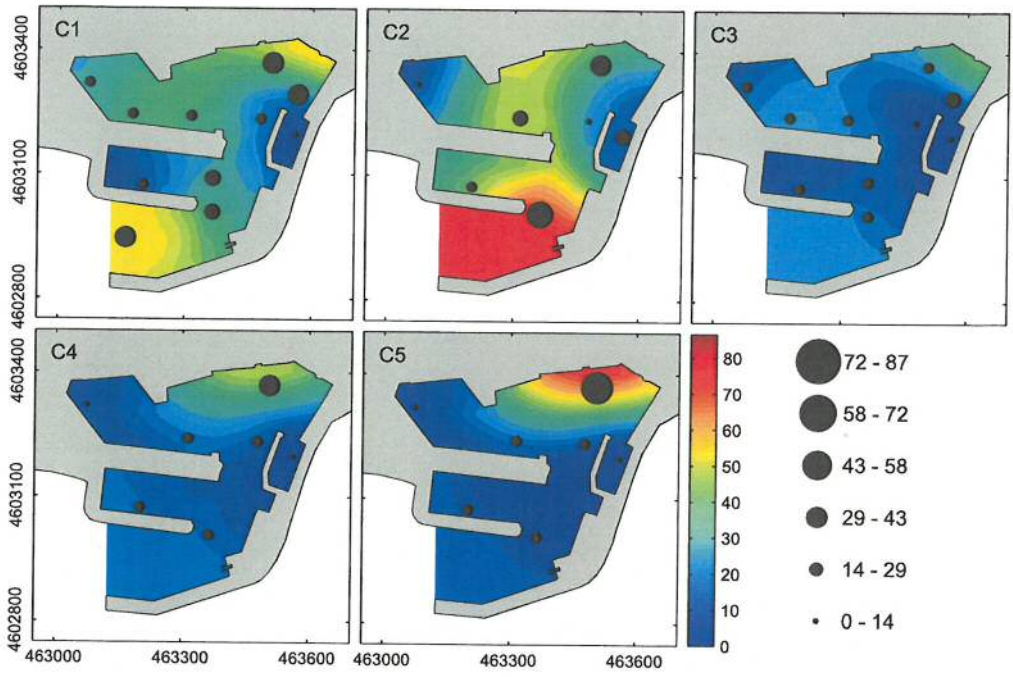


Fig 7. Distribution of the sand fraction (%) in Arenys de Mar harbor during surveys performed from 2005 to 2007. Data are presented as in Fig. 3. Coordinates are UTM (m).

Seiche characteristics

Sea-level spectra (Fig. 8A) for the two periods when the SBE26 sea-level recorder was deployed were estimated using a Hamming window of 512 points, with a half-window overlap (Emery and Thomson, 1997). The computed spectra for the two periods were very similar, although those for 2005–2006 were slightly more energetic, suggesting a more active seiche in that period than in 2007. The main feature of the spectra was a very energetic peak, with a period of 12.8 min, related to the fundamental (Helmholtz) mode of Arenys de Mar harbor. Two lower spectral peaks were also observed, at wave periods of 3.9 min and 2.7 min. The sea-level amplification for the harbor, computed at the sea-level recorder location using the numerical model, is shown in Figure 8B. The agreement in the position of the peaks between the measured spectra and the computed amplification is very reasonable, indicating that the model properly simulated the harbor response.

The spatial distribution of the fundamental resonant mode (12.8 min) obtained from the numerical analysis revealed large sea-level amplification in the inner part of the harbor and one node at the entrance (Fig. 9A), consistent with the theory of natural resonance phenomena. The spatial distribution of the second peak (3.9 min) had two nodes, one at

the entrance and the other at a distance of approximately two-thirds of the harbor's length, as measured from the entrance (Fig. 9B). These corresponded to the first mode of Arenys de Mar harbor whereas the lower spectral peak (2.7 min) reflected a more complicated spatial distribution and corresponded to the second mode (not shown). Similar to the sea-level amplification, currents associated with the harbor response differed significantly from site to site according to their position relative to the oscillation nodes. Jordi et al. (2008) analyzed seiche-driven current variability and associated resuspension events at a location near the SBE26. Here, we used the numerical model to extend their results to our sampling stations.

Figure 10 shows the current amplification at stations 23, 13, and 28, where the vertical distribution of cysts was analyzed, and at station SBE26 for a comparison with the results of Jordi et al. (2008). In the fundamental mode, the currents were highly amplified at the harbor entrance (station 28), whereas those in the first mode

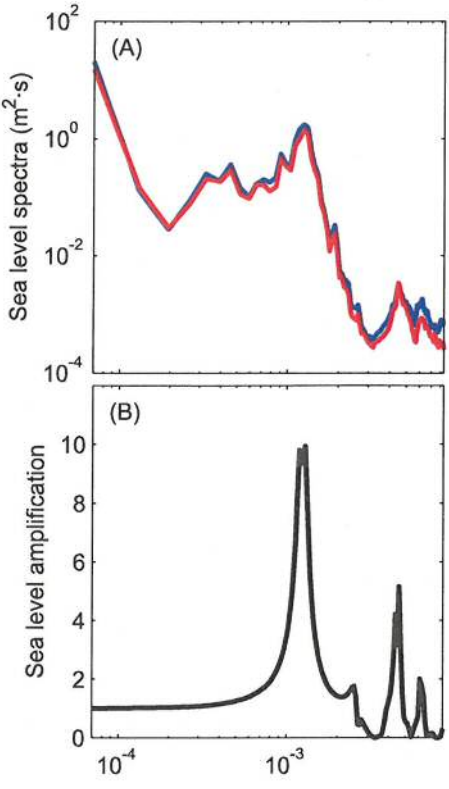


Fig. 8. (A) Sea-level spectral density at the SBE26 location. (B) Sea-level amplification computed with the numerical model at the SBE26 location.

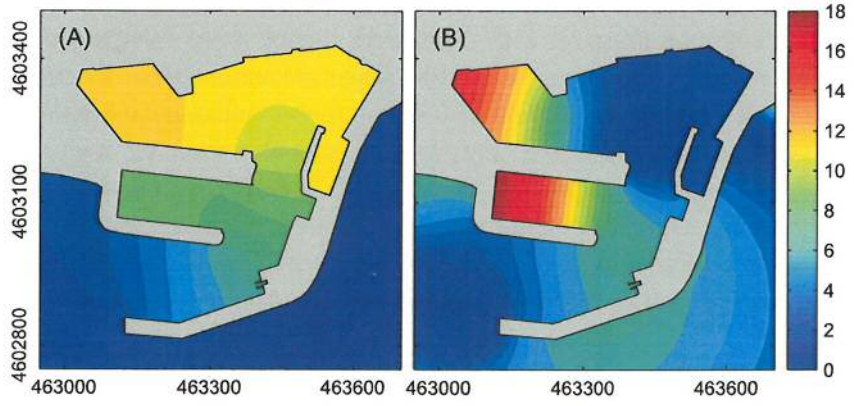


Fig. 9. Spatial distribution of the harbor response (relative sea-level amplification) for (A) the fundamental mode, and (B) the first mode. Coordinates are UTM (m).

were significantly larger in the inner basin (station 23). The currents at SBE26 were strong enough to resuspend the sediment (Jordi et al., 2008); accordingly, those at stations 28 and 23 must be able to do so as well. In contrast, the seiche had little effect on currents inside the confined eastern basin (station 13).

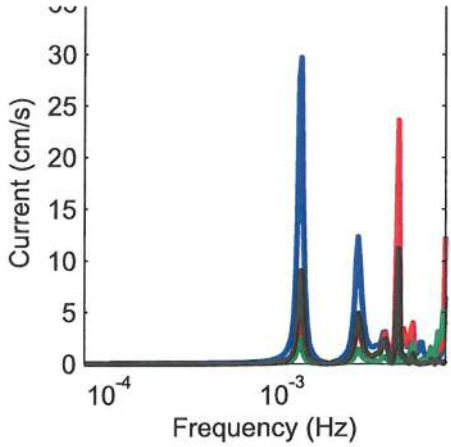


Fig. 10. Currents induced by waves of different frequencies at stations 23 (red), 13 (green), and 28 (blue) and at station SBE26 (black).

Influence of the seiche on the distribution of A. minutum resting cysts

Since clays are the sediment fraction with the highest correlation to resting cysts, only the resuspension of these two classes of particles was analyzed in the one-dimensional vertical model simulations. Figure 11 shows the simulated resuspension in response to a seiche event 10 cm in amplitude and 6 h in duration, i.e., the usual conditions of a strong event in the Arenys de Mar harbor. Resting cysts undergo a higher degree of resuspension than clays due to their lower density. In addition, they remain resuspended in the water column for a longer (about eight times) period of

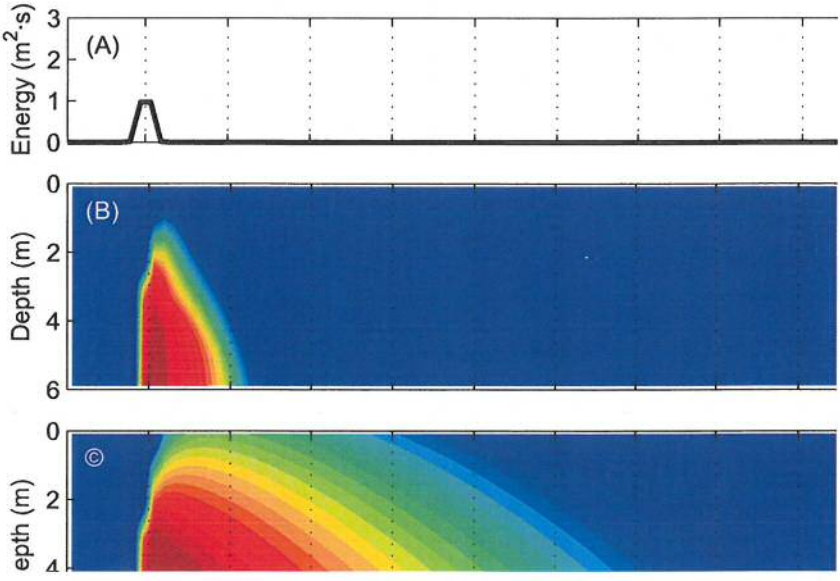


Fig. 11. (A) Energy of a seiche event 10 cm in amplitude and 6 h in duration. (B) Simulation of the seiche-forced resuspension of clays. (C) Simulation of the seiche forced resuspension of resting cysts.

time and are deposited in the surface sediment later than clays. This implies sediment mixing and reallocation of the cysts to the sediment surface.

The vertical distributions of *A. minutum* resting cysts at the selected stations are shown in Figure 12. Surface maxima as well as lower abundances below the surface were recorded at stations 23 and 13. The surface abundances at the two stations were similar, but while there was a progressive decrease with depth at station 23, the abundances remained relatively homogeneous at station 13, where a subsurface peak was detected at a depth of 4 cm. Low, homogeneously distributed cyst abundances were measured throughout the profile at station 28. Since the vertical profiles were obtained prior to development of the bloom, a steady state was assumed and the profiles could be explained by Eq. (2). To test the validity of this equation, the cyst profiles were subjected to an exponential by least-squares, as shown in Figure 12. The highest correlation was found at station 23, and the lowest at station 28.

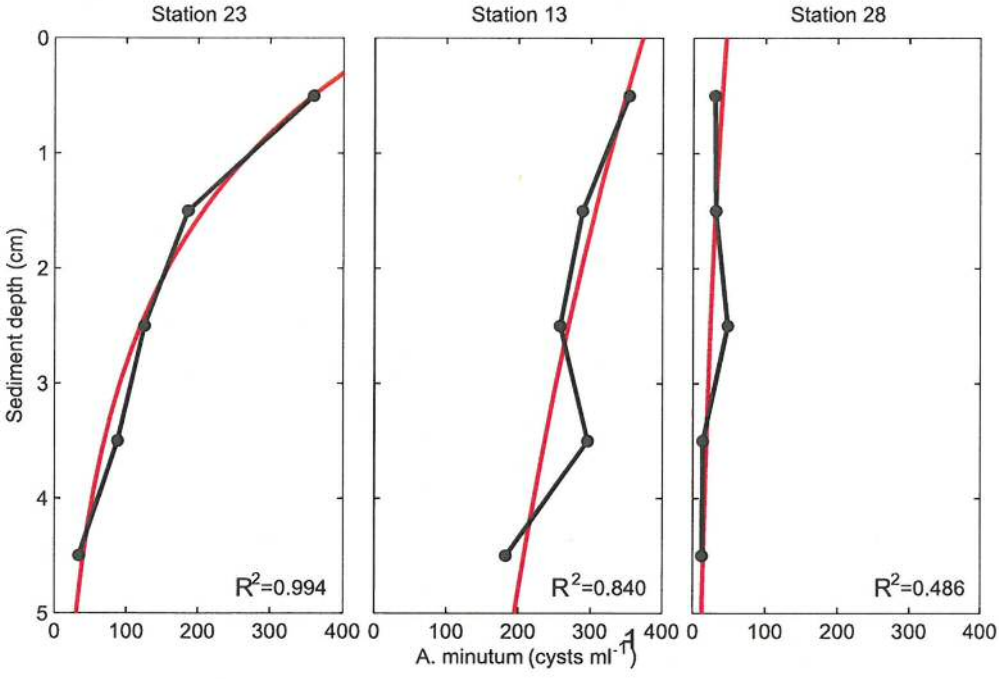


Fig. 12. Vertical profiles of the abundances of *A. minutum* resting cysts (dotted line) and the exponential fit (solid line) at three stations representative of the different seiche effects.

Discussion

The present study evaluated the fate of *A. minutum* resting cysts in the sedimentary regime over three bloom cycles. The spatial and temporal distributions of the resting cysts and of the sediments in the Arenys de Mar harbor were investigated throughout different stages of the vegetative cells bloom. A numerical model was used to assess the distribution of seiche motion and associated currents in the harbor. The following discussion analyzes the distribution dynamics of resting cysts with respect to physical forcing in Arenys de Mar harbor, particularly seiche motion. Jordi et al. (2008) identified seiching as the physical forcing that controls sediment resuspension in the harbor, whereas neither tides, nor wind-driven currents, nor waves are strong enough to resuspend the bottom sediment. They also reported sporadic events of sediment advection from outside waters and freshwater discharges from land-based sources.

Changes in the abundance of cysts present in surface sediments are the net result of biological and physical processes acting on the seedbank (Anderson et al., 1982). Biological processes include benthic animal activity and species-specific characteristics, such as cyst deposition, germination, and mortality, while physical processes include mixing, sedimentation, and resuspension (Keafer et al., 1992). Our data suggest that, immediately after the bloom of vegetative cells, the distribution and abundance of *A. minutum* resting cysts depend mainly on the biological process of cyst formation and cyst deposition. First, the correlation between resting cyst abundances and clay sediment fraction percentage significantly improved (from R^2=0.43 to R^2=0.89) when values of post-bloom surveys were excluded, indicating that cysts are not influenced by processes related to the sediment dynamics at this time of the bloom. Correlations between cysts and sediments need to be interpreted taking into account the period of bloom and the recent cyst deposition. Second, variations recorded over the monitored period showed that resting cyst abundance in the sediment increased substantially after the bloom, suggesting that the horizontal distribution of resting cysts and their respective abundance are a reflection of cyst production. As spatial variations in deposition are the more likely cause of interannual variations in cyst abundance (Anderson et al., 2005), the variability in the increases in cyst abundance after bloom periods was probably due to heterogeneous cyst deposition, determined in large part by the spatial distribution of vegetative cells during cyst production. Indeed, in the post-bloom survey in 2006, resting cyst abundances were highest under the overlying vegetative cell maximum recorded during bloom development (Fig. 3), as Garcés et al. (2004) observed in a cyst survey performed after the bloom in 2002. The differences in total resting cyst abundance observed between the two bloom periods investigated here provide additional evidence for the importance of the biological process of cyst formation. These variations could be related to an unequal efficiency in cyst production during the two bloom periods. Further studies should be carried out to test this hypothIn non-bloom periods, the pool of resting cysts decreases due to germination, mortality, and predation, together with the lack of deposition of new

cysts in the absence of vegetative cells in the water column. The homogeneous decreases measured at nearly all stations indicated that similar losses occurred throughout the harbor. Furthermore, the high correlation between cysts and clays during non-bloom periods confirmed that resting cysts behave as passive particles. Consequently, they are subject to resuspension and thus can be transported by water currents, which favors their dispersal to new areas. In Arenys de Mar harbor, oscillatory currents associated with the seiche are able to resuspend sediments and cysts (Fig. 11), but they transport sediments over rather short distances (Jordi et al., 2008). Other processes, such as wind-driven currents and sediment advection, are likely to be more effective in redistributing suspended sediments and cysts over longer distances. Van Lenning et al. (2007) showed that wind-driven currents favored the accumulation of *A. minutum* vegetative cells in the northeastern region of the harbor during the bloom in 2003, and it is expected that similar currents transport suspended cysts. Sediment advection, although a less-frequent occurrence (Jordi et al., 2008), is also likely to redistribute cysts in the harbor. In fact, exceptions to the general trend of cyst increases and decreases were noted in areas affected by the deposition of coarse sediments, which, in turn, is linked to sediment inputs entering the harbor system. Cysts deposited in the seabed may eventually mix with sediments, which may at least in part explain the observed decreases in cyst abundance. Cyst redistribution by benthic animals is not significant in Arenys de Mar (Garcés et al., 2004); instead, sediment mixing processes are most probably driven by seiche-forced resuspension. Sporadic sediment inputs from external sources can cover sedimented cysts, which are then reallocated to the sediment surface by seiche-forced resuspension, thus tending to reduce decreases in abundance. This is supported by the fact that cyst losses were greater in 2007 (C3–C4 period), when the seiche was less active, than in 2006 (C1–C2 period). However, the time span between the C3 and C4 surveys was longer than that between C1 and C2, which would also account for the greater losses.

In this context, stations 23 and 13 were chosen to study the effects of seiche on the vertical profiles of cyst abundance because they are less influenced by sediment inputs entering the harbor, in contrast to station 28, where there are sand inputs. At the sediment surface, cyst decreases during non-bloom periods were found to be smaller at station 23, where the seiche-induced currents were larger than those at station 13. As is the case for spatial and temporal patterns of cyst abundance, the vertical profiles represent the net balance of gains and losses of surface and subsurface cyst concentrations. Results of the least-squares analysis of *A. minutum* resting cyst profiles (Fig. 12), carried out to test the validity of Eq. (2), showed that the correlation between the exponential fit and the measured cyst abundance was highest at station 23, where the seiche-induced currents are relatively large and coarse sediments are few. At station 13, however, the seiche-induced currents are very low such that the depth of the mixed layer is likely reduced to less than 5 cm, which explains the poor correlation obtained at this station. In addition, sediment deposition can redistribute cysts to depths below the mixed layer. Considering station 28, although the seiche-induced currents at this station are large and should have resulted in strong

mixing, the correlation between the measured cyst abundance and the exponential fit was low due to the deposition of sands, which may cover sedimented cysts and therefore inhibit their resuspension and mixing. Consequently, the homogeneous profile was similar to that recorded at station 13. Similar vertical distributions of cysts were reported by Giannakourou et al. (2005), who found that significant resuspension contributed to the mixing of the upper sediment layers, resulting in more homogeneous cyst profiles and less-pronounced subsurface peaks. After resuspension, homogeneous profiles were more evident at stations with higher sand-fraction percentages, and the presence of subsurface peaks was related to stations where the rate of sediment deposition is high.

With respect to post-bloom periods, Garcés et al. (2004) studied the vertical distribution of *A. minutum* resting cysts in the sediment of Arenys de Mar harbor after the bloom in 2002. Their profiles showed a peak at the surface that decreased notably with sediment depth, similar to the shape predicted by Eq. (2). However, since the cyst profiles were taken after the bloom, they are instead described by Eq. (3), in which differences in the slope of the exponential decrease in cyst number are a result of the sediment mixing rate (D_b). If Eq. (3) is fitted by least squares to the cyst profiles of Garcés et al. (2004), lower slope terms (larger mixing) are obtained at their stations 1, 2, 3, and 4, which were geographically closer to stations 21, 26, 17, and 8 of this study, respectively. By contrast, the slope values were larger (lower mixing) at stations 5, 6, 7, and 8 of their study (closer to our stations 13, 12, and 2). This finding of larger and lower mixing coincides with those areas characterized by larger and lower seiche-induced currents and supports the conclusion that seiching is the main factor controlling the vertical distribution of cysts in Arenys de Mar harbor.

Resuspension not only reallocates resting cysts back to the sediment surface, but also transports resting cysts to the water column, where conditions may be more favorable for germination and growth (Kirn et al., 2005). Vertical simulations of the resuspension of resting cysts and clays showed that dormant stages remain suspended in the water column about eight times longer than sediments. This would expose the cysts to temperature, light, and oxygen conditions that better promote germination compared to the conditions at the sediment surface. In addition, the suspension of cysts may significantly reduce the bloom initiation time compared to germination occurring exclusively from the sediment surface (Nehring, 1996). Furthermore, another effect of resuspension is the release of substances such as nutrients to the water, which stimulates phytoplankton growth (Rengefors et al., 2004). Hence, resuspension not only plays a key role in the distribution of resting cysts, but also could favor the germination of resting cysts and the growth of vegetative cells, giving rise to bloom development.

Conclusions

The spatial and temporal distribution patterns of *A. minutum* resting cysts in Arenys de Mar harbor together with variations in cyst abundance suggest that the abundance of resting cysts after bloom periods mainly depends on the biological process of cyst formation (spatial distribution of vegetative cells and magnitude of resting cyst production). Once deposited, cysts mix with the sediments, where they are influenced by the same hydrodynamic factors that govern the sediments. Seiche, the physical process that controls sediment resuspension in the harbor, also regulates the distribution dynamics of the dormant phase of *A. minutum*. Decreases in cyst abundances during non-bloom periods, in the absence of cyst production, are determined mostly by seiche-forced resuspension and the sporadic events of coarse sediment deposition. Resuspension, as suggested by the vertical profiles of the cysts, tends to reduce cyst losses by reallocating buried cysts to the sediment surface. Seiche-forced resuspension is therefore an important mechanism regulating the distribution and abundance of resting cysts in microtidal or semi-enclosed systems such as harbors or lakes. This may also be the case in more open areas, where resuspension arises from other major physical forcing, i.e., tides, currents, and/or waves. As resuspension could favor the germination of resting cysts and the growth of vegetative cells, further investigation should be addressed to elucidate its possible role in triggering phytoplankton blooms.

Acknowledgements

The authors thank N. Sampedro and A. Reñé for data from the toxic phytoplankton monitoring program, and S. de Diago and N. Maestro for the sediment analysis. B. Casas, K. Van Lenning and X. Novell provided valuable help with the fieldwork. We also are grateful to Creu Roja, Club Nàutic and Cofradia de Pescadors St Telm of Arenys de Mar harbor. This study was financed by the EC-funded Research Project SEED (GOCE-CT-2005-003875). The work of A. Jordi and E. Garcés was supported by a postdoctoral grant and a Ramon y Cajal award, respectively, both from the Spanish Ministry of Science and Innovation.

References

Amorim, A., Dale, B., Godinho, R., Brotas, V., 2001. *Gymnodinium catenatum*-like cysts (Dinophyceae) in recent sediments from the coast of Portugal. Phycologia 40, 572-582.

Andersen, P., Throndsen, J., 2003. Estimating cell numbers. In: Hallegraeff, G.M., Anderson, D.M., Cembella, D.A. (Eds.), Manual on harmful marine microalgae. UNESCO Publishing, Paris, pp. 99-129.

Anderson, D.M., Aubrey, D.G., Tyler, M.A., Coats, D.W., 1982. Vertical and horizontal distributions of dinoflagellate cysts in sediments. Limnology and Oceanography 27, 757-765.

Anderson, D.M., Taylor, C.D., Armbrust, E.V., 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. Limnology and Oceanography 32, 340-351.

Anderson, D.M., Stock, C.A., Keafer, B.A., Nelson, A.B., Thompson, B., McGillicuddy, D.J., Keller, M., Matrai, P.A., Martin, J., 2005. *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep-Sea Research Part II-Topical Studies in Oceanography 52, 2522-2542.

Belmonte, G., Miglietta, A., Rubino, F., Boero, F., 1997. Morphological convergence of resting stages of planktonic organisms: a review. Hydrobiologia 355, 159-165.

Bolch, C.J.S., 1997. The use of sodium polytungstate for the separation and concentration of living dinoflagellate cysts from marine sediments. Phycologia 36, 472-478.

Bravo, I., Garcés, E., Diogene, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. European Journal of Phycology 41, 293-302.

Crank, J., 1975. The Mathematics of Diffusion. Oxford University Press, Oxford.

Dale, B., 1976. Cyst formation, sedimentation, and preservation: Factors affecting dinoflagellate assemblages in recent sediments from Trondheims Fjord, Norway. Review of Palaeobotany and Palynology 22, 39-60.

Dale, B., 1983. Dinoflagellate resting cysts: "benthic plankton". In: Fryxell, G.A. (Ed.), Survival Strategies of the Algae. Cambridge Univ. Press, pp. 69-136.

Emery, W.J., Thomson, R.E., 1997. Data Analysis Methods in Physical Oceanography. Pergamon, New York.

Fritz, L., Triemer, R.E., 1985. A rapid simple technique utilizing Calcofluor white M2R for the visualization of dinoflagellate thecal plates. Journal of Phycology 21, 662-664.

Garcés, E., Bravo, I., Vila, M., Figueroa, R.I., Masó, M., Sampedro, N., 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean). Journal of Plankton Research 26, 637-645.

Giangrande, A., Montresor, M., Cavallo, A., Licciano, M., 2002. Influence of *Naineris laevigata* (Polychaeta: Orbiniidae) on vertical grain size distribution, and dinoflagellate resting stages in the sediment. Journal of Sea Research 47, 97-108.

Giannakourou, A., Orlova, T.Y., Assimakopoulou, G., Pagou, K., 2005. Dinoflagellate cysts in recent marine sediments from Thermaikos Gulf, Greece: Effects of resuspension events on vertical cyst distribution. Continental Shelf Research 25, 2585-2596.

Giró, S., Maldonado, A., 1985. Análisis granulométrico por métodos automáticos: tubo de sedimentación y Sedigraph. Acta Geológica Hispánica 20, 95-102.

Gomis, D., Monserrat, S., Tintore, J., 1993. Pressure-forced seiches of large amplitude in inlets of the Balearic Islands. Journal of Geophysical Research-Oceans 98, 14437-14445.

Guinasso, N.L., Schink, D.R., 1975. Quantitative estimates of biological mixing rates in abyssal sediments. Journal of Geophysical Research-Oceans and Atmospheres 80, 3032-3043.

Jordi, A., Basterretxea, G., Casas, B., Anglès, S., Garcés, E., 2008. Seiche-forced resuspension events in a Mediterranean harbour. Continental Shelf Research 28, 505-515.

Joyce, L., 2005. Dinoflagellate cysts from surface sediments of Saldanha Bay, South Africa: an indication of the potential risk of harmful algal blooms. Harmful Algae 4, 309-318.

Keafer, B.A., Buesseler, K.O., Anderson, D.M., 1992. Burial of living dinoflagellate cysts in estuarine and nearshore sediments. Marine Micropaleontology 20, 147-161.

Kirn, S.L., Townsend, D.W., Pettigrew, N.R., 2005. Suspended *Alexandrium* spp. hypnozygote cysts in the Gulf of Maine. Deep-Sea Research Part II-Topical Studies in Oceanography 52, 2543-2559.

Kremp, A., 2001. Effects of cyst resuspension on germination and seeding of two bloom-forming dinoflagellates in the Baltic Sea. Marine Ecology Progress Series 216, 57-66.

Lynch, D.R., Werner, F.E., 1987. 3-dimensional hydrodynamics on finite-elements. Part 1. Linearized Harmonic Model. International Journal for Numerical Methods in Fluids 7, 871-909.

Lynch, D.R., Werner, F.E., Greenberg, D.A., Loder, J.W., 1992. Diagnostic model for baroclinic, wind-driven and tidal circulation in shallow seas. Continental Shelf Research 12, 37-64.

Montresor, M., Zingone, A., Sarno, D., 1998. Dinoflagellate cyst production at a coastal Mediterranean site. Journal of Plankton Research 20, 2291-2312.

Nehring, S., 1996. Recruitment of planktonic dinoflagellates: Importance of benthic resting stages and resuspension events. Internationale Revue Der Gesamten Hydrobiologie 81, 513-527.

Ostrovsky, I., Yacobi, Y.Z., Walline, P., Kalikhman, I., 1996. Seiche-induced mixing: Its impact on lake productivity. Limnology and Oceanography 41, 323-332.

Persson, A., 2000. Possible predation of cysts - a gap in the knowledge of dinoflagellate ecology?. Journal of Plankton Research 22, 803-809.

Richter, D., Vink, A., Zonneveld, K.A.F., Kuhlmann, H., Willems, H., 2007. Calcareous dinoflagellate cyst distributions in surface sediments from upwelling areas off NW Africa, and their relationships with environmental parameters of the upper water column. Marine Micropaleontology 63, 201-228.

Satta, C.T., Anglès, S., Garcés, E., Lugliè, A., Padedda, B.M., Sechi, N., 2010. Dinoflagellate cysts in recent sediments from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact. Deep-Sea Research Part II-Topical Studies in Oceanography 57, 256-267.

Steidinger, K.A., Garcés, E., 2006. Importance of Life Cycles in the Ecology of Harmful Algae. In: Gráneli, E., Turner, J.T. (Eds.), Ecology of Harmful Algae. Springer-Verlag, Berlin Heidelberg, pp. 37-49.

Tsimplis, M.N., Proctor, R., Flather, R.A., 1995. A 2-dimensional tidal model for the Mediterranean Sea. Journal of Geophysical Research-Oceans 100, 16223-16239.

Usup, G., Azanza, R.V., 1998. Physiology and bloom dynamics of the tropical dinoflagellate *Pyrodinium bahamense*. In: Anderson, D.M., Cembella, A., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Berlin, pp. 81-94.

Van Lenning, K., Vila, M., Masó, M., Garcés, E., Anglès, S., Sampedro, N., Morales-Blake, A., Camp, J., 2007. Short-term variations in development of a recurrent toxic *Alexandrium minutum*-dominated dinoflagellate bloom induced by meteorological conditions. Journal of Phycology 43, 892-907.

Vila, M., Giacobbe, M.G., Masó, M., Gangemi, E., Penna, A., Sampedro, N., Azzaro, F., Camp, J., Galluzzi, L., 2005. A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas. Harmful Algae 4, 673-695.

Villanoy, C.L., Azanza, R.V., Altemerano, A., Casil, A.L., 2006. Attempts to model the bloom dynamics of *Pyrodinium*, a tropical toxic dinoflagellate. Harmful Algae 5, 156-183.

Wang, Z., Qi, Y., Lu, S., Wang, Y., Matsuoka, K., 2004. Seasonal distribution of dinoflagellate resting cysts in surface sediments from Changjiang River Estuary. Phycological Research 52, 387-395.

4

LIFE-CYCLE ALTERNATIONS IN *ALEXANDRIUM MINUTUM* NATURAL POPULATIONS

Marine Ecology Progress Series, submitted

LIFE-CYCLE ALTERNATIONS IN *ALEXANDRIUM MINUTUM* NATURAL POPULATIONS

Sílvia Anglès, Esther Garcés, Albert Reñé, Nagore Sampedro

Institut de Ciències del Mar (CSIC), Pg. Marítim de la Barceloneta, 37-49, 08003 Barcelona, Spain

ABSTRACT

In situ fluxes of germinated cells and of resting cyst production were monitored from October 2005 to August 2008 during recurrent *A. minutum* blooms in Arenys de Mar harbor (northwestern Mediterranean). In addition, vegetative cells in the water and resting cysts in the sediments were quantified. Excystment occurred continuously during the two years studied. Moreover, environmental triggers, consisting of a net increase in irradiance and water temperature, stimulated greater excystment of resting cysts. Periods of high excystment coincided with conditions favorable for vegetative cell growth. During vegetative blooms, resting cyst formation was initiated at periods marked by vegetative cell abundance in the water column of $>2 \times 10^3$ cells l^{-1}, and fluxes of resting cysts were observed to increase as a function of higher vegetative cell abundance. The large extent of the blooms allowed to observe that excystment and encystment processes overlapped for 2 months. Moreover, sustained high excystment fluxes could be explained by the germination of the newly formed resting cysts, which had overcome the mandatory dormancy period and supplied vegetative cells to the same bloom during which they were produced. These germinated cells contributed to the maintenance of the bloom by dividing, and continuously underwent sexual reproduction over long periods. Resting cysts in the sediment were rapidly depleted during periods of high excystment, but their production, although involving only a small fraction of the vegetative population, more than compensated for their loss. The frequent life-stages switches provide an insurance strategy for bloom maintenance of *A. minutum*.

Resum

Es van mesurar els fluxos in situ de cèl·lules germinades i de formació de cists de resistència des d'octubre 2005 a agost 2008 durant proliferacions recurrents d'*Alexandrium minutum* al port d'Arenys de Mar (Mediterrània nord-occidental). A més, es van quantificar les cèl·lules vegetatives a l'aigua i els cists de resistència en els sediments. La germinació de cists de resistència va ocórrer contínuament durant el període on el rang de temperatura va ser permissiu. Desencadenants ambientals, un augment net de la irradiància i la temperatura de l'aigua, van estimular una major germinació dels cists de resistència. A més, aquests períodes d'alta germinació van coincidir amb les condicions favorables perl creixement de cèl·lules vegetatives. Durant les proliferacions, la formació de cists de resistència es va iniciar en els períodes marcats per abundàncies de cèl·lules vegetatives a la columna d'aigua de més de 2×10^3 cèl·lules L^{-1}, i es va observar que els fluxos de cists de resistència augmentaven en funció d'una major abundància de cèl·lules vegetatives. La llarga durada de les proliferacions va permetre observar que els processos d'alta germinació i els d'encistament es superposaven durant 2 mesos. Els fluxos sostinguts d'alta germinació observats podrien ser explicats per la germinació dels nous cists de resistència formats durant la mateixa proliferació. Aquests cists, amb el període de latència obligatòria ja superat, podrien subministrar cèl·lules vegetatives a la mateixa proliferació en què van ser produïts. Aquestes cèl·lules germinades contribuirien al manteniment de la proliferació mitjançant divisió mitòtica i a la vegada entrarien a la fase de reproducció sexual de forma continuada, el qual explicaria la llarga durada de formació de cists de resistència. Els cists de resistència en el sediments es van esgotar ràpidament durant els períodes d'alta germinació, però la seva producció, tot i que només va involucrar una petita fracció de la població vegetativa, va compensar de llarg les seves pèrdues. Els freqüents canvis entre fases del cicle proporcionen una estratègia d'assegurança per al manteniment de la proliferació d'*A. minutum*.

Introduction

Several species of phytoplankton form harmful algal blooms (HABs) in coastal waters worldwide. They cause detrimental effects to human health in addition to significant negative economic and ecologic impacts on aquatic ecosystems, including the closure of shellfish farms, fish kills, and water anoxia and discoloration. Among the many HAB-forming dinoflagellate species, some exhibit a heteromorphic life cycle made up of alternating asexual and sexual stages. The asexual phase is formed by planktonic vegetative cells that divide to increase the population, until they switch to the sexual phase and become gametes. The fusion of two gametes produces a motile diploid planozygote that develops into a resting cyst, in a process referred to as encystment. The resting cyst settles to the benthos and enters into a mandatory dormancy period, during which it physiologically matures in a process conditioned by endogenous factors. Once maturation is completed, the resting cyst undergoes excystment (germination), yielding a motile diploid planomeiocyte that divides and thus re-establishes the asexual phase (Von Stosch, 1973; Walker, 1984). Resting cysts that fail to excyst enter a quiescent state, remaining viable in the sediment for several years until the conditions for germination are adequate. In some species, in addition to this life cycle, other stages have been observed. For example, vegetative cells can form short-living cysts (pellicle cysts) or asexual long-living cysts, whereas planozygotes either divide during the motile stage or encyst as a pellicle cyst (Kremp and Parrow, 2006; Uchida, 1991; Walker, 1984).

The life-cycle stages of phytoplankton species play a key role in bloom occurrence and bloom dynamics. In the benthic phase, resting cysts represent a means for genetic recombination and species dispersal, as well as a survival mechanism for months or even years. At the same time, resting cysts are a seed source in the initiation of vegetative blooms (Dale, 1983). Therefore, shifts between life-cycle stages and the relationships of these stages to the physical, chemical, and biological environment are essential elements in the ecological success of phytoplankton species. As such, excystment and encystment processes are linked to the seasonality of phytoplankton populations (Walker, 1984). Moreover, the factors that trigger these pelagic-benthic transitions vary among the different phytoplankton groups and are highly species-specific (Kremp et al., 2009; Shikata et al., 2008). In the case of dinoflagellates, excystment has been primarily related to temperature, with germination induced within a defined, permissive temperature window but inhibited outside this range. Salinity, light intensity, and endogenous factors also influence excystment to varying degrees, whereas anoxia and darkness have an inhibitory effect (Anderson et al., 1987; Dale, 1983; Pfiester and Anderson, 1987). Encystment has been generally linked to sub-optimal factors for growth, mainly nutrient limitation, but other parameters include temperature, light, bacteria and cell density (Adachi et al., 1999; Pfiester and Anderson, 1987; Uchida, 2001).

Of the HAB-type dinoflagellate species, the widespread *Alexandrium minutum* Halim is a toxin-producer responsible for paralytic shellfish poisoning (PSP) intoxication events. The *A. minutum* group is proposed to comprise two phylogenetic clades, based on LSU ribotypes: the Global clade (strains from Europe and Australia), and the Pacific clade (strains from Asia and New Zealand) (Hansen et al., 2003; Lilly et al., 2005). *A. minutum* blooms occur in shallow systems, such as estuaries, bays, lagoons, and harbors, and are frequently recurrent, a characteristic favored by the presence of resting cysts in the life cycle of this species (Bravo et al., 2010a; Giacobbe et al., 1996; Touzet et al., 2010). Further details of the life cycle, as determined in a laboratory study, showed that sexuality involved more than two genetically different and compatible mating types. Depending on nutrient conditions, the resulting planozygotes then proceeded to either divide or encyst. Resting cysts of natural populations exhibited a mandatory dormancy period of 1–1.5 months (Figueroa et al., 2007). Moreover, under natural as well as laboratory conditions, *A. minutum* also produces pellicle cysts (Bolli et al., 2007; Bravo et al., 2010b).

The cosmopolitan, thermohaline, and euryhaline character of *A. minutum* and its ability to form blooms in densely populated coastal areas have encouraged research on the population dynamics of this species. The biological and environmental factors that influence *A. minutum* life-cycle transitions have been investigated in laboratory experiments, but only encystment has been characterized under natural conditions (e.g., Cannon, 1993; Garcés et al., 2004; Probert, 1999). The present study is the first to quantify in situ excystment of *A. minutum* (Global clade). It also provides global insights into the life-cycle transitions of *A. minutum* by integrating in situ data of excystment and encystment processes, as well as vegetative cells and resting cyst dynamics. The study site was Arenys de Mar harbor (northwestern Mediterranean Sea), where large and intense recurrent blooms of *A. minutum* have been reported (Garcés et al., 2004; Van Lenning et al., 2007; Vila et al., 2001). Blooms usually occur between December and August, reaching cell abundances that frequently exceed 10^6 cells l^{-1}. The particular characteristics of the harbor, i.e., its small dimensions, microtidal regime, high water-residence time, and low turbulence constitute ideal conditions to more precisely study processes that involve direct, straightforward relationships between vegetative cells and resting cysts.

Methods

Study site and sampling stations

Arenys de Mar harbor is an artificial harbor of 0.4 km^2 located on the coast of Catalonia, Spain (northwestern Mediterranean Sea, Fig. 1). It includes shipyards, a fishing port, and yachting docks. Water disturbance caused by ship traffic is very low and such activity consists mainly of leisure and small fishing boats. The harbor has not been dredged for

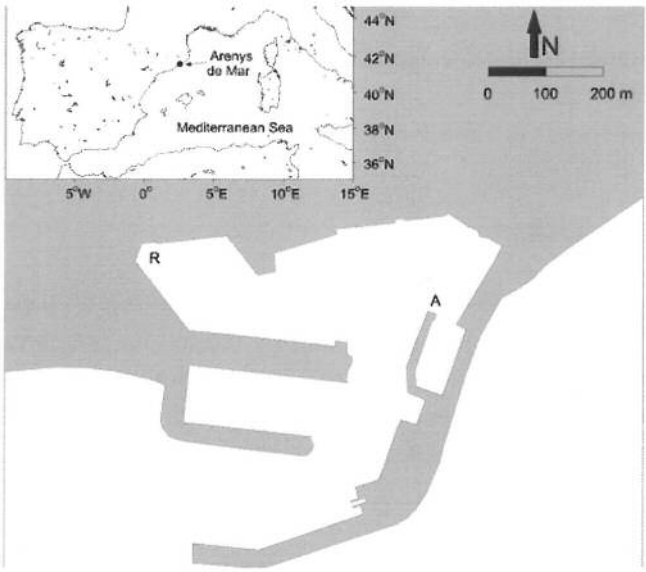


Fig. 1. Locations of Arenys de Mar harbor and the sampling stations.

over 10 years and has an average depth of 4 m, ranging from 1 m in the most confined parts to 6 m at the mouth. The harbor system receives freshwater inflows along its walls and occasional discharges from land drainage after rain episodes. The tidal regime is microtidal, with a spring tide of up to 0.25 m. Two stations, R and A, were monitored during this study (Fig. 1). In situ excystment experiments were conducted at station R because of its shallow depth (2 m) and a resting cyst abundance reflecting the resting cyst dynamics of the harbor sediments (Anglès et al., 2010, referred as station 23). Station R is also the regional phytoplankton monitoring station and long-term data on environmental variables are therefore available. In situ encystment studies were carried out at station A (2 m), as it allowed the convenient deployment of encystment traps and further comparison with data from a previous experiment performed at the same station (Garcés et al., 2004).

Vegetative cells in the water column

Temporal fluctuations of *A. minutum* vegetative cell abundance in the water column were assessed in phytoplankton samples collected 2–4 times per month at station R from October 2005 to May 2008. To estimate rates of resting cyst formation (see details below), phytoplankton samples were collected once or twice weekly at station A from March to April 2006 and from January to June 2007. Samples were taken from the water surface (at 12:00 GMT) and fixed immediately with Lugol's solution. Vegetative cells were quantified by sedimentation of 50-ml subsamples in settling chambers for 24 h followed by counting of an appropriate area, depending on the density, under an inverted microscope (Leica

DMIRB fitted with epifluorescence filters). *A. minutum* cells were identified according to the criteria of Balech (1995), after staining the thecal plates with calcofluor white solution (Fritz and Triemer, 1985).

Environmental variables

Water temperature (°C), salinity, and dissolved oxygen (mg l^{-1}) were measured using a Multiline F/SET-3 WTW probe. Water samples (60 ml) for quantification of dissolved inorganic nutrients (NO_3, NO_2, NH_4, PO_4, and SiO_4; measured in µM) were stored at –20°C until analyzed in an autoanalyzer (Grasshoff et al., 1983). Solar irradiance registrations (W m^{-2}) were collected at a nearby meteorological station.

Resting cysts in the sediment

Sediment cores were collected by a scuba diver from October 2006 to May 2008 at station R to measure the abundance of resting cysts. The cores (20 cm long × 5 cm base diameter) were stored in the dark at 10°C and left to settle overnight before being processed the day after collection. Water above the core was carefully removed and the top 1 cm of the sediment was cut. A subsample of this fraction was sonicated for 2 min, sieved to retain the 10- to 100-µm fraction, and processed by the sodium polytungstate (SPT) density gradient method of Bolch (1997), as modified by Amorim et al. (2001) and Bravo et al. (2006). The resulting sample was rinsed in a 10-µm sieve and collected with filtered seawater. An aliquot of this final sample was used for resting cyst enumeration in 2-ml Utermöhl sedimentation chambers under an inverted microscope. Resting cyst abundance is expressed as cysts cm^{-2} of wet sediment (ws).

Excystment traps

The recruitment of germinated *A. minutum* cells was quantified at station R every 1–2 months from October 2006 to May 2008 using excystment (emergence) traps. The excystment traps were designed after those previously employed to determine zooplankton excystment fluxes (Cáceres, 1998; Hairston et al., 2000), and later successfully used for phytoplankton studies in freshwater lakes (Rengefors et al., 2004). The traps consisted of empty 19-l low-density polyethylene water bottles with the bottom removed (594 cm^2). Two 10-µm mesh windows (8.5 cm diameter) on the sides allowed water flow through the traps while *A. minutum* vegetative cells and larger plankton were excluded. At the mouth of the traps, a 0.25-l clear plastic collection bottle was attached upside down.

The excystment traps were placed at the sampling station by a scuba diver. At the surface, the traps were filled with filtered (0.22 µm) seawater obtained from the same locality, and the bottom was covered by a 10-µm mesh to avoid the flow into the traps of *A. minutum* vegetative cells or other organisms present in the water column. The traps were inserted into the sediment by three metal bars placed around the traps. When the bottom of the traps was in contact with the sediment surface, the 10-µm mesh was withdrawn causing minimal disturbance of the sediment. Then, the bottom of the traps was introduced at a depth of 2-3 cm into the sediment to prevent the flow of cells through the bottom. The collection bottle was removed by a scuba diver after 24 h but before the trap itself was removed. The time at which the traps were deployed and the collection bottles removed was always 12:00 GMT, the time at which vegetative cells are present on the surface layer since they exhibit vertical migration (Delgado et al., 1998). Furthermore, samples of the water inside the traps (100 ml) were collected using a syringe to ensure that all germinated cells had been recruited into the collection bottles. Due to the short sampling time, it was assumed that reproduction of the recruited cells and grazing by zooplankton inside the traps were both minimal. All samples were preserved with Lugol's solution immediately after they had been returned to the surface. Aliquots of 10–50 ml were counted under an inverted microscope following the same procedure described for vegetative cells in the water column.

Excystment rates

The measured recruitment of *A. minutum* cells was used to determine the daily fluxes of germinated cells and to estimate excystment rates. The latter were calculated for each sampling using the following equation:

$$K_{ex} = \frac{V_{ex}}{2C_{ex}} \quad (1)$$

where C_{ex} is the total abundance of resting cysts present in the sediment area under the trap, and V_{ex} the abundance of germinated vegetative cells recruited in the collection bottle (cell d^{-1}). This number was divided by 2 based on the assumption that, after germination, during the 24-h duration of the field recruitment, *A. minutum* planomeiocytes divide once, giving rise to 2 motile vegetative cells (Figueroa et al., 2007; S. Anglès unpubl.). The resting cysts abundance used in the equation was obtained by quantifying the resting cysts in the sediment sample taken on the same day and prior to deployment of the excystment trap. An underlying assumption in estimates of the excystment rates is that all quantified resting cysts are able to germinate. The mandatory dormancy period of 1–1.5 months for newly produced resting cysts was not considered.

Encystment traps

Sedimentation traps to collect *A. minutum* resting cysts and quantify their flux to the sediment were placed at station A. The sampling periods extended from March to April 2006 and from January to June 2007. The traps consisted of two cylindrical collection containers (height 15 cm, diameter 5 cm, aspect ratio 3) moored 0.5 m from the bottom. Sediment traps were collected regularly (see exact dates in Table 3), and samples were kept in the dark at 10°C, without the addition of fixatives. Ten-ml aliquots of the contents were sonicated for 20 s, and 1–3 ml subsamples were subsequently examined under an inverted microscope to enumerate *A. minutum* resting cysts.

Encystment rates

Counted resting cysts of *A. minutum* within the encystment traps were used to determine daily encystment fluxes and encystment rates. The latter was estimated following the method reported in Garcés et al. (2004):

$$K_{\text{en}} = \frac{2C_{en}}{V_{en} + 2C_{en}} \qquad (2)$$

where C_{en} is the resting cysts collected in the encystment traps per day (cyst m^{-2} d^{-1}) and V_{en} the standing stock of vegetative cells above the traps (cells m^{-2}) in the overlying water column (1.5 m). C_{en} was multiplied by 2 since each cyst is produced from 2 cells. V_{en} corresponds to the mean vegetative cell abundance, quantified from the day that the traps were placed until the day they were removed.

Cyst identification

A. minutum cysts in the sediment and in the encystment traps were classified according to shape, color, cytoplasmic content characteristics, and wall thickness. Cysts classified as resting cysts had an almost circular shape in apical view and were reniform in lateral view. They possessed a thick double wall and a yellow accumulation body condensed to a lesser or greater degree (Bolch et al., 1991). Additional morphological details of the resting cysts present in the Arenys de Mar harbor sediments can be found in Satta et al. (2010). Other types of cysts observed were classified as pellicle cysts following Bravo et al. (2010b). These were characterized by a thin wall, and a cytoplasmic content of green, uncondensed chloroplasts. In all cases, only cysts of viable morphology, i.e., with the cellular content intact, were considered.

Calculation of resting cyst losses in the sediment

The loss of resting cysts in the sediment was calculated by rearranging the exponential decay formula as follows:

$$C_t = C_0 e^{-\kappa t} \quad (3)$$

where C_0 is the initial abundance of resting cysts, C_t the remaining abundance, and κ the loss rate. Net loss rates of resting cysts occurring at the sediments were calculated by solving the equation using the resting cyst abundances measured between two consecutive samplings. To calculate the resting cyst losses caused by excystment, C_t was obtained by applying the excystment rate estimated (κ_{ex}) for each sampling, with C_0 the abundance of resting cysts at that time.

Statistical analysis

Simple and multivariate regression analysis was applied to evaluate which environmental variables (abiotic and biotic) measured were most related to the excystment and encystment processes observed. Specifically, to explore the effect of irradiance and water temperature fluctuations on excystment, increases and decreases in monthly mean values between consecutive months were calculated for each variable. The program used was Matlab Statistics Toolbox.

Results

Temporal fluctuation of vegetative cells and environmental variables

The temporal fluctuations of *A. minutum* vegetative cells at station R from October 2005 to May 2008 are shown in Figure 2. Abundances $>1 \times 10^3$ cells l^{-1} were attained for periods lasting several months, generally within winter to mid-summer, and reached maximum values up to 3×10^6 cells l^{-1}. Hereafter, these episodes are considered as "blooms". Cell abundances $<1 \times 10^3$ cells l^{-1}, referred to as "no blooms", occurred sporadically during late-summer and autumn. Environmental variables of water temperature and salinity were 12–27°C and 25–38, respectively (Fig 3A). Solar irradiance registered from the meteorological station (Fig 3B) showed the seasonal pattern typical of the region, with minimum values in winter and maximum values in summer.

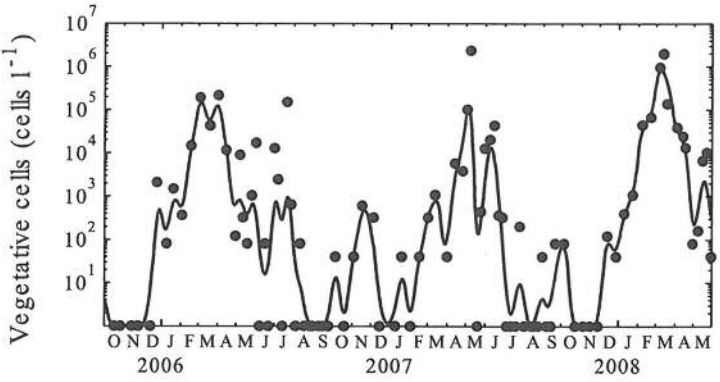


Fig. 2. Fluctuations of *A. minutum* vegetative cells from October 2005 to May 2008 at Arenys de Mar harbor. Solid line represents data filtered by optimal interpolation.

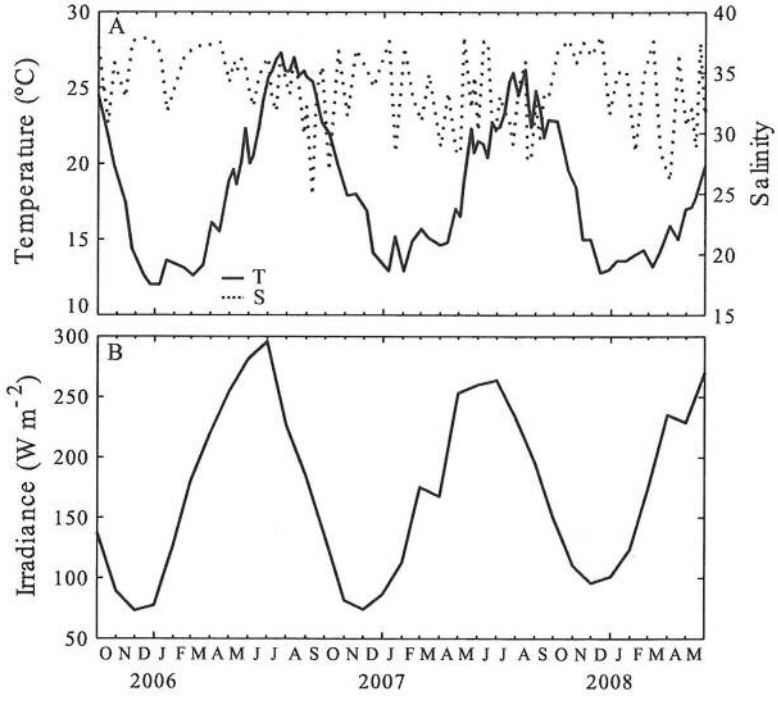


Fig. 3. Environmental variables of (A) water temperature and salinity, and (B) monthly mean solar irradiance.

In situ excystment fluxes and rates

In situ excystment of *A. minutum* occurred throughout the two sampled years (Fig. 4). The fluxes of germinated cells were in the range of 1.6×10^2–1.8×10^7 cells d^{-1} m^{-2} (recorded in November 2007 and March 2008, respectively). Table 1 shows the daily excystment rates estimated for each experiment along with the total abundance of resting cysts in the sediment and germinated cells recruited (cells d^{-1}). Excystment rates ranged from <0.001 to 0.457. In general, excystment fluxes and rates increased progressively from December to June. Based on both excystment fluxes and rates, two types of periods were defined: i) periods of low excystment, that coincided with either the absence of vegetative cells or non-bloom periods (<10^3 cells l^{-1}) in the overlying water column (periods L in Fig.4), and ii) periods of high excystment, that corresponded mainly with blooms of vegetative cells in the water column (periods H in Fig.4). Type L periods were observed from October to December 2006 and from August to November 2007, and type H periods from January to June 2007 and from January to May 2008.

Relationship between in situ excystment and environmental variables

During excystment experiments, the water temperature was in the range of 13–25°C, with fluxes of high excystment periods recorded at temperatures of 13–21°C (Fig. 3A and Fig. 4). This temperature range of high excystment was characterized by yearly variations (15–21°C in January–June 2007, and 13–19°C in January–May 2008). Likewise, temperature range of low excystment varied among years (14–19°C in October–December 2006, in contrast to 18–25°C in August–November 2007). Figure 5 illustrates the relationship of excystment fluxes with increases and decreases in irradiance and water temperature (indicated as ΔI

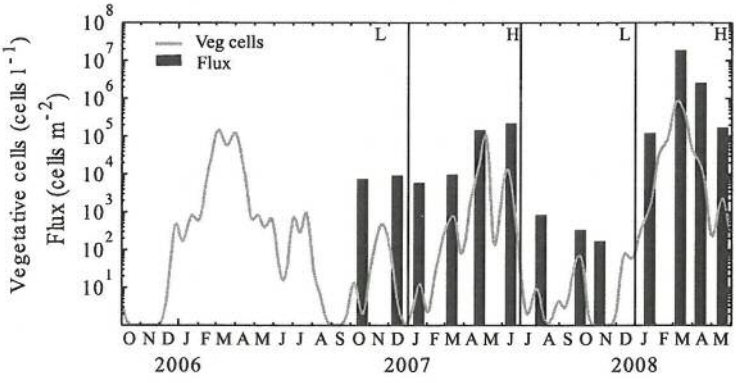


Fig. 4. Fluxes of germinated cells of *A. minutum*, and vegetative cells in the water column as in Fig. 2. Periods L correspond to periods of low excystment fluxes, and periods H to periods of high excystment fluxes. No data from October 2005 to September 2006 on excystment fluxes.

Date	Total resting cysts	Germinated cells	Excystment rate	Resting cysts loss rate	Remaining resting cysts
26 Oct 06	2.4×10^5	4.3×10^2	<0.001		
13 Dec 06	7.4×10^4	5.5×10^2	0.003	0.03	2.3×10^5
17 Jan 07	3.8×10^4	3.5×10^2	0.004	0.02	6.5×10^4
13 Mar 07	1.4×10^4	5.7×10^2	0.019	0.02	2.9×10^4
26 Apr 07	9.5×10^4	8.6×10^3	0.045	+0.04	6.4×10^3
13 Jun 07	2.3×10^6	1.2×10^4	0.002	+0.07	1.1×10^4
4 Aug 07	4.9×10^5	5×10^1	<0.001	0.03	2×10^6
9 Oct 07	2.1×10^5	2×10^1	<0.001	0.01	4.8×10^5
7 Nov 07	1.4×10^5	1×10^1	<0.001	0.02	2.1×10^5
25 Jan 08	2.1×10^5	7×10^3	0.016	+0.01	1.4×10^5
13 Mar 08	1.2×10^6	1.1×10^6	0.457	+0.04	9.5×10^4
15 Apr 08	8.9×10^5	1.5×10^5	0.084	0.01	1
21 May 08	3.9×10^5	1×10^4	0.012	0.02	4.2×10^4

Table 1. Dates of deployment of the excystment traps, total abundance of resting cysts in the sediment area under the excystment traps (cysts), germinated cells recruited by the collection bottles (cells d^{-1}), excystment rates as obtained from Eq. (1), estimated net loss rates of resting cysts calculated by Eq. (3), and remaining resting cysts (cysts) after applying the excystment rate to Eq. (3). (+) indicates loss rates of resting cysts with a negative sign, i.e., indicating gains.

and ΔT, respectively). Excystment fluxes were significantly correlated with both ΔI and ΔT individually (R^2=0.50, p=0.007, and R^2=0.38, p=0.027, respectively). Although irradiance and temperature parameters covariate, multivariate regression analysis was applied to both parameters, which showed a correlation of R^2=0.55 (p=0.004). Periods of high excystment coincided with increases in irradiance and water temperature, conditions that occur typically from late winter to spring.

Salinity varied greatly during excystment experiments (Fig. 3A and Fig. 4) but did not correlate significantly with excystment since high excystment fluxes occurred at both the lower (28) and the upper (38) values. Nutrient concentrations and dissolved oxygen levels measured during low and high excystment fluxes are shown in Table 2, along with values for periods of non-bloom and bloom conditions for comparison. Dissolved inorganic nitrogen forms (expressed as DIN), composed mainly of NO_3, were the dominant nutrients. Nutrient concentrations did not correlate significantly with excystment fluxes, and they did not appear to limit excystment. Dissolved oxygen values were much higher than those defining hypoxic conditions and they did not significantly correlate with excystment fluxes as well.

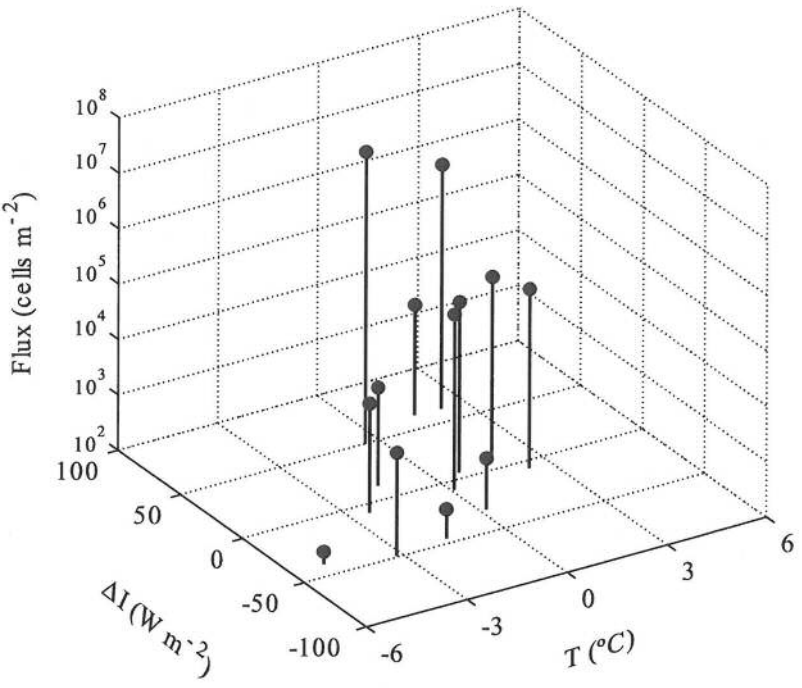


Fig. 5. Influence of increases and decreases in monthly mean irradiance (ΔI) and water temperature (ΔT) on fluxes of germinated cells of *A. minutum*.

In situ encystment fluxes and rates

Resting cyst deposition in the encystment traps deployed at station A was observed during March-April 2006 and April-June 2007 (Table 3). Pellicle cysts were also noted in the traps during the two periods sampled; their presence coincided with peaks of very high abundance (>10^5 cells l^{-1}). Pellicle cysts counted in the sediment traps of 2006 accounted

Conditions	DIN	PO_4	SiO_4	DO
No bloom	131.2 (±124.4)	1.7 (±5.6)	39.2 (±31.9)	5.2–10.5
Bloom	126.7 (±103.7)	0.7 (±0.4)	34.9 (±23.5)	5.8–13.1
Low Ex	158.0 (±205.6)	0.6 (±0.4)	38.9 (±35.9)	6.4–8.9
High Ex	169.5 (±167.0)	0.7 (±0.5)	38.1 (±36.3)	7.9–13.0

Table 2. Means (±SD) of nutrient concentrations (DIN, PO_4, SiO_4; µM) and ranges of dissolved oxygen (DO; mg l^{-1}) measured at station R during conditions of no bloom (n=55), bloom (n =60), low excystment (Low Ex, n =6) and high excystment (High Ex, n =7).

Date	Vegetative cells	Resting cysts flux	Encystment rate
3 Mar 06	2.5×10^6		
15 Mar 06	3.2×10^6	2.6×10^5	13.9
20 Mar 06	1.5×10^7	1.2×10^5	1.6
27 Mar 06	3×10^7	8.5×10^5	5.4
31 Mar 06	6.2×10^7	5.9×10^6	16.0
3 Apr 06	7.5×10^7	3×10^6	7.4
10 Apr 06	5.5×10^7	nd	nd
24 Apr 06	1.4×10^6	nd	nd
28 Apr 06	1.1×10^6	9×10^4	14.1
20 Jan 07	0		
31 Jan 07	0	0	
8 Feb 07	0	0	
20 Feb 07	0	0	
13 Mar 07	0	0	
16 Mar 07	0	0	
24 Mar 07	0	0	
1 Apr 07	0	0	
13 Apr 07	6	0	
18 Apr 07	2.4×10^6	0	
26 Apr 07	6.1×10^6	1.9×10^5	5.8
3 May 07	4.9×10^7	1.1×10^5	0.4
14 May 07	4.8×10^7	1.3×10^6	5.3
20 May 07	6.2×10^6	7×10^4	2.2
25 May 07	4×10^6	2.5×10^5	11.1
31 May 07	1.1×10^8	4.5×10^6	7.5
8 Jun 07	1.2×10^8	4.4×10^5	0.7
15 Jun 07	2.2×10^7	1.7×10^5	1.5
22 Jun 07	8.8×10^6	0	
28 Jun 07	3.4×10^6	0	

Table 3. Dates of sampling of the sediment traps, standing stock of vegetative cells in the overlying water column (cells m^{-2}), flux of resting cysts collected in the trap (cyst $m^{-2} d^{-1}$), and encystment rates as obtained from Eq. (2), expressed in percentages. nd: not determined.

for 17–32% of the total abundance of cysts (data not shown). During both years, fluxes of resting cysts varied but were generally higher when the abundance of vegetative cells in the overlying water column was greater. The encystment rates estimated also varied during the bloom period, but in general were consistent with a continuous encystment of a low proportion of the vegetative cell population (Table 3).

Relationship between in situ encystment and environmental variables

The values of the environmental variables temperature, salinity, and nutrients recorded at station A during encystment experiments are shown in Table 4. For comparison, the values for the same variables recorded during periods of resting cyst deposition in the encystment traps and during the period in 2007 when resting cysts were absent are provided as well. Temperature values recorded during encystment differed between the two blooms studied, being lower in 2006 than in 2007, and showed a wider range in the latter year. Although there was a progressive increase in temperature during both encystment periods, it did not correlate with encystment fluxes. There were no significant variations in salinity during the encystment periods, with values

Periods	T	S	DIN	PO4	SiO4
En 2006	12.3–15.6	37.9–38.2	3.0 (±1.3)	3.9 (±3.5)	1.7 (±0.8)
En 2007	16.5–21.4	38.0–38.2	3.7 (±2.2)	0.2 (±0.1)	2.3 (±0.8)
No En	14.6–16.5	37.5–38.3	3.5 (±3.7)	0.2 (±0.2)	1.2 (±1.0)

Table 4. Ranges of temperature (T; °C) and salinity (S), and means (±SD) of nutrient concentrations (DIN, PO4, SiO4; µM) measured at station A during the periods of encystment (En) in 2006 (n=9) and 2007 (n=8), and no encystment (No En, n=8) in 2007.

always around 38. Therefore, there was no evidence for an influence of salinity on encystment. Similarly, there was no significant correlation between nutrient concentrations and encystment. Simple regression analysis was applied to the flux of resting cysts formed and the corresponding standing stock of vegetative cells in the water column: i) for data measured in the present work and ii) for previous data regarding vegetative cells and resting cyst fluxes reported by Garcés et al. (2004) and Bravo et al. (2010b). The coefficient of determination (R^2) was determined to be 0.81 ($p<0.0001$, $n=25$) (Fig. 6). Published data corresponding to encystment rates >0.70 ($n=6$), determined at the end of the bloom, were not considered in the present correlation, as discussed in Garcés et al. (2004).

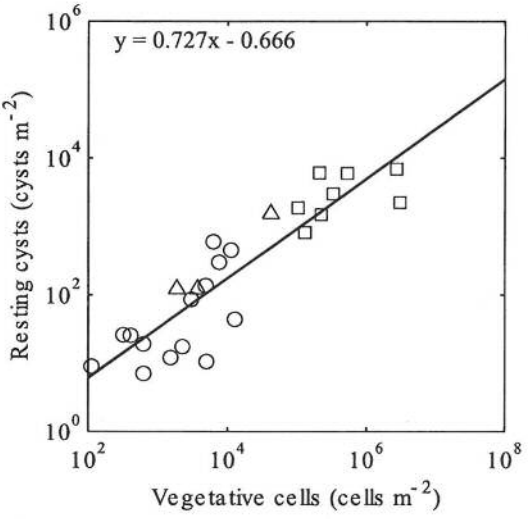


Fig. 6. Regression between fluxes of resting cysts production and the corresponding standing stock of vegetative cells in the water column. Circles correspond to data quantified in the present work, squares to data from Garcés et al. (2004), and triangles to data from Bravo et al. (2010b).

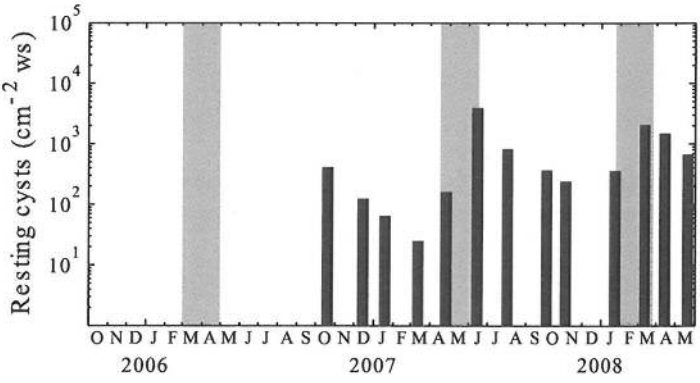


Fig. 7. Resting cyst abundance in the sediments from October 2006 to May 2008 at station R. Gray shaded bars correspond to periods of resting cyst production. No data from October 2005 to September 2006.

Resting cyst abundance in the sediment

Resting cysts of *A. minutum* in surface sediments from station R (Fig. 7) varied in abundance, from as low as 25 cysts cm^{-2} ws (March 2007) to a maximum of 3938 cysts cm^{-2} ws (June 2007). The fluctuation in resting cyst abundance was highly related to the preceding occurrence of vegetative cell blooms, with the highest abundance always measured after the blooms (June 2006 and 2007, and March 2008, Fig. 2). By contrast, in non-bloom periods, resting cyst abundance in the sediment progressively decreased. The increase of resting cysts in the sediments coincided chronologically with resting cyst deposition detected in the encystment traps deployed at station A (gray shaded bars in Fig. 7). Therefore, increases in resting cyst abundance in the sediments are considered as indicative of resting cyst production. In situ net loss rates of resting cysts in the sediment ranged from 0.01 to 0.03 (Table 1). During periods of resting cyst production, the rates had a negative sign, indicating gains in abundance. As suggested by the number of remaining cysts, calculated after applying the excystment rates obtained for each sampling, the losses of resting cysts that could be ascribed to excystment were lower than the in situ net losses, except during periods of high excystment.

Discussion

Excystment and encystment are key processes in the life strategy of many phytoplankton species. These processes, as well as the factors that regulate them, have been linked to the seasonality of their populations. By integration of the two processes, we provide insights on *A. minutum* life-cycle transitions to help understand the dynamics and ecology of this species that occurs worldwide. In the following discussion, we analyze first each of these processes separately, and then we examine the relationship between them, along with the planktonic and benthis phase dynamics. Figure 8 summarizes the periods of bloom development, high excystment, and encystment observed in this study.

Excystment of *A. minutum* resting cysts occurred throughout the two years examined. Similarly, continuous germination was observed in other dinoflagellates in studies using in situ excystment traps (Ishikawa and Taniguchi, 1996; Ishikawa et al., 2007). In our study, continuous germination is an explanation for the presence of vegetative cells throughout the year, particularly during no-bloom periods. As shown in investigations conducted in Arenys de Mar harbor, there is always a low abundance of vegetative cells in the water between bloom periods (Van Lenning et al., 2007; Vila et al., 2001; this study). The resting cysts present in the sediment were able to germinate within a broad temperature window (13–25ºC). However, since the annual range of water temperature in Arenys de Mar harbor is similar to that allowing excystment, we cannot rule out that *A. minutum* is also able to geminate at lower or higher temperatures. Likewise, salinity during germination varied widely (28–38), and a specific value did not seem to favor higher excystment.

Despite the fact that excystment was continuous throughout the year, the estimated excystment rates indicated a pattern of alternating low and high excystment periods. However, it should be mentioned that the excystment rates corresponding to March–April 2008 are unrealistic (0.457 and 0.084, respectively). These rates have been estimated from remarkably high excystment fluxes quantified during the maximum vegetative cell peak of the bloom occurring in 2008 and the consecutive month. Furthermore, they were the only two occasions when vegetative cells were detected in the water sampled inside the excystment traps. Small proportions of pellicle cysts are present in encystment traps during peaks marked by very high abundances of vegetative cells (Garcés et al., 2004, this study), which have a very short dormancy of 1–17 days (Bravo et al., 2010b). We hypothesize that the two unusually high recruitments of germinated cells were the result of a combination of fluxes supplied by both pellicle and resting cysts. However, since the methodological procedures used for resting cyst collection and quantification in the sediment were not adequate for the detection of pellicle cysts, it was impossible to determine the proportion of recruited cells corresponding to each type. Accordingly, the germination fluxes for these samplings should be regarded solely as high excystment fluxes, and the estimated excystment rates should not be considered.

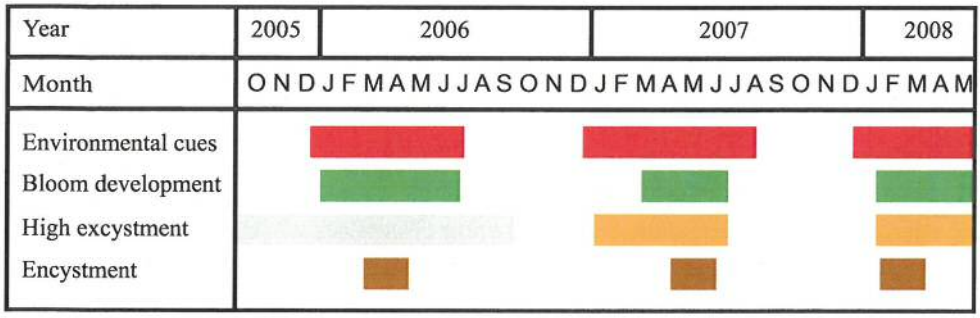


Fig. 8. Summary of *A. minutum* blooms: timing of vegetative bloom development, high excystment and encystment processes. Marked periods of environmental cues represent irradiance and water temperature increases. Gray bar represents periods of excystment not investigated.

The pattern of alternating low and high excystment periods was indicative of a slight seasonality. Of the environmental parameters recorded, combined variations in irradiance and water temperature were significantly related to excystment. Excystment fluxes and rates began to rise when irradiance and water temperature increased. As both parameters decreased, high excystment fluxes and rates were no longer maintained and started to drop. The development of *A. minutum* blooms in Arenys de Mar harbor has been reported to coincide with a progressive increase in both irradiance and water temperature (Van Lenning et al., 2007). Therefore, increases in irradiance and water temperature can be interpreted as not only cueing the excystment of a large fraction of resting cysts, which provide inoculum for the development of blooms, but also signaling the onset of conditions favorable for the growth of vegetative cells.

In other *Alexandrium* species, annual oscillations in the germination of resting cysts from deep ocean areas have been attributed to an endogenous annual clock that stimulates excystment at those times of year when both temperature and irradiance of the surface water are favorable for vegetative cell growth (*A. fundyense* from the Gulf of Maine, Anderson and Keafer, 1987; Matrai et al., 2005). To our knowledge, an endogenous annual clock has not been reported for *A. minutum,* and it is unlikely to exist in the population comprising our case study. The endogenous clock is an ecological adaptation of populations from deep areas, where external cues of temperature and light are either very subtle or non-existent. In contrast, resting cysts of *Alexandrium* spp. inhabiting shallow ponds lack an endogenous clock, since populations from this type of environment are more responsive to external cues, which are able to reach bottom sediments. Moreover, the control of excystment in changing environments by a clock-type mechanism would be a disadvantage (Anderson, 1998). Nevertheless, further studies should be addressed to support this hypothesis.

In our study, encystment was not clearly related to sub-optimal environmental factors. These results are in accordance with the studies available in the literature conducted by

means of sediment traps during *A. minutum* blooms that have addressed that issue (i.e. Garcés et al. 2004; Pitcher et al. 2007). Indeed, according to the environmental parameters of temperature, salinity, and nutrients described in those studies, encystment seems to occur at different values and within different ranges, and to be of long duration (from 2 weeks to several months). The common finding is that resting cyst production starts at a defined vegetative cell abundance in the overlying water column. Although the influence of environmental or endogenous parameters cannot be discarded, cyst production depends largely on vegetative cells abundance, with higher fluxes of resting cysts corresponding to greater abundances of cells in the water column. As indicated by the regression analysis, vegetative cell abundance explains 81% of the variability in cyst production quantified in different studies (Bravo et al., 2010b; Garcés et al., 2004; this study). A possible role of cell contact in the performance of sexual reproduction by hypothetical cell-to-cell communication has been pointed out in the literature. Cell abundance is crucial for the mating of gametes as well as the encystment of the resulting planozygotes since mating success increases as cell encounters become more likely (Uchida, 1996, 2001; Wyatt and Jenkinson, 1997). Indeed, Wyatt and Jenkinson (1997) suggested a requisite threshold in cell abundance of $>10^4$ cells l^{-1}, which, according to our data, may be even lower ($>2 \times 10^3$ cells l^{-1}).

The large extent of the blooms allowed to observe that excystment and encystment processes were overlapped (see Fig. 8). When the vegetative cell bloom reached the maintenance phase, high excystment fluxes were maintained, while at the same time resting cyst production started. Considering the length of the mandatory dormancy period (1–1.5 months), the extent of the high excystment and encystment fluxes (>3 months and 2 months respectively), newly formed resting cysts were fully capable of germinating during the same bloom in which they were produced. At the same time, the germination of these recently produced resting cysts could explain the prolonged high excystment (summary of bloom development, high excystment, and encystment periods during this study shown in Fig. 8). Dale (1983) suggested that a linear time-temperature relationship governs the length of the mandatory dormancy of resting cysts (higher temperature, shorter dormancy period), which seems to be consistent for *Alexandrium* species (Anderson, 1980; Castell-Pérez et al., 1998; Turpin et al., 1978). The reported mandatory dormancy period of *A. minutum* from Arenys de Mar harbor was determined with resting cysts stored at 4°C; thus, it is unclear to what extent the length of the dormancy period of the newly produced resting cysts was influenced by water temperature during our study. Based on the temperatures at which the resting cysts were formed, it is likely that these recently formed resting cysts had a foreshortened mandatory dormancy period and excysted soon after their formation, thus contributing to the bloom. This possibility is the reason why the dormancy period was not considered in the estimation of excystment rates, although it should be noted that it could have resulted in underestimated excystment rates for periods of overlapped excystment and encystment, as in the case of June 2007.

Resting cysts in the benthic stage are affected by physical and biological factors, in the form of sediment mixing by resuspension, burial, or bioturbation, as well as by germination, deposition, mortality, degradation, and grazing of resting cysts. According to our data, continuous germination is a low but constant cause of the loss of resting cysts in the sediments, and is more severe during high excystment periods. Based on the remaining resting cysts estimated from the excystment rates for these periods, it seems that resting cysts at the sediment surface are rapidly depleted. However, the cyst stock is replenished through the formation of resting cysts by the vegetative cells in the overlying column. In addition to resting cyst production, the regular events of sediment resuspension caused by seiches (natural oscillations occurring in harbors, estuaries, and other semi-enclosed basins) have been shown to prevent resting cyst burial (Anglès et al., 2010; Jordi et al., 2008).

Resting cysts insure the maintenance of *A. minutum* natural populations. The length of dormancy and the ability to sense the onset of favorable conditions are essential to the ecological success of a species (Montresor et al., 1996). The relatively short mandatory dormancy period of *A. minutum* and the coupling of high-low excystment with favorable-unfavorable periods for vegetative growth determine the success of blooms of this species. Continuous germination may guarantee a background population. The presence of these vegetative cells together with the excystment dynamics provides the source for bloom development at the onset of the growth season, resulting in an optimized strategy that allows *A. minutum* to reach higher abundances more rapidly and thus earlier, as demonstrated in a mathematic population model (Estrada et al., 2010). In shallow systems, the size of the inoculum for initiation of blooms is relatively small and the magnitude of the bloom may depend more on the growth of the vegetative population (Anderson, 1998). In our study, the minimum inoculum size recorded during initial bloom phases was ~5 cysts cm^{-2}. Although further germination had little effect on the size of the bloom, continuous high excystment during periods favorable for growth may have contributed to the maintenance of vegetative cell abundance for longer periods. The ability to produce resting cysts at relatively low cell abundance underlines an advantageous life-cycle strategy in which the production of resting cysts is ensured. Recently formed resting cysts are able to excyst within the same bloom, and germinated vegetative cells divide and again undergo sexual reproduction. This indicates a multivoltine population strategy consisting of frequent, rapid switches between vegetative and dormant stages within the bloom. This, in turn, underlines a high degree of genetic recombination, with its corresponding advantages for the ecological success of the species, such as increased resistance to parasites and viruses, among others.

Acknowledgements

The authors thank K. Van Lenning, X. Novell, and J. Riba for assistance with field work. K. Rengefors provided an original excystment trap to be re-designed for this study. A. Jordi provided valuable help with the graphs. Thanks to I. Bravo for facilitating her data of encystment. Thanks are extended to Creu Roja, Club Nàutic, and Cofradia de Pescadors Sant Telm of Arenys de Mar harbor for their support. Data from the meteorological station were supplied by the Catalan Meteorological Service (Meteocat). This study was financed by the EC-funded research project SEED (GOCE-CT-2005-003875). The work of E. Garcés was supported by a Ramon y Cajal award from the MICINN.

References

Adachi, M., Kanno, T., Matsubara, T., Nishijima, T., Itakura, S., Yamaguchi, M., 1999. Promotion of cyst formation in the toxic dinoflagellate *Alexandrium* (Dinophyceae) by natural bacterial assemblages from Hiroshima Bay, Japan. Marine Ecology Progress Series 191, 175-185.

Amorim, A., Dale, B., Godinho, R., Brotas, V., 2001. *Gymnodinium catenatum*-like cysts (Dinophyceae) in recent sediments from the coast of Portugal. Phycologia 40, 572-582.

Anderson, D.M., 1980. Effects of temperature conditioning on development and germination of *Gonyaulax tamarensis* (Dinophyceae) hypnozygotes. Journal of Phycology 16, 166-172.

Anderson, D.M., Keafer, B.A., 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*. Nature 325, 616-617.

Anderson, D.M., Taylor, C.D., Armbrust, E.V., 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. Limnology and Oceanography 32, 340-351.

Anderson, D.M., 1998. Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: Anderson, D.A., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Berlin-Heidelberg, pp. 29-48.

Anderson, D.M., Stock, C.A., Keafer, B.A., Nelson, A.B., Thompson, B., McGillicuddy, D.J., Keller, M., Matrai, P.A., Martin, J., 2005. *Alexandrium fundyense* cyst dynamics in the Gulf of Maine. Deep-Sea Research Part II: Topical Studies in Oceanography 52, 2522-2542.

Anglès, S., Jordi, A., Garcés, E., Basterretxea, G., Palanques, A., 2010. *Alexandrium minutum* resting cyst distribution dynamics in a confined site. Deep-Sea Research Part II: Topical Studies in Oceanography 57, 210-221.

Balech, E., 1995. The genus *Alexandrium* Halim. Sherkin Island Marine Station, Sherkin Island, Co. Cork, Ireland.

Bolch, C.J.S., 1997. The use of sodium polytungstate for the separation and concentration of living dinoflagellate cysts from marine sediments. Phycologia 36, 472-478.

Bolli, L., Llaveria, G., Garcés, E., Guadayol, Ò., van Lenning, K., Peters, F., Berdalet, E., 2007. Modulation of ecdysal cyst and toxin dynamics of two *Alexandrium* (Dinophyceae) species under small-scale turbulence. Biogeosciences 4, 559-567

Bravo, I., Garcés, E., Diogene, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. European Journal of Phycology 41, 293-302.

Bravo, I., Fraga, S., Figueroa, R. I., Pazos, Y., Massanet, A., Ramilo, I., 2010a. Bloom dynamics and life cycle strategies of two toxic dinoflagellates in a coastal upwelling system (NW Iberian Peninsula). Deep Sea Research Part II: Topical Studies in Oceanography 57, 222-234.

Bravo, I., Figueroa, R. I., Garcés, E., Fraga, S., Massanet, A., 2010b. The intricacies of dinoflagellate pellicle cysts: The example of *Alexandrium minutum* cysts from a bloom-recurrent area (Bay of Baiona, NW Spain). Deep Sea Research Part II: Topical Studies in Oceanography 57, 166-174.

Caceres, C.E., 1998. Interspecific variation in the abundance, production, and emergence of *Daphnia* diapausing eggs. Ecology 79, 1699-1710.

Cannon, J.A., 1993. Germination of the toxic dinoflagellate, *Alexandrium minutum*, from sediments in the Port River, South Australia. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier Sciences Publishers, pp. 103-107.

Castell-Perez, C., Roy, S., Levasseur, M., Anderson, D.M., 1998. Control of germination of *Alexandrium tamarense* (Dinophyceae) cysts from the lower St. Lawrence estuary (Canada). Journal of Phycology 34, 242-249.

Dale, B., 1983. Dinoflagellate resting cysts: "benthic plankton". In: Fryxell, G.A. (Ed.), Survival Strategies of the Algae. Cambridge Univ. Press, pp. 69-136.

Delgado, M., Garcés, E., Vila, M., Camp, J., 1998. Control of diel vertical migration of *Alexandrium minutum* by light and dark cycles. In: Reguera, B., Blanco, J., Fernandez, M.L., Wyatt, T. (Eds.), VIII International Conference on Harmful Algae. Xunta de Galicia and IOC-UNESCO, Vigo, pp. 160-162.

Estrada, M., Solé, J., Anglès, S., Garcés, E., 2010. The role of resting cysts in *Alexandrium minutum* population dynamics. Deep-Sea Research Part II: Topical Studies in Oceanography 57, 308-321.

Figueroa, R.I., Garces, E., Bravo, I., 2007. Comparative study of the life cycles of *Alexandrium tamutum* and *Alexandrium minutum* (Gonyaulacales, Dinophyceae) in culture. Harmful Algae 43, 1039-1053.

Fritz, L., Triemer, R.E., 1985. A rapid simple technique utilizing Calcofluor white M2R for the visualization of dinoflagellate thecal plates. Journal of Phycology 21, 662-664.

Garcés, E., Bravo, I., Vila, M., Figueroa, R.I., Masó, M., Sampedro, N., 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean). Journal of Plankton Research 26, 637-645.

Giacobbe, M.G., Oliva, F.D., Maimone, G., 1996. Environmental factors and seasonal occurrence of the dinoflagellate *Alexandrium minutum*, a PSP potential producer, in a Mediterranean lagoon. Estuarine Coastal and Shelf Science 42, 539-549.

Grasshoff, K., Ehrhardt, M., Kremling, K., 1983. Methods of sea water analysis. Verlag Chemie, Germany.

Hairston, N.G., Hansen, A.M., Schaffner, W.R., 2000. The effect of diapause emergence on the seasonal dynamics of a zooplankton assemblage. Freshwater Biology 45, 133-145.

Hansen, G., Daugbjerg, N., Franco, J.M., 2003. Morphology, toxin composition and LSU rDNA phylogeny of *Alexandrium minutum* (Dinophyceae) from Denmark, with some morphological observations on other European strains. Harmful Algae 2, 317-335.

Ishikawa, A., Taniguchi, A., 1996. Contribution of benthic cysts to the population dynamics of *Scrippsiella* spp. (Dinophyceae) in Onagawa Bay, northeast Japan. Marine Ecology Progress Series 140, 169-178.

Ishikawa, A., Hattori, M., Imai, I., 2007. Development of the "plankton emergence trap/chamber (PET Chamber)", a new sampling device to collect in situ germinating cells from cysts of microalgae in surface sediments of coastal waters. Harmful Algae 6, 301-307.

Jordi, A., Basterretxea, G., Casas, B., Anglès, S., Garcés, E., 2008. Seiche-forced resuspension events in a Mediterranean harbour. Continental Shelf Research 28, 505-515.

Kremp, A., Parrow, M.W., 2006. Evidence for asexual resting cysts in the life cycle of the marine peridinoid dinoflagellate, *Scrippsiella hangoei*. Journal of Phycology 42, 400-409.

Kremp, A., Rengefors, K., Montresor, M., 2009. Species-specific encystment patterns in three Baltic cold-water dinoflagellates: The role of multiple cues in resting cyst formation. Limnology and Oceanography 54, 1125-1138.

Lilly, E.L., Halanych, K.M., Anderson, D.M., 2005. Phylogeny, biogeography, and species boundaries within the *Alexandrium minutum* group. Harmful Algae 4, 1004-1020.

Matrai, P., Thompson, B., Keller, M., 2005. Circannual excystment of resting cysts of *Alexandrium* spp. from eastern Gulf of Maine populations. Deep Sea Research Part II: Topical Studies in Oceanography 52, 2560-2568.

Montresor, M., Marino, D., 1996. Modulating effect of cold-dark storage on excystment in *Alexandrium pseudogonyaulax* (Dinophyceae). Marine Biology 127, 55-60.

Pfiester, L.A., Anderson, D.M., 1987. Dinoflagellate reproduction. In: Taylor, F.J.R. (Ed.), The Biology of Dinoflagellates. Blackwell Scientific Publications, Oxford, pp. 611-648.

Pitcher, G.C., Cembella, A.D., Joyce, L.B., Larsen, J., Probyn, T.A., Sebastian, C.R., 2007. The dinoflagellate *Alexandrium minutum* in Cape Town harbour (South Africa): Bloom characteristics, phylogenetic analysis and toxin composition. Harmful Algae 6, 823-836.

Probert, I., 1999. Sexual reproduction and ecophysiology of the marine dinoflagellate *Alexandrium minutum* Halim. University of Westminister, London, p. 99.

Rengefors, K., Gustafsson, S., Stahl-Delbanco, A., 2004. Factors regulating the recruitment of cyanobacterial and eukaryotic phytoplankton from littoral and profundal sediments. Aquatic Microbial Ecology 36, 213-226.

Satta, C.T., Anglès, S., Garcés, E., Lugliè, A., Padedda, B.M., Sechi, N., 2010. Dinoflagellate cysts in recent sediments from two semi-enclosed areas of the Western Mediterranean Sea subject to high human impact. Deep-Sea Research Part II: Topical Studies in Oceanography 57, 256-267.

Shikata, T., Nagasoe, S., Matsubara, T., Yoshikawa, S., Yamasaki, Y., Shimasaki, Y., Oshima, Y., Jenkinson, I.R., Honjo, T., 2008. Factors influencing the initiation of blooms of the raphidophyte *Heterosigma akashiwo* and the diatom *Skeletonema costatum* in a port in Japan. Limnology and Oceanography 53, 2503-2518.

Touzet, N., Farrell, H., Ní Rathaille, A., Rodriguez, P., Alfonso, A., Botana, L.M., Raine, R., 2010. Dynamics of co-occurring *Alexandrium minutum* (Global Clade) and *A. tamarense* (West European) (Dinophyceae) during a summer bloom in Cork Harbour, Ireland (2006). Deep Sea Research Part II: Topical Studies in Oceanography 57, 268-278.

Turpin, D.H., Dobell, P.E.R., Taylor, F.J.R., 1978. Sexuality and cyst formation in Pacific strains of the toxic dinoflagellate *Gonyaulax tamarensis*. Journal of Phycology 14, 235-238.

Uchida, T., 1991. Sexual reproduction of *Scrippsiella trochoidea* isolated from Muroran Harbor Hokkaido. Nippon Suisan Gakkaishi 57, 1215.

Uchida, T., Matsuyama, Y., Yamaguchi, M., Honjo, T., 1996. The life cycle of *Gyrodinium instriatum* (Dinophyceae) in culture. Phycological Research 44, 119-123.

Uchida, T., 2001. The role of cell contact in the life cycle of some dinoflagellate species. Journal of Plankton Research 23, 889-891.

Van Lenning, K., Vila, M., Masó, M., Garcés, E., Anglès, S., Sampedro, N., Morales-Blake, A., Camp, J., 2007. Short-term variations in development of a recurrent toxic *Alexandrium minutum*-dominated dinoflagellate bloom induced by meteorological conditions. Journal of Phycology 43, 892-907.

Vila, M., Camp, J., Garcés, E., Masó, M., Delgado, M., 2001. High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean. Journal of Plankton Research 23, 497-514.

Vila, M., Giacobbe, M.G., Masó, M., Gangemi, E., Penna, A., Sampedro, N., Azzaro, F., Camp, J., Galluzzi, L., 2005. A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas. Harmful Algae 4, 673-695.

von Stosch, H.A., 1973. Observations on vegetative reproduction ans sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. British Phycological Journal 8, 105-134.

Walker, L.M., 1984. Life histories, dispersal, and survival in marine, planktonic dinoflagellates. In: Steindinger, K.A., Walker, L.M. (Eds.), Marine Plankton Life Cycle Strategies.

Wyatt, T., Jenkinson, I.R., 1997. Notes on *Alexandrium* population dynamics. Journal of Plankton Research 19, 551-575.

5

In situ life-cycle stages of *Alexandrium tamarense* species complex during a bloom development in Long Island (USA)

In preparation

In situ life-cycle stages of *Alexandrium tamarense* species complex during a bloom development in Long Island (USA)

Sílvia Anglès[1], Esther Garcés[1], Christopher J. Gobler[2]

[1] Institut de Ciències del Mar (CSIC), Pg. Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain

[2] Stony Brook University, School of Marine and Atmospheric Sciences, Southampton, NY 11968, USA

Abstract

Knowledge of the specific life-cycle dynamics during bloom development is essential for understanding and forecasting the onset, evolution and future occurrence of bloom events. In this study, quantification of life-cycle stages of the *A. tamarense* complex from the onset to the decline of a bloom in Northport Harbor (Long Island, USA) was conducted in the water column and in the sediments. Moreover, in situ excystment and encystment were quantified through the deployment of excystment and encystment traps, respectively. During April–June 2008, a bloom of the *A. tamarense* complex developed for 6 weeks and reached maximum vegetative cell abundance of 1.3×10^6 cells l^{-1}. Despite very low abundance of resting cysts in the sediment at the onset of the bloom, excystment of these resting cysts provided inoculum of vegetative cells for bloom development. Furthermore, all mature resting cysts in the surface sediments germinated during the development of the bloom. Sexual reproduction started at the exponential phase of the bloom. First detection of planozygotes in the water column occurred at vegetative cell abundances of ~10^4 cells l^{-1}, which implied a threshold of cell density for successful sexual reproduction. Planktonic planozygotes, and nonmotile planozygotes and resting cysts in the encystment traps were detected concurrently during the first vegetative bloom peak. Since planozygotes are known to swim in the water column for 1–2 weeks before settling to the sediments, the increasing trends in abundance observed for planktonic planozygotes, and nonmotile planozygotes and resting cysts in the encystment traps likely reflected the accumulation of planozygotes that had progressively formed during the bloom. The estimated encystment rates and planozygote percentages respect to vegetative cells were low, indicating that a small proportion of the vegetative cell population is involved in sexual reproduction. However, encystment was certainly a crucial process to replenish the seed stock of the *A. tamarense* complex in the area. For the first time, formation of pellicle cysts in the field was observed, which coincided with high cell densities during the bloom.

Resum

El coneixement de la dinàmica del cicle de vida de les espècies durant el desenvolupament de les proliferacions algals és essencial per entendre i predir l'aparició, evolució i futurs episodis de proliferacions. En aquest estudi, es varen quantificar in situ les fases del cicle de vida del complex *Alexandrium tamarense* tant en la columna d'aigua com en els sediments des de l'inici fins l'acabament d'una proliferació a Northport Harbor (Long Island, EUA). A més, es van quantificar l'excistament i enquistament mitjançant trampes dissenyades per cada un dels processos. Durant l'abril-juny de 2008, es va desenvolupar una proliferació del complex *A. tamarense* durant 6 setmanes i va arribar fins a una màxima abundància de cèl·lules vegetatives de 1.3×10^6 cèl·lules l^{-1}. Malgrat una presència molt escassa de cists de resistència en el sediment a l'inici de la proliferació, l'excistament d'aquests cists de resistència varen donar lloc a l'inòcul de les cèl·lules vegetatives per al desenvolupament de la proliferació. A més, tots els cists de resistència madurs en els sediments superficials varen germinar durant el desenvolupament de la proliferació. La reproducció sexual es va iniciar a la fase exponencial de la proliferació. La primera detecció de planozigots a la columna d'aigua es va produir quan les abundàncies de cèl·lules vegetatives varen arribar a $\sim 10^4$ cèl·lules l^{-1}, el que indica un llindar de densitat de cèl·lules vegetatives per a una reproducció sexual amb èxit. Els planozigots planctònics, així com els planozigots immòbils i els cists de resistència en les trampes d'encistament es van detectar al mateix temps que el primer pic de la proliferació vegetativa. Les tendències creixents en l'abundància observada de planozigots planctònics, i planozigots immòbils i cists de resistència en les trampes d'encistament reflectiren l'acumulació de planozigots que s'havien anat formant progressivament durant la proliferació, ja que es coneix que els planozigots són capaços de nedar en la columna d'aigua durant 1-2 setmanes abans de dipositar-se en els sediments. Les taxes estimades de encistament i els percentatges de planozigots respecte les cèl·lules vegetatives varen ser baixes, el que indica que tan sols una petita proporció de la població de cèl·lules vegetatives està involucrada en la reproducció sexual. Tot i així, l'encistament va ser el procés clau per emplenar les existències de cists de resistència del complex *A. tamarense* en els sediments superficials de l'àrea. Es va observar per primera vegada, la formació de cists peliculars en el camp, que va coincidir amb alta densitat de cèl·lules vegetatives durant la proliferació.

Introduction

The life cycle of dinoflagellates typically involves an alternation between a motile planktonic stage, the vegetative cell, and a nonmotile benthic stage, the resting cyst. Vegetative cells constitute the planktonic phase by dividing asexually. At a given time, vegetative cells reproduce sexually by forming gametes, two of which fuse to form a motile planozygote. After its maturation in the water column, which can last up to several days, the planozygote loses its motility and settles to the sediment. The nonmotile planozygote undergoes encystment and produces a resting cyst. Once formed, the resting cyst enters into a mandatory dormancy period of different species-specific lengths, during which excystment (or germination) is physiologically inhibited (von Stosch, 1973; Walker, 1984). After this period, the resting cyst is mature and able to germinate if environmental conditions (e.g. temperature, oxygen, and light) are favorable (Pfiester and Anderson, 1987). Otherwise, the resting cyst remains dormant in the sediment, where it survives for several years due to its resistant wall and storage products. Resting cysts in the sediments constitute a 'seed bed', which allows further blooms at the region where they are present (Dale, 1983; Steidinger, 1975). Several dinoflagellate species can also produce pellicle cysts, which are produced by vegetative cells. Unlike resting cysts, pellicle cysts possess a thin wall, and can germinate within short time since they are formed (Bravo et al., 2010; Dale, 1977). Furthermore, in dinoflagellates there are even more complicated life cycles to be found (Figueroa and Bravo, 2005; Figueroa et al., 2006; 2008).

The genus *Alexandrium* includes species which cause paralytic shellfish poisoning (PSP). Blooms associated with *Alexandrium* species frequently cause public health, economic, and ecological impacts worldwide. Humans are most commonly sickened by PSP toxins by the consumption of filter feeding shellfish which have bioaccumulated toxic Alexandrium cells (Shumway, 1990). On an ecosystem level, PSP toxins concentrate within the food chain, impacting multiple trophic levels such as zooplankton, fish larvae, adult fish, birds and marine mammals (Doucette et al., 2005). On the US east coast, PSP is a substantial and recurrent problem from Maine to Massachusetts, but also occurs sporadically in Connecticut, New York, and New Jersey (Anderson et al., 1994). However, there is uncertainty regarding the taxonomy of the organism causative of the PSP events, and thus the organism is referred as the *Alexandrium tamarense* species complex. This complex, which includes the morphotypes *A. tamarense*, *A. catenella* and *A. fundyense*, is composed by a number of ribotypes according to their geographic origin and toxicity (Scholin et al., 1994). In the US northeastern coast, both the morphotypes *A. tamarense* and *A. fundyense* have the North American ribotype (Scholin et al., 1994), or ribotype I (Lilly et al., 2007). In this study, the term *A. tamarense* complex is used to refer to the causative organism of a bloom in Northport Harbor (Long Island, USA).

Blooms of the *A. tamarense* complex in Long Island are small-scale events confined to embayments in contrast to the recurrent large-scale, widespread outbreaks of this species occurring from Maine to Massachusetts. The occurrence of these two types of blooms is considered to be unconnected (Anderson, 1997). Blooms of the *A. tamarense* complex in Long Island were first reported in the early 1980s, with abundances usually below 10^4 cells l^{-1} from early March to mid-June in several embayments (Schrey et al., 1984). At the same time, presence of resting cysts was reported at a few dispersed locations in Long Island (Anderson et al., 1982). The planktonic populations in the isolated embayments are thought to be initiated by germination of the resting cysts from local seed beds at the beginning of the annual growth cycle. The following events of sexual reproduction, influenced by biotic and abiotic factors, ultimately determine resting cyst beds that will provide the bloom inoculum in subsequent years (Anderson, 1998). Therefore, knowledge of the specific life-stages dynamics during bloom development is essential for understanding and forecasting the onset, evolution and future occurrence of bloom events. With this purpose, the present study focused on the appearance of life-cycle stages of the *A. tamarense* complex from the onset to the decline of a bloom in Northport Harbor by observations in the water column and in the sediments. Moreover, resting cysts in the sediment were quantified to evaluate their role in the onset of the vegetative cells bloom, and their abundance at end of the bloom as result of sexual reproduction. In addition, we obtained field-based information on the transitions between the stages, specifically excystment and encystment.

Methods

Identification and quantification of life-cycle stages in the water column

The study was conducted in Northport Harbor, located in the southeastern part of Northport Bay, on the north shore of Long Island (New York, USA, Fig. 1). This shallow system (2 – 4 m) is well mixed without major sources of freshwater discharged and was never stratified with respect to temperature or salinity during this study. Surface samples from the water column were collected 1–2 times per week from April through June at three stations (2, 7, and 8) for the evaluation of life cycle stages (vegetative cells and planozygotes). Samples were Lugol's-preserved after collection, settled in counting chambers and enumerated in an appropriate area on an inverted microscope (Nikon Eclipse TS 100). Vegetative cells of the A. tamarense complex were identified according to the criteria of Balech (1995) after staining with Calcofluor-White solution (Fritz and Triemer, 1985) and observed with UV fluorescence. Planozygotes were identified based on their size and color, since planozygotes of the A. tamarense complex are larger and darker than vegetative cells (Anderson et al., 1983).

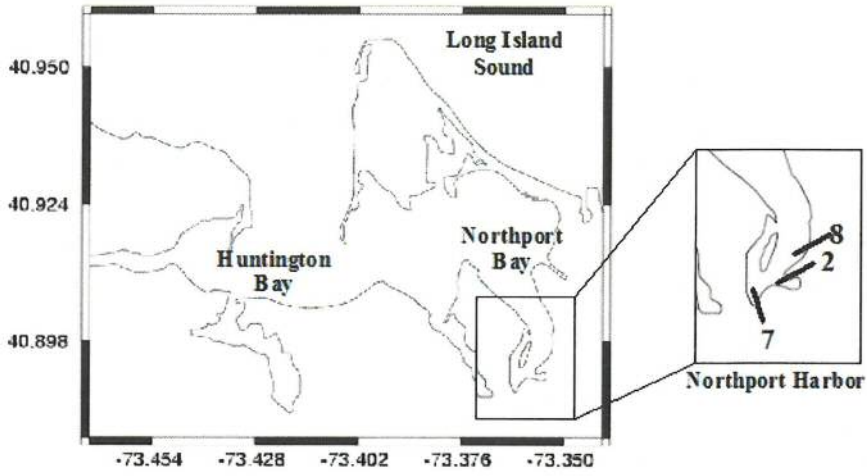


Fig.1. Location of Northport Harbor and the sampling sites monitored during this study.

In situ excystment

Two excystment traps were deployed at stations 2, 7, and 8 to monitor excystment of *A. tamarense* species complex. The excystment traps were identical to those previously used for *A. minutum* germination studies in Anglès et al. (submitted), with some modifications. The traps consisted of empty 20-l low density polyethylene water bottles with the bottom removed and two holes on the sides covered with 20-µm mesh. At the mouth of each trap, about 40 cm above the sediment surface, a 500-ml polyethylene terephthalate (PET) clear plastic collection bottle was attached upside down. The excystment traps were filled with filtered seawater (0.22 µm) at the surface and were submerged by a scuba diver with the bottom completely covered by a 20-µm mesh. The traps were inserted into the sediment at the sampling stations by 3 bars placed around the bottom. When the bottom was in contact with the sediment, the 20-µm mesh was rapidly but carefully pulled out. The excystment traps were deployed on 25 April and were not removed until 13 June, with the exception of excystment traps at station 8, which were lost after 7 May. Collection bottles were retrieved every 6 to 16 days (see dates in Table 2) by a Scuba diver. After removal of each collection bottle, a new bottle filled with filtered seawater was attached to the excystment trap. The content of the collection bottles was preserved with Lugol's solution immediately after bottles were returned to the surface. Aliquots of 10–50 ml were counted in an inverted microscope at 200× magnification using Utermöhl chambers. For this study, we utilized the excystment trap data as a qualitative indication of the time period of when excystment was occurring and when excystment ended, not to quantify a precise excystment rate.

Quantification of resting cysts in the sediment

Sediment samples from Northport Harbor were obtained by a scuba diver. Two sediment cores (20 cm long × 5 cm base diameter) were collected at stations 2, 7, and 8 on 25 April when the excystment traps were deployed. When the traps were retrieved at stations 2 and 7 on 13 June, two cores were collected from the sediment under the traps and two additional cores were collected from outside the traps. Samples were stored in the dark at 4°C and left to settle for 24 h before immediate processing. The water above the cores was carefully removed and the top 1-cm was cut. A subsample of this portion was sonicated and sieved to retain the 10- to 100-µm fraction. A 5-ml subsample of the slurry was processed by the SPT density gradient methodology of Bolch (1997) as modified by Amorim et al. (2001) and Bravo et al. (2006). The resulting sample was rinsed with filtered seawater on a 10-µm mesh sieve and collected to a 5-10 ml volume. Aliquots of 2 ml of this sample were sedimented in settling chambers to enumerate resting cysts of *A. tamarense* complex with an inverted microscope (Nikon Eclipse TS 100). Resting cyst abundance is expressed in cysts ml^{-1} of wet sediment (ws).

Fluxes of life-cycle stages to the sediments

Sedimentation of life-cycle stages was monitored by means of encystment traps placed at station 2, as it was located at the entrance of a marina and allowed the convenient deployment of the traps. The traps consisted of two cylindrical collection containers (height 22.3 cm, diameter 10.5 cm, aspect ratio 2.1) suspended 1.3 m from a floating dock of the marina. Traps were collected and replaced every 2 to 4 days from 1 May to 13 June (see exact dates in Table 3). Samples were kept in the dark without adding preservatives, and processed immediately upon arrival to the laboratory. A 400-ml subsample of the content of each container was sonicated and sieved to retain the 10- to 100-µm fraction. Aliquots of 2 ml of the sieved sample were sedimented in settling chambers to enumerate *A. tamarense* complex life stages with an inverted microscope (Nikon Eclipse TS 100). *A. tamarense* complex planozygotes and cysts were identified based on morphology, shape, color, wall thickness and size.

Resting cysts enumerated within the encystment traps were used for cyst flux and encystment rates estimates. The encystment rate was calculated by the following equation (Anglès et al., submitted; Garcés et al., 2004):

$$K_{en} = \frac{2C_{en}}{V_{en} + 2C_{en}} \qquad (1)$$

where C_{en} is the resting cysts collected in the encystment traps per day (cyst m^{-2} d^{-1}) and V_{en} the standing stock of vegetative cells above the traps (cells m^{-2}) in the overlying water column (1.3 m). C_{en} was multiplied by 2 since each cyst is produced from 2 cells. V_{en} corresponds to the mean vegetative cell abundance, quantified from the day the traps were placed until the day they were removed.

Results

Morphological characteristics of the life stages

The vegetative cells of the *A.tamarense* complex identified with Calcofluor-White solution presented a ventral pore in the first apical plate (1'), and a pore in the posterior sulcal plate (sp). Cells appeared mainly as single cells or formed occasionally short chains of 2–3 cells (Fig. 2A and 2B). According to these morphological features, these cells belonged to the *A. tamarense* morphotype. The characteristics of the planozygote in the water column and its morphological difference with respect to the vegetative cell are shown in Figure

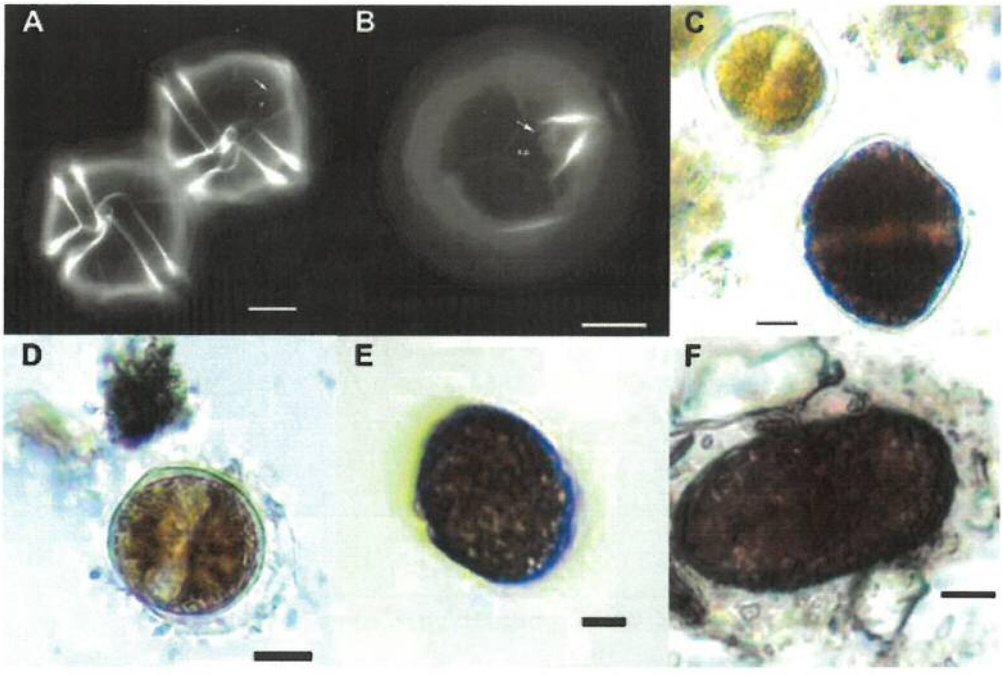


Fig. 2. Light micrographs of *A. tamarense* complex life stages. Vegetative cells stained with Calcofluor-White solution in water sample: (A) chain of two vegetative cells, with ventral pore in the first apical plate (1'), and (B) posterior sulcal plate (sp) with pore; vegetative cell (left) and planozygote (right), Lugol's-fixed water sample (C); life stages from the encystment trap, alive sample: (D) pellicle cyst; (E) planozygote, and (F) resting cyst. Scale bar 10 µm.

2C. Life-cycle stages observed in the sediment traps included nonmotile planozygotes, pellicle cysts and resting cysts (Fig. 2D–F). Pellicle cysts were characterized by a thin wall, and a cytoplasmic content of green, uncondensed chloroplasts. Resting cysts in the encystment traps were dark and densely granulated, with a scarcely visible brown–yellow accumulation body.

Life-cycle stages in the water column

Bloom development of the *A. tamarense* complex lasted for six weeks. The first peak of the bloom occurred on 16 May at 1.3×10^6 cells l^{-1}, and a second, less pronounced bloom peak occurred on 26 May (5.2×10^5 cells l^{-1}) (Fig. 3A). Planozygotes were first observed in the water column on 14 May, coinciding with the exponential phase of the bloom when

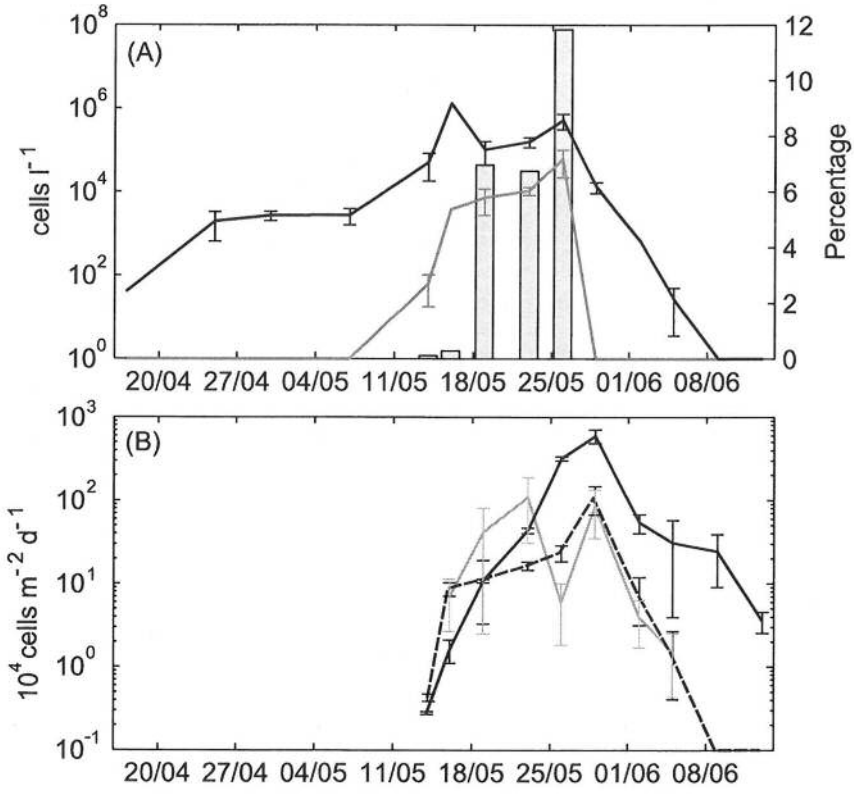


Fig. 3. (A) Life-stages fluctuations of the *A. tamarense* complex in the water column: vegetative cells (black solid line), planozygotes (gray solid line), and percentages of planozygotes respect to vegetative cells (bars). Data are mean values calculated from abundances at stations 2, 7, and 8 (n=3). Error bars represent SD. (B) Life-stages fluctuations of the *A. tamarense* complex in the encystment traps: resting cysts (black solid line), planozygotes (black dashed line), and pellicle cysts (gray solid line). Values are mean abundances (n=2). Error bars represent SD

the vegetative cell abundance increased from 2.8×10^3 cells l^{-1} (7 May) to 5.1×10^4 cells l^{-1} (14 May). After this date, planozygotes progressively increased until the second peak of the bloom (26 May). Following this date, planozygotes were no longer detected in the water column. Planozygotes represented between 0.1 to 11.8 % of the total planktonic *A. tamarense* complex vegetative cells during the bloom with the lowest percentages being present during the exponential phase of the bloom and first vegetative bloom peak (14–16 May; Fig. 3A). After this first peak, a sharp increase in the percentage values of planozygotes was observed, which attained the highest percentage during the second vegetative peak (26 May).

Resting cysts in sediments

Resting cysts of the *A. tamarense* complex in the sediment were quantified to evaluate their role in the onset of the vegetative cells bloom, and their abundance at end of the bloom as result of sexual reproduction. On 25 April, resting cyst mean abundance at the three stations was very low, ranging from 2 to 25 cysts ml^{-1} wet sediment (ws; Table 1). On 5 June, at the end of the bloom, mean resting cyst abundance in the sediment outside the area covered by the excystment traps increased notably to 1272 and 4078 cysts ml^{-1} ws at stations 2 and 7, respectively. Conversely, resting cysts of the *A. tamarense* complex were undetected in the sediment under the excystment traps at both stations.

Dates	Sites		
	2	7	8
25 Apr	3(±1)	2(±0)	25(±4)
5 Jun (outside trap)	1272(±392)	4078(±687)	-
5 Jun- (inside trap)	0	0	-

Table 1. Mean abundance (±SD, n=2) of resting cysts in the sediments (cysts ml^{-1} ws) from Northport Harbor. For site 8, no data are available on 5 June.

In situ excystment

Presence of germinated cells of the *A. tamarense* complex was observed in the excystment trap samples at the three stations only from 25 April to 23 May (Table 2). Specifically, germinated cells were found at station 2 and 8 during the sampling period from 25 April to 7 May, while station 7 presented germinated cells from 7 to 23 May. At station 8, data was unavailable after 7 May due to the lost of the two excystment trap replicates.

Sampling dates	Sites		
	2	7	8
25 Apr – 7 May	45831±15898	0	24800±22345
7 – 23 May	0	2300±990	-
23 – 29 May	0	0	-
29 May – 5 Jun	0	0	-

Table 2. Mean abundance (±SD, n=2) of germinated cells in the excystment traps (cells l^{-1}) deployed in Northport Harbor. For site 8, no data are available from 7 May to 5 June.

Life-cycle stages in the encystment traps, and in situ encystment of resting cysts

Nonmotile planozygotes, pellicle cysts, and resting cysts of the *A. tamarense* complex were all observed in the encystment traps deployed at station 2 (Fig. 3B). Pellicle cysts were observed coinciding with the first vegetative bloom peak, and attained their higher abundances when vegetative cell densities in the water column were $>10^5$ cells l^{-1} (Fig. 3B). Nonmotile planozygotes and resting cysts were first observed on 14 May at low abundances. Their presence also coincided with the first detection of planozygotes in the water column (Fig. 3A). Both planozygote and resting cyst abundances increased until reaching their highest values in late May, a few days after both the second bloom peak and the maximum planozygote abundance in the water column. Following this date, sedimentation of resting cysts decreased gradually, whereas planozygotes displayed a sharper decline. Estimated encystment rates of resting cysts increased from low values during the exponential phase to higher values during the decline of the bloom (Table 3).

Sampling dates	Standing stock of cells above trap	Resting cyst flux	Percentage of encystment
1 – 14 May	2391	0.1	0.01
14 – 16 May	100093	2.9	0.006
16 – 19 May	116118	10.8	0.02
19 – 23 May	26387	43.4	0.3
23 – 26 May	31428	317.4	1.8
26 – 29 May	24303	594.3	5.0
29 May – 2 Jun	1199	54.2	8.8
2 – 5 Jun	78	30.9	45.9
5 – 9 Jun	30	24.2	63.3
9 – 13 Jun	0	3.55	100

Table 3. Standing stock of vegetative cells in the water column (1.3 m) above the encystment traps (10^4 cells m^{-2}), resting cyst flux (10^4 cysts m^{-2} d^{-1}), and encystment rates expressed in percentages.

Discussion

The present study describes the in situ life-cycle stages of the largest *A. tamarense* complex bloom ever recorded on the east coast of the US south of Massachusetts. The presence of germinated cells of the *A. tamarense* complex in the excystment traps confirmed that germination of resting cysts within the harbor was involved in the occurrence of the bloom. Excystment of resting cysts of the *A. tamarense* complex has been shown to occur within a temperature window of 5–21°C (Anderson and Morel, 1979; Anderson, 1998). This temperature range is consistent with that recorded during excystment in Northport Harbor (10–21°C; Hattenrath et al., 2010). Excystment was found to coincide with the initial stages of the vegetative cell bloom. This finding indicates that, despite their very low abundance, resting cysts in the sediment provided inoculum of vegetative cells for bloom development. The environmental conditions favorable for vegetative cell growth, namely anthropogenic nitrogen loading and ideal temperatures, ultimately determined the magnitude of the bloom (Hattenrath et al., 2010). The absence of resting cysts in the sediments covered by the excystment traps detected after the bloom suggests that all mature resting cysts present in the surface sediments germinated during the development of the bloom. Since the resting cyst abundances at the monitored stations were among the highest of other studied areas in the harbor (Hattenrath et al., 2010), it is likely that mature resting cysts were also depleted elsewhere in the harbor.

Our study combining observation of life stages in water samples and encystment traps allowed monitoring the transition from the vegetative cell to the resting cyst stage during the bloom development. Planozygotes in the water column were detected when vegetative cell population attained abundances of ~10^4 cells l^{-1}. This suggests that successful sexual reproduction is predicated upon the achievement of this threshold of cell density. The requirement of a minimum cell density for sexual reproduction has already been suggested and supported by several studies (Anglès et al., submitted; Garcés et al., 2004; Wyatt and Jenkinson, 1997). Furthermore, the appearance of planozygotes during the exponential phase of the bloom gives support to the hypothesis that sexual reproduction begins during the earlier stages of the bloom when vegetative cells are actively growing and dividing (Anglès et al., submitted; Anderson et al., 1983; Garcés et al., 2004; Wall et al., 1970).

Recently formed planozygotes of the *A. tamarense* complex are able to remain swimming in the water column for 1–2 weeks before settling to the sediments, where they form resting cysts (Anderson et al., 1983). Nonmotile planozygotes and resting cysts were first observed in the traps coinciding with the detection of planktonic planozygotes. These findings indicate that planozygote formation most likely occurred several days prior. The subsequent steady increase in planktonic planozygotes as well as in nonmotile planozygotes and resting cysts in the encystment traps likely reflected the accumulation of planozygotes that had progressively formed during the bloom. Indeed, nonmotile

planozygotes settled into the encystment traps during approximately 10 days after their disappearance from the water column.

The observed delay between planozygote formation in the water column and detection of resting cysts in the traps could bias estimate of the encystment percentages. Nevertheless, it can be concluded that encystment rates were generally low until the end of the bloom. The percentages estimated at the decline of the bloom may be overestimates, however, since they were strongly influenced by the low vegetative cell numbers in the water column at that moment and the accumulation of planozygotes. Similarly, the percentage of planozygotes relative to the vegetative cells was low. Both the low encystment rates and planozygote percentages suggest that a small proportion of the vegetative cell population was involved in sexual reproduction. This finding is consistent with other studies of life-cycle stages of *Alexandrium* in European embayments (Anglès et al., submitted; Garcés et al., 2004). In contrast, other field studies report higher encystment rates and planozygote percentages for the *A. tamarense* complex (Anderson et al., 1983). One reason for this discrepancy could be an underestimation of planozygotes. In this study we used morphological features to quantify planozygotes in the water column. Differences in cell size have been previously used to distinguish between planozygotes and vegetative cells (e.g., Anderson et al., 1983; Anderson and Lindquist, 1985; Probert, 1999). It should be noted, however, that this approach may underestimate small-sized or non-mature planozygotes, as reported in other *Alexandrium* species (Figueroa et al., 2007). These authors solved the identification of planozygotes by use of flow cytometry to measure the DNA content. However, this methodology, tested in laboratory cultures, is currently not applicable to field studies for our target species. Another reason for the underestimation of planozygotes could be the aggregation of stages in thin layers or pycnoclines, which has been reported to be significant for gametes to fuse in some environments (Persson et al., 2008). Our sampling method, based on discrete water surface samples, could overlook cell aggregations and underestimate total planozygote abundance.

Encystment has been definitely proved as a strategy to replenish the seed stock of the *A. tamarense* complex in the area. The cumulative resting cyst flux to the sediments would result in a resting cyst production of 3×10^7 resting cyst m^{-2}. This value is consistent with our quantification of cysts following the bloom (1000–4000 cysts ml^{-1} ws = $1–4 \times 10^7$ resting cyst m^{-2}). These resting cyst abundances were two-to-three orders of magnitude greater than densities found at the start of the bloom, likely reflecting the extreme density achieved by this bloom. These resting cysts constituted a significant seed bed for the subsequent dense blooms which occurred in this system in 2009 and 2010 (C. Gobler, pers. obs.).

In this study, the deployment of encystment traps also allowed the detection of pellicle cysts for the first time in field populations. As noted in a recent review by Bravo et al. (2010), observations of pellicle cysts of the *A. tamarense* complex have been limited to culture

conditions in which nutrients were deficient and changes in temperature were applied. In addition, formation of pellicle cysts by the *A. tamarense* complex also occurred after the passage of vegetative cells through the digestive tract of shellfish (referred as *A. fundyense*; Hegaret et al., 2008; Persson et al., 2006) Pellicle cysts were observed during the bloom and coincided with the highest peaks in the abundance of vegetative cells ($>10^5$ cells l^{-1}) as observed in other *Alexandrium* field populations (Anglès et al., submitted; Garcés et al., 2004). Further studies should be conducted to ascertain the possible role of pellicle cysts in bloom dynamics of the *A. tamarense* complex.

Acknowledgements

We thank the people who helped in fieldwork: T. Hattenrath, A. Marcoval, and C. Wall. This research was funded by a fellowship from the AGAUR (Generalitat de Catalunya) and by the New York State Department of Environmental Conservation. The work of E. Garcés was supported by a Ramon y Cajal contract from the MICINN.

References

Amorim, A., Dale, B., Godinho, R., Brotas, V., 2001. *Gymnodinium catenatum*-like cysts (Dinophyceae) in recent sediments from the coast of Portugal. Phycologia 40, 572-582.

Anderson, D.M., Morel, F.M.M., 1979. The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. Estuarine and Coastal Marine Science 8, 279-293.

Anderson, D.M., Kulis, D.M., Orphanos, J.A., Ceurvels, A.R., 1982. Distribution of the toxic dinoflagellate *Gonyaulax tamarensis* in the southern New England region. Estuarine Coastal and Shelf Science 14, 447-458.

Anderson, D.M., Chisholm, S.W., Watras, C.J., 1983. Importance of life-cycle events in the population dynamics of *Gonyaulax tamarensis*. Marine Biology 76, 179-189.

Anderson, D.M., Kulis, D.M., Doucette, G.J., Gallagher, J.C., Balech, E., 1994. Biogeography of toxic dinoflagellates in the Genus *Alexandrium* from the northeastern United States and Canada. Marine Biology 120, 467-478.

Anderson, D.M., 1997. Bloom dynamics of toxic *Alexandrium* species in the northeastern US. Limnology and Oceanography 42, 1009-1022.

Anderson, D.M., 1998. Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: Anderson, D.A., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Berlin-Heidelberg, pp. 29-48.

Anglès, S., Garcés, E., Reñé, A., Sampedro, N. Life-cycle alternations in *Alexandrium minutum* natural populations. Submitted to Marine Ecology Progress Series.

Balech, E., 1995. The genus *Alexandrium* Halim. Sherkin Island Marine Station, Sherkin Island, Co. Cork, Ireland.

Bolch, C.J.S., 1997. The use of sodium polytungstate for the separation and concentration of living dinoflagellate cysts from marine sediments. Phycologia 36, 472-478.

Bravo, I., Garcés, E., Diogene, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. European Journal of Phycology 41, 293-302.

Bravo, I., Figueroa, R.I., Garcés, E., Fraga, S., Massanet, A., 2010. The intricacies of dinoflagellate pellicle cysts: The example of *Alexandrium minutum* cysts from a bloom-recurrent area (Bay of Baiona, NW Spain). Deep Sea Research Part II: Topical Studies in Oceanography 57, 166-174.

Dale, B., 1977. Cysts of the toxic red-tide dinoflagellate *Gonyaulax excavata* (Braarud) Balech from Oslofjorden, Norway. Sarsia 63, 29-34.

Dale, B., 1983. Dinoflagellate resting cysts: "benthic plankton". In: Fryxell, G.A. (Ed.), Survival Strategies of the Algae. Cambridge Univ. Press, pp. 69-136.

Doucette, G.J., Turner, J.T., Powell, C.L., Keafer, B.A., Anderson, D.M., 2005. Trophic accumulation of PSP toxins in zooplankton during *Alexandrium fundyense* blooms in Casco Bay, Gulf of Maine, April-June 1998. I. Toxin levels in A-fundyense and zooplankton size fractions. Deep-Sea Research Part Ii-Topical Studies In Oceanography 52, 2764-2783.

Figueroa, R.I., Bravo, I., 2005. Sexual reproduction and two different encystment strategies of *Lingulodinium polyedrum* (Dinophyceae) in culture. Journal of Phycology 41, 370-379.

Figueroa, R.I., Bravo, I., Garcés, E., 2006. Multiple routes of sexuality in *Alexandrium taylori* (Dinophyceae) in culture. Journal of Phycology 42, 1028-1039.

Figueroa, R.I., Bravo, I., Ramilo, I., Pazos, Y., Moroño, A., 2008. New life-cycle stages of *Gymnodinium catenatum* (Dinophyceae): laboratory and field observations. Aquatic Microbial Ecology 52, 13-23.

Fritz, L., Triemer, R.E., 1985. A rapid simple technique utilizing Calcofluor white M2R for the visualization of dinoflagellate thecal plates. Journal of Phycology 21, 662-664.

Garcés, E., Bravo, I., Vila, M., Figueroa, R.I., Masó, M., Sampedro, N., 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean). Journal of Plankton Research 26, 637-645.

Hattenrath, T.K., Anderson, D.M., Gobler, C.J., 2010. The influence of anthropogenic nitrogen loading and meteorological conditions on the dynamics and toxicity of *Alexandrium fundyense* blooms in a New York (USA) estuary. Harmful Algae 9, 402-412.

Lilly, E., Halanych, K., Anderson, D., 2007. Species boundaries and global biogeography of the *Alexandrium tamarense* complex (Dinophyceae). Journal of Phycology 43, 1329-1338.

Persson, A., Smith, B.C., Wikfors, G.H., Quilliam, M., 2006. Grazing on toxic *Alexandrium fundyense* resting cysts and vegetative cells by the eastern oyster (*Crassostrea virginica*). Harmful Algae 5, 678-684.

Pfiester, L.A., Anderson, D.M., 1987. Dinoflagellate Reproduction. In: Taylor, F.J.R. (Ed.), The Biology of Dinoflagellates. Blackwell Scientific Publications, Oxford, pp. 611-648.

Probert, I., 1999. Sexual reproduction and ecophysiology of the marine dinoflagellate *Alexandrium minutum* Halim. University of Westminster, London, p. 99.

Scholin, C.A., Herzog, M., Sogin, M.L., Anderson, D.M., 1994. Identification of group and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). II. Sequence analysis of a fragment of the LSU rRNA gene. Journal of Phycology 30, 999-1011.

Schrey, S.E., Carpenter, E.J., Anderson, D.M., 1984. The abundance and distribution of the toxic dinoflagellate, *Gonyaulax tamarensis*, in Long-Island estuaries. Estuaries 7, 472-477.

Shumway, S.A., 1990. A review of the effects of algal blooms on shellfish and aquaculture. Journal of the World Aquaculture Society 21, 65-104.

Steidinger, K.A., 1975. Implications of dinoflagellate life cycles on initiation of *Gymnodinium breve* red tides. Environmental letters 9, 129-139.

von Stosch, H.A., 1973. Observations on vegetative reproduction ans sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. British Phycological Journal 8, 105-134.

Walker, L.M., 1984. Life histories, dispersal, and survival in marine, planktonic dinoflagellates. In: Steindinger, K.A., Walker, L.M. (Eds.), Marine Plankton Life Cycle Strategies.

Wyatt, T., Jenkinson, I.R., 1997. Notes on *Alexandrium* population dynamics. Journal of Plankton Research 19, 551-575.

General discussion

General discussion

In semi-enclosed systems characterized by low hydrodynamism and water column stabilization, the biological processes may prevail over the physical ones. Life-cycle features in population dynamics are thus more relevant in these systems compared to open coastal ones. Studies of life-cycle features, including planktonic- and benthic-phase dynamics, and measurement of in situ encystment and excystment are therefore more tractable in these semi-enclosed systems.

Study of the planktonic-phase dynamics of target species in diverse embayments

For a better understanding of the benthic-phase dynamics, accurate investigations on the planktonic-benthic phase coupling are needed. Studies of planktonic phase dynamics are typically based on the sampling and measurement of several physical, chemical, and biological variables. The effort required by this type of analysis, particularly the labor-intensive microscopy techniques for phytoplankton identification, hampers the ability to obtain data of adequate resolution to address the high spatio-temporal variability of vegetative cell abundance.

To overcome this limitation, several technologies are currently being explored (Babin et al., 2005, 2008). In this thesis, the LISST-100X particle-size analyzer was used to examine the high spatio-temporal variability of a phytoplankton population dominated by the high-biomass species *A. taylori* at La Fosca beach (**Paper 1**). The high-resolution temporal data allowed the high variability in abundance to be related over a short time scale to the diel vertical migration of the phytoplankton population. LISST-100X measurements of vegetative cells coupled with simulations obtained using the physical numerical model showed that surface-water circulation, determined by the prevailing wind regime, influences vegetative cell distribution within the surface waters of the beach. This finding has significant implications in terms of the distribution of resting cysts following a bloom, as discussed below.

The good agreement between LISST-100X measurements and microscopy counts supports the utility of the analyzer for measuring cell abundance in phytoplankton populations. Nonetheless, the LISST-100X does not provide autonomous information on species composition, and complementary microscopy analysis is still needed. During our study

in La Fosca beach, the size fraction method was applied to LISST-100X measurements to discriminate successfully between the two dominant phytoplankton species. These initial findings encouraged application of the instrument in the other embayments investigated in this thesis, specifically, in Arenys de Mar harbor. However, the phytoplankton community accompanying the target species (*A. minutum*) was highly diverse and some of the vegetative cells were non-spherically shaped, resulting in overlaps in cell sizes that hindered species discrimination. In addition, the presence of sediment particles in the water column, a frequent occurrence in the harbor, posed a likely source of error.

For future studies aimed at species identification, the Imaging Flow-Cytobot, still commercially unavailable, is a promising tool. This is a submersible particle analyzer and imager that combines aspects of microscopy and flow cytometry (Olson and Sosik, 2007; Sosik and Olson, 2007), yielding autonomous and real-time detection of phytoplankton species at a high temporal resolution. Use of the Imaging Flow-Cytobot allowed the observation and detailed analysis of bloom dynamics at the species level (Campbell et al., 2010).

Studies of the vegetative-phase dynamics described in **Papers 3** and **4** relied on microscopy, which was performed at a spatio-temporal frequency sufficient to assess the bloom dynamics of the target species. The vegetative cell dynamics detected at the routinely monitored stations provided a good baseline for the timing of both resting cysts and vegetative cells spatial surveys (**Paper 3**), and for intensifying the sampling effort in bloom situations (**Papers 3** and **4**).

Identification and quantification of the benthic phase, and investigation of its dynamics

Resting cyst diversity

A prerequisite to understanding the benthic phase is knowledge of the resting-cyst assemblages present in the sediments. By focusing on the seed bank in the sediments of the northwestern Mediterranean, this thesis has contributed to the scant knowledge of resting cyst diversity in sediments from this area (**Paper 2**). Previous studies of the harbors along the Catalan coast focused on the genus *Alexandrium* showed the presence of resting cysts belonging to eight species (Bravo et al., 2006, 2008; Garcés et al., 2004). However, the potential diversity of resting cysts of other dinoflagellate species was not addressed.

Diversity estimates based on resting cysts are much higher than those based on the presence of vegetative cells (Boero et al., 1996). The comparison of the two coastal embayments (Arenys de Mar harbor and the Gulf of Olbia) showed that the diversity

of cyst-producing species in their respective seed banks was high, hosting a greater number of species than recorded in the vegetative population of both sites. In addition, 10 morphotypes not previously described in the literature were identified. The vegetative cells that germinated from some of these morphotypes probably correspond to new species and the descriptions of them are undergoing.

The abundances of resting cysts were remarkably higher in Arenys harbor than in Olbia. One of the reasons for this difference may be the restricted hydrodynamics to which harbors are subjected. This would support the hypothesis that semi-enclosed systems are potential reservoirs of resting cysts, being harbors areas more prone to benthic-stage accumulation. Based on these findings, mapping the distribution of benthic stages of phytoplankton species in sediments from semi-enclosed areas, mainly harbors, would be a useful survey tool in combination with traditional planktonic sampling. This would allow monitoring of spreading events, detection of new species, and assessment of potential risk for HABs.

In addition to classic microscopy analysis, PCR probes for the detection of resting cysts have been recently developed. Sediment samples of the two sites including the ones considered in **Paper 2** were analyzed by microscopy and the results used to calibrate a new method of detection of resting cysts based on PCR assays (Penna et al., 2010). These molecular techniques yielded comparative or even more sensitive results than obtained by microscopy and thus may provide a useful methodology for faster analysis of species composition, without requiring expertise in taxonomic identification. However, the quantification of resting stages by PCR assays remains unresolved for the large majority of dinoflagellate species, with the exception of a few *Alexandrium* species (Erdner et al., 2010; Kamikawa et al., 2005).

Resting cyst dynamics

Detailed information on resting cysts dynamics is acquired by mapping the resting cysts of a target species in space and time at a relatively high frequency. As passive particles, resting cysts in the water column and in the sediments are influenced by the same forcing processes that control the dynamics of passive particles. Resting cysts disperse from a "point source," which influences their distribution and thus must be taken into account in studies of dinoflagellate populations and, especially, HABs (Wyatt, 2002). The spatial distribution of vegetative cell blooms throughout their entire duration (integrated in time) is called the "bloom shadow." Resting stages are known to be present where blooms develop, but their maximum accumulation may not be directly underneath the bloom shadow. Instead, resting cysts are found in deep basins at the continental slope or in close, shallow protected areas (e.g., Joyce, 2005; Villanoy et al., 2006; Wang et al., 2004). Although these observations have been confirmed, the specific mechanisms involved in resting cyst

distribution in the sediments are not fully known. Accordingly, this thesis analyzed the spatio-temporal distribution of *A. minutum* resting cysts in Arenys de Mar harbor in relation to the point source of resting-cyst formation (the distribution of vegetative cells), seiche-forced resuspension, and sediment granulometry. The spatial distribution of the vegetative cell bloom during the production of resting cysts within the harbor largely determined the sediment area where the cysts initially settled. In Arenys de Mar harbor, the preferential regions for vegetative cell accumulations are the northwestern and northeastern parts of the harbor (**Paper 3**; Garcés et al., 2004; Van Lenning et al., 2007; Vila et al., 2005), which coincides with the two preferential areas of resting cyst distribution observed following a bloom (**Paper 3**). Since wind-driven currents influence the distribution of vegetative cells at the water surface (**Paper 1**), the resting cyst distributions must likewise be the result of wind regimes favoring the accumulation of vegetative cells during bloom development (**Paper 3**).

During the periods in which resting cysts were not produced, they accumulated in areas where fine sediment fractions, mainly clays, tended to collect (**Paper 3**), confirming that hydrodynamic and sedimentary processes largely controlled their secondary dispersal (Dale, 1976). Seiches were identified for the first time as a major mechanism of sediment and resting cyst resuspension in marine semi-enclosed systems (**Paper 3**). This is a significant contribution to a better understanding of bloom dynamics in semi-enclosed systems where seiches may exert an influence, since resuspension is considered to favor the germination of resting cysts and the growth of vegetative cells. Moreover, seiche-forced resuspension was observed to influence the horizontal and vertical distributions as well as the abundance of resting cysts in the sediment.

In additional studies not presented in this thesis, sediment surveys were performed at La Fosca beach. The results showed that resting cysts of *A. taylori* were scarcely present in the beach sediments following a bloom (**Paper 1**). Later, during a survey conducted several months after the bloom, resting cysts were absent. The absence of resting cysts in La Fosca sediments is consistent with the beach's mainly sandy sediments. Instead, resting stages may collect in areas where fine sediments tend to accumulate, such as deep basins offshore or nearby zones protected from water movement. Unlike Arenys de Mar harbor, La Fosca beach is affected by wave-induced currents that generate turbulence in the bottom boundary layer. This may resuspend both resting cysts and fine sediment particles, favoring their current-mediated transport to areas more protected from bottom-boundary turbulence. In open waters, resting cysts may collect in areas far removed from their planktonic source, as a result of transport and concentration by hydrographic and sedimentary processes. Conversely, coastal embayments with restricted water circulation, such as harbors, may have a regularly repeated cycle whereby resting cysts in bottom sediments form seed beds. These produce local blooms which in turn produce resting cysts.

The coastline of the Catalan coast and many other coastal areas has been, and still is, modified through the construction of harbors, semi-enclosed beaches, and protective barriers. The increasing tendency in the construction of semi-enclosed systems such as harbors due to human activities is favoring the existence of reservoirs of resting cysts, including those of toxic species (**Paper 2**).

Assessment of the planktonic-benthic transitions in target species of selected sites

The study of in situ excystment and encystment has shown that these processes play a key role in the annual recurrence, initiation and maintenance of blooms, and in the success of the species.

Germination strategies in dinoflagellates are species-specific. In a study using germination traps, Ishikawa and Taniguchi (1996, 1997) identified three basic excystment patterns within cyst-producing species in the same community: "sporadic", "incessant", and "synchronous". As described in their study, species with a sporadic germination pattern lacked a clear seasonality in excystment, and their excystment rate was always very low. The incessant germination pattern occurred in species with continuous germination characterized by marked peaks in a particular season but which did not contribute remarkably to the planktonic population. Finally, in the synchronous germination pattern excystment was limited to a particular season depending on the species. As suggested by the authors of the study, the latter germination strategy resulted in synchronous contributions to the vegetative cell population, whereas the former two were an opportunistic strategy for maintaining the planktonic population throughout the year.

In this thesis, the excystment patterns of the two *Alexandrium* species were of the incessant (*A. minutum*, **Paper 4**) and synchronous (*A. tamarense* complex, **Paper 5**) types. In the case of *A. minutum*, the excystment peak (high excystment, **Paper 4**) was coincident with an increase in the irradiance and water temperature. In contrast to the observations of Ishikawa and Taniguchi (1996, 1997) for species undergoing incessant germination, the high excystment period coincided with the vegetative cells bloom, although the contribution of this germination had no effect on its magnitude. At the same time, continuous germination of *A. minutum* favored the maintenance of a background planktonic population throughout the year. These findings suggest that the incessant germination strategy of *A. minutum*, which included excystment peaks at a particular season, served to maintain the population and acted as a safety mechanism for re-inoculating vegetative cells. Synchronous germination of the *A. tamarense* complex was influenced by water temperature, as demonstrated in several previous studies (Anderson et al., 1979; Anderson 1980). Furthermore, the timing of excystment coincided with the

vegetative cell bloom (**Paper 5**). In this species, limiting the excystment period to the occurrence of favorable vegetative growth conditions increases the chances of bloom success, but during a restricted period.

The encystment process initiated at early bloom stages in the two *Alexandrium* species, with encystment fluxes observed over the bloom phases of exponential growth, maintenance and decline. The proportion of vegetative cells involved in encystment was low. Although physico-chemical factors did not appear to modulate encystment of either species, there was a high correlation between resting cyst and vegetative cell abundances for *A. minutum* (**Paper 4**). Accordingly, the magnitude of the encystment fluxes determines the abundance of *A. minutum* resting cysts found in the sediments of the harbor, as described in **Paper 3**. In the *A. tamarense* complex, this relationship between resting cyst and vegetative cell abundances was not observed. This could be due to the accumulation of planozygotes in the water column, which, once formed, can remain swimming for 1–2 weeks before encystment (**Paper 5**; Anderson et al., 1983). In contrast, the correlation between resting cyst and vegetative cell abundances was readily observed for *A. minutum* since planozygotes of this species have been reported to swim for up to 2-3 days after their formation (Figueroa et al., 2007; Probert, 1999).

A threshold density of vegetative cells may be a prerequisite for an effective sexual reproduction (Wyatt and Jenkinson, 1997). These authors suggested a threshold of $>10^4$ cells l^{-1}. In this thesis, resting cyst formation was observed when vegetative cell abundance reached 10^3–10^4 cells l^{-1} for both species. Several semi-enclosed systems are characterized by calm waters and long residence times, factors that favor cell aggregation and thus enable low thresholds (e. g., *A. minutum* in Arenys de Mar harbor, **Paper 4**). Conversely, the threshold density might be more difficult to attain in other systems with higher hydrodynamism (*A. tamarense* complex in Northport Harbor, **Paper 5**). In these systems, cell aggregation strategies of the organisms may be of major importance to reach the vegetative cell density necessary to ensure resting cyst formation.

During this thesis, other life-cycle stages have been described during field experiments. Pellicle cysts formation in natural conditions was observed in both *A. minutum* and *A. tamarense* complex. These pellicle cysts were observed during the bloom and they coincided with peaks of high abundance of vegetative cells ($>10^5$ cells l^{-1}). According to the recent review from Bravo et al. (2010), this thesis reports for the first time pellicle cyst formation by the *A. tamarense* complex in the field. Further studies should be addressed to ascertain the possible role of pellicle cysts in bloom dynamics of both *Alexandrium* species.

To explore the role of resting cysts in dinoflagellate population dynamics, data obtained on in situ excystment and encystment (**Paper 4**), and resting cyst abundance (**Papers 3** and **4**) were used to parameterize numerical models describing the population dynamics of *A. minutum* in Arenys de Mar harbor (Estrada et al., 2010). Most models relate phytoplankton

species abundance only to the physical and chemical parameters of the water column, disregarding the role of life stages. The results obtained by Estrada et al. emphasized the need to incorporate life-cycle traits in future models of population dynamics, mainly those aimed at the management of HABs.

Integration of the life cycle into dinoflagellate population dynamics

By combining planktonic- and benthic-phase dynamics with analyses of in situ transitions, this thesis has provided new insights into dinoflagellate population dynamics, and, in turn, a new description of the life-cycle strategy of an *Alexandrium* species.

In some species with abundant cyst stocks, such as *A. minutum*, continuous germination is a means to exploit environmental windows favorable for growth, with losses of few resting cysts. In contrast, for species such as the *A. tamarense* complex, in which resting cyst abundance in seed beds may be scarce, it is better to reserve resting cysts for periods optimal for growth. The plasticity of the organism is also a factor that must be taken into account, since species of high plasticity (e.g., *A. minutum*) are expected to be capable of growing within a wider environmental window than species with restricted environmental requirements. In any case, for species relying on seed beds, seeding success will be greater if it coincides with the occurrence of favorable growth conditions in the overlying waters. A certain degree of synchrony in excystment (high excystment periods in species with continuous germination) is therefore advantageous. This synchrony may imply some type of excystment cues that match germination with favorable environmental growth factors (irradiance and temperature in *A. minutum* and temperature in *A. tamarense* complex).

Once exponential growth is initiated, further germination may have little effect on bloom magnitude, but maintained excystment may contribute to the increase and maintenance of vegetative cell abundance. This is of major importance since vegetative populations must achieve a sufficient cell density to reach the threshold required to produce resting cysts. In fact, an essential function of the planktonic phase of *Alexandrium* species may well be the provision of recruits to the cyst bed (Wyatt and Jenkinson, 1997). In *A. minutum*, the relatively short mandatory dormancy period of recently formed resting cysts (one month, Figueroa et al. 2007) implies that, given the long duration of the vegetative bloom, these newly produced resting cysts are able to excyst within the same bloom in which they were formed. The germinated vegetative cells divide and undergo sexual reproduction and thus employ a life-cycle strategy consisting of frequent switches between planktonic and benthic stages within the bloom season. In the *A. tamarense* complex, the long mandatory dormancy period (3-6 months, Anderson, 1998) is indicative of a life-cycle strategy adapted to survival under seasonal environmental conditions less favorable for growth.

The stock of resting cysts is affected by physical and biological factors (resuspension, burial, bioturbation, germination, deposition, mortality, degradation, and grazing; Anderson et al., 1982; Giangrande et al., 2002; Persson, 2000). The above-mentioned study by Estrada et al. (2010) revealed the importance of knowing the rate of loss of resting cysts in seed beds. In a semi-enclosed system with high cyst stock, losses in resting cyst abundance were partly explained by incessant germination, whereas seiche-forced resuspension prevented resting cyst burial (**Papers 3** and **4**). In contrast, in a semi-enclosed system with low cyst stock, resting cysts losses at the surface sediments were mainly due to synchronic germination (**Paper 5**). This latter system is exposed to tidal- and wave-induced currents, which generate turbulence in the bottom boundary layer. These processes could have a significant role in resuspending resting cysts from deeper sediment layers.

The morphological and physiological characteristics of resting cysts increase their survival (i.e., thick walls, protuberances, spines, mucous layers, and intracellular storage products). Nonetheless, they remain vulnerable to loss factors still poorly understood, such as natural mortality, predation, bacteria, fungi, anoxia, and toxic compounds present in the sediments. Given the implications of resting-cyst abundance in the germination potential of seed beds, future investigations of loss factors of resting cysts should be addressed.

Throughout their life cycles, dinoflagellate species have developed different ecological strategies depending on the environment to which they are adapted. By means of life-cycle alternations, species adjust their life cycle to environmental factors optimal to their growth and persistence. If conditions allow, these species will form intense and recurrent blooms—a clear confirmation that their life-cycle strategy works perfectly.

General conclusions

General conclusions

1) The LISST-100X particle-size analyzer provides high-frequency in situ data that allows the temporal and spatial variability of phytoplankton blooms to be described. Nevertheless, use of the LISST-100X may be limited to specific characteristics of the phytoplankton community and of the system to be studied.

2) Semi-enclosed systems are significant reservoirs of biodiversity, hosting a greater number of cyst-producing species than recorded in the vegetative population.

3) Ten resting cyst morphotypes not previously described in the literature were identified. Some of them correspond to new dinoflagellate species that have yet to be described.

4) In a semi-enclosed system, wind-driven currents influenced the distribution of vegetative cells at the water surface, and as a consequence the resting cyst distribution in the sediment.

5) The spatio-temporal distribution of resting cysts in the sediments was related to biological behavior (resting cyst production) and physical processes (e. g., seiche-forced resuspension, tidal- and wave-induced currents).

6) *Alexandrium minutum* and the *A. tamarense* complex presented two different types of germination strategies: incessant and synchronous, respectively. The incessant germination is an opportunistic strategy for maintaining the planktonic population throughout the year, whereas the synchronous germination pattern limits the bloom occurrence to a particular season.

7) The encystment process, and hence sexual reproduction, initiated at early bloom stages in the two *Alexandrium* species, with encystment fluxes observed over the bloom phases of exponential growth, maintenance and decline.

8) The proportion of vegetative cells involved in encystment was low. Maximum encystment rates were 16% and 9% for *A. minutum* and the *A. tamarense* complex, respectively.

9) Encystment started at cell abundances of 10^3–10^4 cells l^{-1}. The magnitude of the encystment fluxes determined the abundance of *A. minutum* resting cysts found in the sediments, whereas in the *A. tamarense* complex this relationship between resting cyst and vegetative cell abundances was not observed.

10) Pellicle cysts formation by *A. minutum* and the *A. tamarense* complex coincided with high vegetative cell abundances during the bloom. The possible role of this life-cycle stage in the population dynamics of the two species is poorly understood.

11) Morphological features enabled the quantification of planktonic planozygotes of the *A. tamarense* complex, and allowed monitoring the transition from the vegetative cell to the resting cyst stage more accurately. However, this approach was not suitable for *A. minutum*.

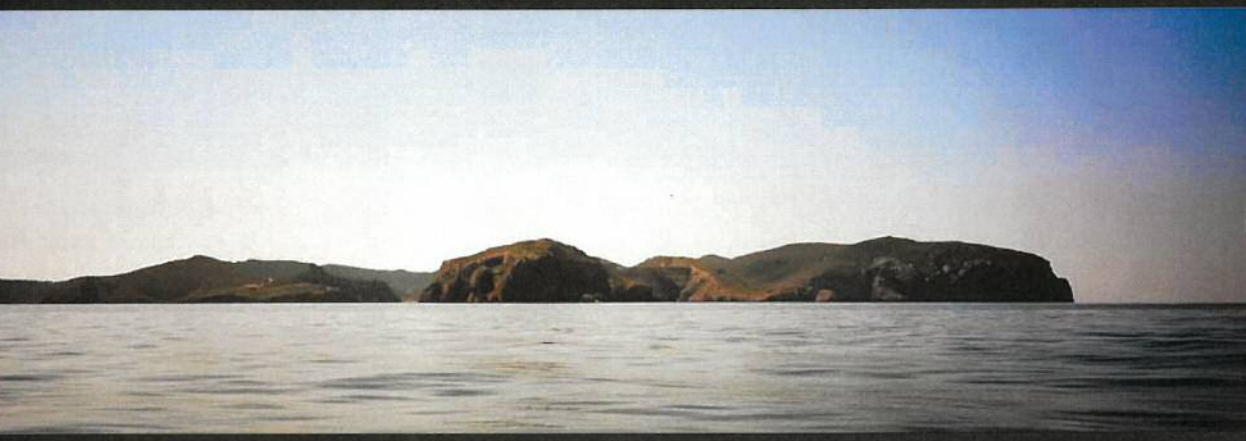
12) Losses of resting cysts in the surface sediments of an enclosed area with high cyst stock were partly explained by germination, even though this germination was incessant. In contrast, synchronic germination was a significant factor of resting cyst losses in a semi-enclosed area with a low cyst stock.

13) In *A. minutum*, recently formed resting cysts were able to excyst within the same bloom, and germinated vegetative cells divided and again underwent sexual reproduction. This feature was not observed during the bloom of the *A. tamarense* complex.

14) Planktonic-benthic transitions in dinoflagellates are species-specific. Through them, species develop their ecological strategy, adjusting their life cycle to environmental conditions and ensuring their persistence.

15) Life-cycle features enable species to colonize new niches such as the artificial constructions that are being created by humans.

References



References of General introduction and discussion

Adachi, M., Kanno, T., Matsubara, T., Nishijima, T., Itakura, S., Yamaguchi, M., 1999. Promotion of cyst formation in the toxic dinoflagellate *Alexandrium* (Dinophyceae) by natural bacterial assemblages from Hiroshima Bay, Japan. Marine Ecology Progress Series 191, 175-185.

Amorim, A., Dale, B., 2006. Historical cyst record as evidence for the recent introduction of the dinoflagellate *Gymnodinium catenatum* in the north-eastern Atlantic. African Journal of Marine Science 28, 193-197.

An, K.H., Lassus, P., Maggi, P., Bardouil, M., Truquet, P., 1992. Dinoflagellate cyst changes and winter environmental conditions in Vilaine Bay, Southern Brittany. (France). Botanica Marina 35, 61-67.

Anderson, D.M., Wall, D., 1978. Potential importance of benthic cysts of *Gonyaulax tamarensis* and *G. excavata* in initiating toxic dinoflagellate blooms. Journal of Phycology 14, 224-234.

Anderson, D.M., Morel, F.M.M., 1979. The seeding of two red tide blooms by the germination of benthic *Gonyaulax tamarensis* hypnocysts. Estuarine and Coastal Marine Science 8, 279-293.

Anderson, D.M., 1980. Effects of temperature conditioning on development and germination of *Gonyaulax tamarensis* (Dinophyceae) hypnozygotes. Journal of Phycology 16, 166-172.

Anderson, D.M., Aubrey, D.G., Tyler, M.A., Coats, D.W., 1982. Vertical and horizontal distributions of dinoflagellate cysts in sediments. Limnology and Oceanography 27, 757-765.

Anderson, D.M., Chisholm, S.W., Watras, C.J., 1983. Importance of life-cycle events in the population dynamics of *Gonyaulax tamarensis*. Marine Biology 76, 179-189.

Anderson, D.M., Stolzenbach, K.D., 1985. Selective retention of two dinoflagellates in a well-mixed estuarine embayment: the importance of diel vertical migration and surface avoidance. Marine Ecology Progress Series 25, 39-50.

Anderson, D.M., Keafer, B.A., 1987. An endogenous annual clock in the toxic marine dinoflagellate *Gonyaulax tamarensis*. Nature 325, 616-617.

Anderson, D.M., Taylor, C.D., Armbrust, E.V., 1987. The effects of darkness and anaerobiosis on dinoflagellate cyst germination. Limnology and Oceanography 32, 340-351.

Anderson, D.M., 1998. Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: Anderson, D.A., Cembella, A.D., Hallegraeff, G.M. (Eds.), Physiological Ecology of Harmful Algal Blooms. Springer-Verlag, Berlin-Heidelberg, pp. 29-48.

Anderson, D.M., Pitcher, G., Estrada, M., 2005. The comparative "systems" approach to HAB research. Oceanography 18, 148-157.

Babin, M., Cullen, J.J., Roesler, C.S., Donaghay, P.L., Doucette, G.J., Kahru, M., Lewis, M.R., Scholin, C.A., Sieracki, M.E., Sosik, H.M., 2005. New approaches and technologies for observing Harmful Algal Blooms. Oceanography 18, 213-227.

Babin, M., Roesier, C.S., Cullen, J.J., 2008. Real-time Coastal Observing Systems for Marine Ecosystem Dynamics and Harmful Algal Blooms. Oceanographic Methodology Series. UNESCO Publishing, pp. 807.

Band-Schmidt, C.J., Lechuga-Devéze, C.H., Kulis, D.M., Anderson, D.M., 2003. Culture studies of *Alexandrium affine* (Dinophyceae), a non-toxic cyst forming dinoflagellate from Bahía Concepción, Gulf of California. Botanica Marina 46, 44-54.

Basterretxea, G., Garcés, E., Jordi, A., Masó, M., Tintoré, J., 2005. Breeze conditions as a favoring mechanism of *Alexandrium taylori* blooms at a Mediterranean beach. Estuarine Coastal and Shelf Science 62, 1-12.

Bibby, B.T., Dodge, J.D., 1972. The encystment of a freshwater dinoflagellate: a light and electron microscopical study. British Phycological Journal 7, 85-100.

Binder, B.J., Anderson, D.M., 1987. Physiological and environmental control of germination in *Scrippsiella trochoidea* (Dinophyceae) resting cysts. Journal of Phycology 23, 99-107.

Blanco, E.P., Lewis, J., Aldridge, J., 2009. The germination characteristics of *Alexandrium minutum* (Dinophyceae), a toxic dinoflagellate from the Fal estuary (UK). Harmful Algae 8, 518-522.

Boero, F., Belmonte, G., Fanelli, G., Piraino, S., Rubino, F., 1996. The continuity of living matter and the discontinuities of its constituents: do plankton and benthos really exist? Trends in Ecology & Evolution 11, 177-180.

Bolch, C.J., Hallegraeff, G.M., 1990. Dinoflagellate cysts in recent marine sediments from Tasmania, Australia. Botanica Marina 33, 173-192.

Braarud, T., 1945. Morphological observations on marine dinoflagellate cultures. Avhandling at der Norske Vitenskalepige Akadademi, Oslo, 11, 1-18.

Braarud, T., 1962. Species distribution in marine phytoplankton. Journal of the Oceanographical Society of Japan 20, 628-649.

Bravo, I., Garcés, E., Diogene, J., Fraga, S., Sampedro, N., Figueroa, R.I., 2006. Resting cysts of the toxigenic dinoflagellate genus *Alexandrium* in recent sediments from the Western Mediterranean coast, including the first description of cysts of *A. kutnerae* and *A. peruvianum*. European Journal of Phycology 41, 293-302.

Bravo, I., Vila, M., Maso, M., Figueroa, R.I., Ramilo, I., 2008. *Alexandrium catenella* and *Alexandrium minutum* blooms in the Mediterranean Sea: Toward the identification of ecological niches. Harmful Algae 7, 515-522.

Bravo, I., Figueroa, R.I., Garcés, E., Fraga, S., Massanet, A., 2010. The intricacies of dinoflagellate pellicle cysts: The example of *Alexandrium minutum* cysts from a bloom-recurrent area (Bay of Baiona, NW Spain). Deep Sea Research Part II: Topical Studies in Oceanography 57, 166-174.

Burkholder, J.M., Azanza, R.V., Sako, Y., 2006. Importance of life cycles in the ecology of harmful microalgae. In: Gráneli, E., Turner, J.T. (Eds.), Ecology Of Harmful Algae. Springer-Verlag, Berlin, pp. 53-64.

Calbet, A., Vaque, D., Felipe, J., Vila, M., Sala, M.M., Alcaraz, M., Estrada, M., 2003. Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate *Alexandrium minutum*. Marine Ecology Progress Series 259, 303-309.

Cannon, J.A., 1993. Germination of the toxic dinoflagellate, *Alexandrium minutum*, from sediments in the Port River, South Australia. In: Smayda, T.J., Shimizu, Y. (Eds.), Toxic Phytoplankton Blooms in the Sea. Elsevier Sciences Publishers, pp. 103-107.

Campbell, L., Olson, R., Sosik, H., Abraham, A., Henrichs, D., Hyatt, C., 2010. First harmful *Dinophysis* (Dinophyceae, Dinophysiales) bloom in the US is revealed by Automated Imaging Flow Cytometry. Journal of Phycology 46, 66-75.

Cembella, A.D., Ibarra, D.A., Diogene, J., Dahl, E., 2005. Harmful Algal Blooms and their assessment in fjords and coastal embayments. Oceanography 18, 158-171.

Chapman, D.V., Livingstone, D., Dodge, J.D., 1981. An electron microscope study of the excystment and early development of the dinoflagellate *Ceratium hirundinella*. British Phycological Journal 16, 183-194.

Dale, B., 1976. Cyst formation, sedimentation, and preservation: Factors affecting dinoflagellate assemblages in recent sediments from Trondheims Fjord, Norway. Review of Palaeobotany and Palynology 22, 39-60.

Dale, B., 1977. Cysts of the toxic red-tide dinoflagellate *Gonyaulax excavata* (Braarud) Balech from Oslofjorden, Norway. Sarsia 63, 29-34.

Dale, B., 1983. Dinoflagellate resting cysts: "benthic plankton". In: Fryxell, G.A. (Ed.), Survival Strategies of the Algae. Cambridge University Press, pp. 69-136.

Ellegaard, M., Lundholm, N., Ribeiro, S., Ekelund F., Andersen, T. J., 2008. Long-term survival of dinoflagellate cysts in anoxic marine sediments. Eighth International Conference on Modern and Fossil. Dinoflagellates. 4-10 May 2008 in Montreal, Canada, pp. 13.

Eppley, R.W., Holm-Hansen, O., Strickland, J.D.H., 1968. Some observations on the vertical migration of dinoflagellates. Journal of Phycology 4, 333-340.

Erdner, D.L., Percy, L., Keafer, B., Lewis, J., Anderson, D.M., 2010. A quantitative real-time PCR assay for the identification and enumeration of *Alexandrium* cysts in marine sediments. Deep-Sea Research Part II: Topical Studies in Oceanography 57, 279-287.

Erdtman, G., 1949. Palynological aspects of the pioneer phase in the immigration of the Swedish flora. 11. Identification of pollen grains in Late Glacial samples from Mt. Omberg, Ostrogothia. Svensk Botanisk Tidskrift 43, 46-55.

Erdtman, G., 1950. Fynd av *Hyslrichosphaeror furcafa* i Gullmaren. Geologiska Föreningen i Stockholmen Förhandlingar 72, 221.

Erdtman, G., 1954. On pollen grains and dinoflagellate cysts in the Firth of Gullmaren, SW Sweden. Botanica Notiser, pp. 103-111.

Estrada, M., Solé, J., Anglès, S., Garcés, E., 2010. The role of resting cysts in *Alexandrium minutum* population dynamics. Deep-Sea Research Part II: Topical Studies in Oceanography 57, 308-321.

Evitt, W. R. 1961. Observations on the morphology of fossil dinoflagellates. Micropaleontology 7, 385-420.

Evitt, W. R., Lentin, J. K., Millioud, M. E., Stover, L. E., Williams, G. L., 1977. Dinoflagellate cyst terminology. Canadian Geological Survey Paper 76-24, 1-11.

Fensome, R. A., Taylor, F. J. R., Norris, G., Sarjeant, W. A. S., Wharton, D. I. and Williams, G. L., 1993. A classification of fossil and living dinoflagellates. Micropaleontology Special Publication Number 7. Sheridan Press, pp. 351.

Field, C.B., Behrenfeld, M.J., Randerson, J.T., Falkowski, P., 1998. Primary production of the Biosphere: Integrating terrestrial and oceanic components. Science 281, 237-240.

Figueroa, R.I., Bravo, I., 2005. Sexual reproduction and two different encystment strategies of *Lingulodinium polyedrum* (Dinophyceae) in culture. Journal of Phycology 41, 370-379.

Figueroa, R.I., Bravo, I., Garces, E., 2005. Effects of nutritional factors and different parental crosses on the encystment and excystment of *Alexandrium catenella* (Dinophyceae) in culture. Phycologia 44, 658-670.

Figueroa, R.I., Bravo, I., Garcés, E., 2006a. Multiple routes of sexuality in *Alexandrium taylori* (Dinophyceae) in culture. Journal of Phycology 42, 1028-1039.

Figueroa, R.I., Bravo, I., Garcés, E., Ramilo, I., 2006b. Nuclear features and effect of nutrients on *Gymnodinium catenatum* (Dinophyceae) sexual stages. Journal of Phycology 42, 67-77.

Figueroa, R.I., Rengefors, K., Bravo, I., 2006c. Effects of parental factors and meiosis on sexual offspring of *Gymnodinium nolleri* (Dinophyceae). Journal of Phycology 42, 350-362.

Figueroa, R.I., Garces, E., Bravo, I., 2007. Comparative study of the life cycles of *Alexandrium tamutum* and *Alexandrium minutum* (Gonyaulacales, Dinophyceae) in culture. Harmful Algae 43, 1039-1053.

Gaines, G., Elbrächter, M., 1987. Heterotrophic Nutrition. In: Taylor, F.J.R. (Ed.), The Biology of Dinoflagellates. Blackwell Scientific Publications, Oxford, pp. 224-268.

Garcés, E., Delgado, M., Masó, M., Camp, J., 1998. Life history and in situ growth rates of *Alexandrium taylori* (Dinophyceae, Pyrrophyta). Journal of Phycology 34, 880-887.

Garcés, E., Masó, M., Camp, J., 1999. A recurrent and localized dinoflagellate bloom in Mediterranean beach. Journal of Plankton Research 21, 2373-2391.

Garcés, E., 2002. Temporary cyst in dinoflagellates. In: Garcés, E., Zingone, A., Montresor, M., Reguera, B., Dale, B. (Eds.), LIFEHAB: Life histories of microalgal species causing harmful blooms. Office for the Official Publications of the European Communities, Luxembourg, pp. 46-48.

Garcés, E., Bravo, I., Vila, M., Figueroa, R.I., Masó, M., Sampedro, N., 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean). Journal of Plankton Research 26, 637-645.

GEOHAB, 2001. Global Ecology and Oceanography of Harmful Algal Blooms, Science Plan. In: Glibert, P., Pitcher, G. (Eds.). Scientific Committee on Oceanic Research and Intergovernmental Oceanographic Commission, Baltimore and Paris, pp. 87.

Giangrande, A., Montresor, M., Cavallo, A., Licciano, M., 2002. Influence of *Naineris laevigata* (Polychaeta: Orbiniidae) on vertical grain size distribution, and dinoflagellate resting stages in the sediment. Journal of Sea Research 47, 97-108.

Godhe, A., McQuoid, M.R., 2003. Influence of benthic and pelagic environmental factors on the distribution of dinoflagellate cysts in surface sediments along the Swedish west coast. Aquatic Microbial Ecology 32, 185-201.

Graham, L.E., Wilcox, L.W., 2000. Algae. Prentice Hall, Upper Saddle River, NJ pp. 640.

Hallegraeff, G.M., 1993. A review of Harmful Algal Blooms and their apparent global increase. Phycologia 32, 79-99.

Hansson, L., 1996. Behavioural response in plants: adjustment in algal recruitment Induced by herbivores. Proceedings: Biological Sciences 263, 1241-1244.

Head, M.J., 1996. Modern dinoflagellate cysts and their biological affinities. In: Jansonius, J., McGregor, D.C., (Eds.), Palynology: Principles and Applications. The American Association of Stratigraphic Palynologists, Dallas, pp. 1197–1248.

Huber, G. and Nipkow, F., 1922. Experimentelle Untersuchungen über die Entwicklung von *Ceratium hirundinella* O.F.M. Zeitschrift für Botanik 14, 337–71.

Huber, G. and Nipkow, F., 1923. Experimentelle Untersuchungen über Entwicklung und Formbildung von *Ceratium hirundinella* O.F. Müller. Flora 116, 114215.

Ishikawa, A., Taniguchi, A., 1996. Contribution of benthic cysts to the population dynamics of *Scrippsiella* spp. (Dinophyceae) in Onagawa Bay, northeast Japan. Marine Ecology Progress Series 140, 169-178.

Ishikawa, A., Taniguchi, A., 1997. In situ germination patterns of cysts, and bloom formation of some armored dinoflagellates in Onagawa Bay, north-east Japan. Journal of Plankton Research 19, 1783-1791.

Joyce, L.B., 2004. Dinoflagellate cysts in recent marine sediments from Scapa Flow, Orkney, Scotland. Botanica Marina 47, 173-183.

Joyce, L.B., 2005. Dinoflagellate cysts from surface sediments of Saldanha Bay, South Africa: an indication of the potential risk of harmful algal blooms. Harmful Algae 4, 309-318.

Kamikawa, R., Hosoi-Tanabe, S., Nagai, S., Itakura, S., Sako, Y., 2005. Development of a quantification assay for the cysts of the toxic dinoflagellate *Alexandrium tamarense* using real-time polymerase chain reaction. Fisheries Science 71, 987-991.

Keafer, B.A., Buesseler, K.O., Anderson, D.M., 1992. Burial of living dinoflagellate cysts in estuarine and nearshore sediments. Marine Micropaleontology 20, 147-161.

Kim, Y.O., Park, M.H., Han, M.S., 2002. Role of cyst germination in the bloom initiation of *Alexandrium tamarense* (Dinophyceae) in Masan Bay, Korea. Aquatic Microbial Ecology 29, 279-286.

Kita, T., Fukuyo, Y., Tokuda, H., Hirano, R., 1985. Life history and ecology of *Goniodoma pseudogoniaulax* (Pyrrhophyta) in a rockpool. Bulletin of Marine Science 37, 643-651.

Kremp, A., Heiskanen, A.S., 1999. Sexuality and cyst formation of the spring-bloom dinoflagellate *Scrippsiella hangoei* in the coastal northern Baltic Sea. Marine Biology 134, 771-777.

Kremp, A., Parrow, M.W., 2006. Evidence for asexual resting cysts in the life cycle of the marine peridinoid dinoflagellate, *Scrippsiella hangoei*. Journal of Phycology 42, 400-409.

Margalef, R., 1994, Diversity and biodiversity– Their possible meaning in relation with the wish for sustainable development. Anais da Academia Brasileira de Ciências 66, 3-14

Margalef, R., 2002,Diversidad y biodiversidad. In: Pineda, F., De Miguel, J. M., Casado, M. A., Montalvo, J. (Eds.), La Diversidad Biológica de España. Prentice Hall, Madrid, pp. 432.

Matrai, P., Thompson, B., Keller, M., 2005. Circannual excystment of resting cysts of *Alexandrium* spp. from eastern Gulf of Maine populations. Deep Sea Research Part II: Topical Studies in Oceanography 52, 2560-2568.

Matsuoka, K., Joyce, L.B., Kotani, Y., Matsuyama, Y., 2003. Modern dinoflagellate cysts in hypertrophic coastal waters of Tokyo Bay, Japan. Journal of Plankton Research 25, 1461-1470.

Moestrup, Ø., Codd, G.A., Elbrächter, M., Faust, M.A., Fraga, S., Fukuyo, Y., Cronberg, G., Halim, Y., Taylor, F.J.R., Zingone, A., 2004. IOC taxonomic reference list of toxic algae. In: Moestrup, Ø. (Ed.), Intergovernmental Oceanographic Commission of UNESCO.

Nehring, S., 1994. Spatial distribution of dinoflagellate resting cysts in recent sediments of Kiel Bight, Germany (Baltic Sea). Ophelia 39, 137-158.

Nordli, E., 1951. Resting spores in *Gonyaulax polyedra* Stein. Nytt Magasin for Naturvidenskapene 88, 207-21.

Olson, R., Sosik, H., 2007. A submersible imaging-in-flow instrument to analyze nano- and microplankton: Imaging FlowCytobot. Limnology and Oceanography Methods 5, 195-203.

Penna, A., Battocchi, C., Garcés, E., Anglès, S., Cucchiari, E., Totti, C., Kremp, A., Satta, C., Giacobbe, M.G., Bravo, I., Bastianini, M., 2010. Detection of microalgal resting cysts in European coastal sediments using a PCR-based assay. Deep-Sea Research Part II: Topical Studies in Oceanography 57, 288-300.

Persson, A., 2000. Possible predation of cysts—a gap in the knowledge of dinoflagellate ecology?. Journal of Plankton Research 22, 803-809.

Persson, A., Smith, B.C., Wikfors, G.H., Alix, J.H., 2008. Dinoflagellate gamete formation and environmental cues: Observations, theory, and synthesis. Harmful Algae 7, 798-801.

Pfiester, L.A., Anderson, D.M., 1987. Dinoflagellate Reproduction. In: Taylor, F.J.R. (Ed.), The Biology of Dinoflagellates. Blackwell Scientific Publications, Oxford, pp. 611-648.

Pitcher, G.C., Cembella, A.D., Joyce, L.B., Larsen, J., Probyn, T.A., Sebastian, C.R., 2007. The dinoflagellate *Alexandrium minutum* in Cape Town harbour (South Africa): Bloom characteristics, phylogenetic analysis and toxin composition. Harmful Algae 6, 823-836.

Prakash, A., 1967. Growth and toxicity of a marine dinoflagellate *Gonyaulax tamarensis*. Journal of the Fisheries Research Board of Canada 7, 1589-1606.

Probert, I., 1999. Sexual reproduction and ecophysiology of the marine dinoflagellate *Alexandrium minutum* Halim. University of Westminister, London, p. 99.

Rengefors, K., Anderson, D.M., Pettersson, K., 1996. Phosphurus uptake by resting cysts of the marine dinoflagellate *Scrippsiella trochoidea*. Journal of Plankton Research 18, 1753-1765.

Rengefors, K., Anderson, D.M., 1998. Environmental and endogenous regulation of cyst germination in two freshwater dinoflagellates. Journal of Phycology 34, 568-577.

Rengefors, K., Karlsson, I., Hansson, L., 1998. Algal cyst dormancy: a temporary escape from herbivory. Proceedings of the Royal London Society, Series B, Biological Sciences 265, 1353-1358.

Rintala, J.M., Spilling, K., Blomster, J., 2007. Temporary cyst enables long-term dark survival of *Scrippsiella hangoei* (Dinophyceae). Marine Biology 152, 57-62.

Sgrosso, S., Esposito, F., Montresor, M., 2001. Temperature and daylength regulate encystment in calcareous cyst-forming dinoflagellates. Marine Ecology Progress Series 211, 77-87.

Shumway, S.A., 1990. A review of the effects of algal blooms on shellfish and aquaculture. Journal of the World Aquaculture Society 21, 65-104.

Smayda, T.J., 1997a. What is a bloom? A commentary. Limnology and Oceanography 42, 1132-1136.

Smayda, T.J., 1997b. Harmful algal blooms: their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography 42, 1137-1153.

Smayda, T.J., Reynolds, C.S., 2003. Strategies of marine dinoflagellate survival and some rules of assembly. Journal of Sea Research 49, 95-106.

Sosik, H., Olson, R., 2007. Automated taxonomic classification of phytoplankton sampled with imaging-in-flow cytometry. Limnology and Oceanography Methods 5, 204-216.

Steidinger, K.A., 1975. Implications of dinoflagellate life cycles on initiation of *Gymnodinium breve* red tides. Environmental letters 9, 129-139.

Steidinger, K.A., Haddad, K., 1981. Biologic and hidrographic aspects of red tides. BioScience 31.

Steidinger, K.A., Garcés, E., 2006. Importance of life cycles in the ecology of harmful microalgae. In: Gráneli, E., Turner, J.T. (Eds.), Ecology Of Harmful Algae. Springer-Verlag, Berlin, pp. 37-49.

Taylor, F.J.R., 1975. Non-helical transverse flagella in dinoflagellates. Phycologia 14, 45-47.

Taylor, F.J.R., 1987. Ecology of Dinoflagellates. In: Taylor, F.J.R. (Ed.), The Biology of Dinoflagellates. Blackwell Scientific Publications, Oxford, pp. 399-501.

Taylor, F.J.R., Hoppenrath, M., Saldarriaga, J.F., 2008. Dinoflagellate diversity and distribution. Biodiversity and Conservation 17, 407-418.

Tyler, M.A., Coats, D.W., Anderson, D.M., 1982. Encystment in a dynamic environment: Deposition of dinoflagellate cysts by a frontal convergence. Marine Ecology Progress Series 7, 163-178.

Uchida, T., 1991. Sexual reproduction of *Scrippsiella trochoidea* isolated from Muroran Harbor Hokkaido. Nippon Suisan Gakkaishi 57, 1215.

Uchida, T., Matsuyama, Y., Yamaguchi, M., Honjo, T., 1996. The life cycle of *Gyrodinium instriatum* (Dinophyceae) in culture. Phycological Research 44, 119-123.

Uchida, T., 2001. The role of cell contact in the life cycle of some dinoflagellate species. Journal of Plankton Research 23, 889-891.

Valero, M., Richerd, S., Perrot, V., Destombe, C., 1992. Evolution of alternation of haploid and diploid phases in life cycles. Trends in Ecology & Evolution 7, 25-29.

Van Lenning, K., Vila, M., Masó, M., Garcés, E., Anglès, S., Sampedro, N., Morales-Blake, A., Camp, J., 2007. Short-term variations in development of a recurrent toxic *Alexandrium minutum*-dominated dinoflagellate bloom induced by meteorological conditions. Journal of Phycology 43, 892-907.

Vila, M., Camp, J., Garcés, E., Masó, M., Delgado, M., 2001. High resolution spatio-temporal detection of potentially harmful dinoflagellates in confined waters of the NW Mediterranean. Journal of Plankton Research 23, 497-514.

Vila, M., Giacobbe, M.G., Masó, M., Gangemi, E., Penna, A., Sampedro, N., Azzaro, F., Camp, J., Galluzzi, L., 2005. A comparative study on recurrent blooms of *Alexandrium minutum* in two Mediterranean coastal areas. Harmful Algae 4, 673-695.

Villanoy, C.L., Azanza, R.V., Altemerano, A., Casil, A.L., 2006. Attempts to model the bloom dynamics of *Pyrodinium*, a tropical toxic dinoflagellate. Harmful Algae 5, 156-183.

von Stosch, H.A., 1969. Dinoflagellaten aus der Nordsee I. über *Chaconina niei* Loeblich (1968), *Gonyaulax grindleyi* Reinecke (1967) und eine Methode zur Darstellung von Peridineenpanzern. Helgoländer wiss. Meeresunters 19, 558-568.

von Stosch, H.A., 1972. La signification cytologique de la cyclose nucléaire dans le cycle de vie des dinoflagellés. Memoires Publiciés par la Société Botanique de France, 201-212.

von Stosch, H.A., 1973. Observations on vegetative reproduction ans sexual life cycles of two freshwater dinoflagellates, *Gymnodinium pseudopalustre* Schiller and *Woloszynskia apiculata* sp. nov. British Phycological Journal 8, 105-134.

Walker, L.M., 1984. Life histories, dispersal, and survival in marine, planktonic dinoflagellates. In: Steindinger, K.A., Walker, L.M. (Eds.), Marine Plankton Life Cycle Strategies, pp. 19-34.

Wall, D., 1965. Modern hystrichospheres and dinoflagellate cysts from the Woods Hole Region. Grana Palynologica 6, 297-314.

Wall, D., Dale, B., 1966. "Living fossils" in Western Atlantic plankton. Nature, 211, 1025-1026.

Wall, D., 1971. Biological problems concerning fossilizable dinoflagellates. Geoscience and Man 3, 1-15.

Wall, D., Guillard, R.R.L., Dale, B., Swift, E., Watabe, N., 1970. Calcitic resting cysts in *Peridinium trochoideum* (Stein) Lemmermann, an autotrophic marine dinoflagellate. Phycologia 9, 151-156.

Wang, Z., Qi, Y., Lu, S., Wang, Y., Matsuoka, K., 2004. Seasonal distribution of dinoflagellate resting cysts in surface sediments from Changjiang River Estuary. Phycological Research 52, 387-395.

Wyatt, T., Jenkinson, I.R., 1997. Notes on *Alexandrium* population dynamics. Journal of Plankton Research 19, 551-575.

Wyatt, T., 2002. How can we combine the population dynamics of life history stages? In: Garcés, E., Zingone, A., Montresor, M., Reguera, B., Dale, B. (Eds.), LIFEHAB: Life histories of microalgal species causing harmful blooms. Office for the Official Publications of the European Communities, Luxembourg, pp. 112-115.