HOST PARASITE INTERACTIONS

THE *PARVILUCIFERA SINERAE* MODEL IN MARINE MICROALGAE

Master's thesis
by
Elisabet Alacid Fernández

Project director
Dr. Esther Garcés
Institut de Ciències del Mar, CSIC

Project tutor
Dr. Montserrat Vidal
Universitat de Barcelona

Faculty of Biology of Universitat de Barcelona

MASTER IN MARINE SCIENCES:
OCEANOGRAPHY AND MARINE ENVIRONMENT MANAGEMENT

September 2012

Abstract

Microparasites of *Parvilucifera* genus have been proposed as control agent in dinoflagellate HABs mitigation. But the control that parasites exert on its host depends on the degree of their specific interactions. We used the dinoflagellate parasite *Parvilucifera sinerae* and its host *Alexandrium minutum* to study biological and behavioral life-history traits, as well as the specificity degree of their interactions. In the present study, complete life cycle stages and zoospores patterns were first time described in *Parvilucifera* genus, as well as *P. sinerae* generation time and prevalence as a function of inoculums size. Furthermore, strain specificity of *P. sinerae* in *Alexandrium* species and host genotype by parasite genotype interactions in *A. minutum* are also presented. *P.sinerae* generation time lasted 4 days, a single flagellate infection appearing as a spherical mature sporangia containing about 150-200 infective zoospores and parasite prevalence increasing exponentially as a function of zoospores:host ratio used demonstrate a rapid generation time. *P. sinerae* is a generalist parasitoid of dinoflagellates which is specialized in *Alexandrium* genus, as we found that 90% of *Alexandrium* strains tested were susceptible to infection. This specialization may drive changes in community composition and microalgae succession in natural environment. However, resistance at intra-species level was found in some *Alexandrium* species, as *A. minutum, A. andersonii*, *A. tamutum* and *A. tamarense* complex (Group III). The experiments performed here indicate that level of host resistance or parasite virulence in such parasite-host system depends on the genotype-specific interactions of both, the parasite and the host. The rapid generation time and the existence of genotype-by-genotype interactions suggest that *P. sinerae* could benefit from evolutionary advantages in front of its host, allowing for parasite local adaptation within the bloom system.

Keywords: Perkinsozoa, dinoflagellates, host-parasite interactions, generation time, prevalence, resistance, virulence, adaptation.

TABLE OF CONTENTS

INTRODUCTION

Background and the state of the art of the scientific knowledge

Parasitism is an interespecific interaction where one organism (the parasite) spends the whole or part of its life feeding in or on a single individual of another species (the host) (Price 1980). The parasite's benefits gives rise to the host harm, termed virulence, where important fitness traits of the host are often negatively affected by the parasite. Parasites include a very diverse group of organisms that have managed to spread across a large diverse taxonomic host groups, being one of the most common mode of life on earth. Due to its ubiquities in nature, the diversity of life cycles displayed and host-parasite interactions, understand the factors that control these associations become a relevant issue cause its implications from an ecological and evolutionary point of view (Agosta et al., 2010).

The relevance of the parasitism on protists in marine planktonic ecosystems has received little attention until recently, despite their highly interesting potential to understand the rules that govern host parasite relations. Interactions among planktonic protists represent a complex web of great relevance given that: i) the major part of the organisms are affected by parasites in one way or another, ii) they play a top-down control role in their host populations affecting population dynamics and succession (Toth et al., 2001), iii) they can act as a selective pressure within the host populations being of prime importance for the evolution of their hosts (Buckling and Rainey 2002).

Photosynthetic protists (microalgae) are responsible for much of the primary production that occurs in the world's ocean and most of them are phytoplankton. Phytoplankton dynamics depends on both abiotic (such us temperature, light) and biotic factors (as growth, predation and parasitism). A wide variety of organisms act as parasites of phytoplankton, while prokaryotic pathogens (viruses and bacteria) infects, especially smaller phytoplankton species as chrysophytes, prymnesiophytes, prasinophytes, raphydophytes and cyanobacteria, they appear to be much less prevalent in dinoflagellates and diatoms whose are well known to be infected by eukaryotic parasites (see

reviews by Elbrächter and Schnepf 1998; Brussaard 2004; Ibelings et al., 2004; Park et al., 2004).

In marine environment, dinoflagellates are an important group of phytoplankton whose scientific interest has risen because of the increased frequency of harmful algae blooms connected with water eutrophication and species introduction (Zingone and Enevoldsen 2000; Hackett et al., 2004). A number of dinoflagellate species are known to produce potent neurotoxins associated with those blooms which has expanded during the last decades over the world (Hallegraeff 2004). The economic, public health, and ecosystem impacts of this HAB events take a variety of forms and include human intoxications and death from contaminated shellfish or fish, loss of natural and cultured seafood resources, impairment of tourism and recreational activities, alterations of marine trophic structure, and death of marine mammals, fish, and seabirds (Van Dolah 2000; Zingone and Enevoldsen 2000). Numerous dinoflagellate species act as a host of eukaryotic parasites as fungi, perkinsozoa, amoebae, euglenoids, kinetoplastids and other flagellates (Elbrächter and Schnepf 1998; Norén et al., 1999). This microalgal parasites have been proposed as biological control agents of toxic marine dinoflagellate blooms because its role as control of host populations in many occasions (Taylor 1968; Chambouvet et al., 2008).

The capacity of parasitoids to control their hosts is highly dependent on the parasitic fitness mechanisms underlying the parasitic specificity. While some parasites are generalists, many other are specialists, able to infect only one or a few host species, usually defined as a host range. Nevertheless, specialization can also occur at intraspecific level, as local adaptation of parasites to their sympatric host population (Kaltz and Shykoff 1998), and within populations where some parasite strains are able to infect some host genotypes while others are resistant (Carius et al., 2001; Lambrechts et al., 2005). This specificity could differ substantially in the strength of the selection pressure they can exert on their hosts determining the dynamic, diversity and evolution of host populations. In coevolutionary host-parasite systems each species constitutes an ever changing environment to which its opponent has to adapt (host-parasite arms-race). Indeed, at the same time that parasites drive changes in their hosts

who try to escape of the infection (resistance) lead to the evolution of the own parasites in terms of virulence to enhance their ability to infect hosts. Forces that govern coevolution in parasite-host systems are one of the major questions in this topic.

Parasitism in the marine ecosystems on protists host-parasite interactions and the processes that drive them must to be further studied in order to address a wide variety of ecological issues and also because its relevance due to the selective pressure that parasites exerts on their hosts leading to the coevolution of both. Thus, makes this research relevant from an ecologic, phylogenetic and evolutionary point of view.

Host-parasite Interactions

Parasites may be directly or indirectly involved in host population dynamics and extinctions, maintenance of genetic diversity, sexual selection, evolution of genetic systems, and evolution of sexual recombination, for example. Parasites possess features that make them attractive as explanatory factors in the evolution and ecology of their hosts. These features include their high abundance in nearly every ecosystem, their host range, their adverse effects on their hosts, and density dependence during horizontal transmission (Anderson and May 1979; Price 1980). Additionally, hosts are the environment for the parasites and thus define their niche. Most parasites are not viable outside of their hosts for extended periods and therefore parasite and their host form an inseparable unit.

Parasites can infect and exploit different type of host referring to different variants (i.e., genotypes or phenotypes) within the same host species or to different host species, thus, parasites can be generalists or specialists. This is one of the most intriguing features in parasitism, the range of host used, and it is of practical interest in ecology understanding what limits host range may aid and what are the species preferences to infect in the use of parasites as control agents in dinoflagellates outbreaks. Because the machinery required for infection, exploitation, and transmission is likely to vary from one host to another, the selective pressures acting on parasites in different hosts may also

vary (Gandon 2004). Parasite virulence varies across parasites isolates (strains, genotypes) and host clones (Restif and Koella 2003). Host clones originating from within or between populations differ in the degree with which they express disease symptoms, and parasite isolates vary greatly in the extent to which they cause damage to the same host clones (Little and Ebert 2000). Genetic variation for parasite virulence is most pronounced across populations, which often follows a certain pattern which is discussed in the context of local adaptation, where local parasites cause more harm to their hosts than parasites isolates from other populations. These are consistent with the idea that parasites evolve local adaptation to the hosts they have encountered recently. Locally adapted parasites show higher levels of damage to their local hosts and also have higher levels of transmission stage production. It has been suggested that the key variable for the host or parasite local adaptation is the relative speed of evolution of the two antagonists (Gandon et al., 2002).

Most models of evolutionary processes in host-parasite systems assume that the evolution of attack or defense strategies is governed by the balance of their evolutionary costs and benefits from the point of view of either the parasite or the host and thus hold the other partner constant. In other words, they consider that traits of infection such as host resistance or parasite virulence are determined by the genotype of either the host or the parasite, but not both (i.e., the studies of Frank (1996), Boots and Haraguchi (1999)). However, recently more attention has been paid to coevolutionary processes in which both the host and the parasite are considered to evolve (Gandon et al., 2002). This can lead to an epidemiological feedback in which virulence and resistance traits are controlled by the two partners, and the response to evolutionary pressure changes the epidemiological situation that is responsible to evolutionary pressure (Restif and Koella 2003). There are two models in which the outcome of infection is determined by the specific combination of the host and parasite genotypes, gene-for-gene and matching-allele models that show that the compatibility of host-parasite systems is often based on genotype-by-genotype interactions. In such systems some hosts are compatible with a subset of parasite genotypes, whereas other hosts are compatible with another subset (Lambrechts et al., 2006). Most models of parasite evolution assume a trade-off

between virulence and parasite reproductive capacity (Bull 1994). According to the trade-off model hypothesis, high virulence can also reduce host survival and therefore the duration of transmission; thus, theoretical models predict an optimal intermediate virulence that maximizes transmission while taking this trade-off into account. Relationships between parasite and hosts became more complicated in a natural population, where it is expected to be heterogeneous. Regoes et al. (2000) found that host heterogeneity may be an important determinant for the evolution of virulence and its influence depends on the nature of trade-offs of the parasite virulence in the different host types.

The host-parasite model system

The Parasite.-*Parvilucifera* genus, first described by Norén et al. (1999) is a genus of parasites recently included in the taxon Perkinsozoa (Alveolate phylum), that combines characteristics of dinoflagellates and apicomplexans comprising the genus *Parvilucifera*, *Perkinsus* and *Colpodella*. *Parvilucifera* is an intracellular parasite of dinoflagellate species with part of the life cycle confined to the host and part as a free-living flagellate, which ends the infection with the host dead, consequently we will use the term parasitoid. Three species of *Parvilucifera* genus have been described until now, despite the real diversity of *Parvilucifera* genus has been most probably underestimated, due to most of reports of these species have been done in base of morphological features. The first species described belonging to this genus was *P. infectans* which was isolated from the dinoflagellate genus *Dinophysis* on the west coast of Sweden (Norén et al., 1999); Leander and Hoppenrath (2008) described *P. prorocentri*, and Figueroa et al. (2008) described *P. sinerae* from a toxic dinoflagellate specie *Alexandrium minutum* in the Catalan coast (NW Mediterranean sea). *Parvilucifera* genus has a mobile stage (the zooid) and a benthic stage (the sporangium) in its life cycle. The three species share morphological features of both stages (see Leander and Hoppenrath 2008; Garcés and Hoppenrath 2010), however, *P. sinerae* and *P. infectans* have more in common than *P. prorocentri*, which seems to be more related with *Perkinsus* genus.

These species show differences of specificity in the range of species they can infect. *P. infectans* and *P. sinerae* are known to infect several dinoflagellate species with a broad host range (Norén et al., 1999; Garcés et al., submitted), and seems to be specialized on Dinophysiales, Gonyaulacales, and Peridinales groups. While both species are not able to infect prorocentrales, *P. prorocentri* is known to infect only *Prorocentrum fukuyoi* (Leander and Hoppenrath 2008). Despite the morphological and physiological differences explained within the three species, molecular phylogenetic analyses using SSU rDNA sequences indicate a strongly supported clade in *Parvilucifera*, rather than erect a new clade for *P. prorocentri* (Hoppenrath and Leander 2009).

Given the capacity of *Parvilucifera* to infect toxic dinoflagellates, furthermore to be linked to declines of these microalgae populations, have been proposed as potential agents for controlling HAB events (Delgado 1999; Chambouvet et al., 2008). Its effective use in natural environments for this purpose will depend on prior knowledge of the ecology of the parasite. Specifically, the stages of the parasite life cycle, their specificity, virulence, and other questions related with host-parasite interactions remain to be determined.

In this study we are focused in *P. sinerae*. Some work has been done in this species (Figueroa et al., 2008; Figueroa et al., 2010; Garcés and Hoppenrath 2010) but the whole infection process and the intermediate stages remain to be described.

The host.- The genus *Alexandrium* (Dinophyceae) includes 32 described species of dinoflagellates, some of them, as *A. minutum*, produce potent neurotoxins which cause paralytic shellfish poisoning (PSP) in many coastal waters (Hallegraeff et al., 1988; Lilly et al., 2002) and thus represent a risk for public health and a source of economic impact on aquaculture. *A. minutum* has been regularly detected during the last decade in different areas of the Mediterranean Sea, blooms related to the production of PSP toxin were reported in this area (Casabianca et al., 2011; Vila et al., 2005).

Alexandrium often suffers parasite attacks, especially from the genus *Parvilucifera* (Norén et al., 1999; Figueroa et al., 2008; Garcés et al., submitted) that exert a strong selective pressure in their host. In example, Figueroa et al.

(2010) founded that the capability to alternate between asexual and sexual reproduction of *Alexandrium* is regulated during *Parvilucifera* infection, a trade-off between asexual fast growth, and sexual reproduction (new gene combinations). Despite of this, the role of the infection over an algal bloom dynamics remains unknown, as well as the specificity at inter and intra-specific level. In this study we focus in susceptibility of *Alexandrium* genus to *P. sinerae* infection and the specificity of this parasitoid at intra-species level in *A. minutum*.

Main goals of this project

This project aims to advance in the knowledge of marine protists parasites ecology analyzing host-parasite interactions in the model comprising the dinoflagellate species *Alexandrium minutum* Halim (1960) (the host) and the perkinsozoid *Parvilucifera sinerae* Figueroa (2008) (the parasite).

The goals of this project are: i) to identify the different stages of *P. sinerae* infection in the host *A. minutum*; ii) to define biological aspects of this host-parasite interaction examining the influence of inoculum size on parasite prevalence, determine duration of infection cycle estimating total parasite generation time and the time of each stage of infection; iii) to examine host specificity of *P. sinerae* under culture conditions in *Alexandrium* genus from different geographical origin, and iv) to study how host and parasite genotypes affect infectivity in a cross-infection experiment.

MATERIAL AND METHODS

Laboratory cultures

Parvilucifera sinerae culture (strain ICMB 852) was established from an almost monospecific bloom of *Alexandrium minutum* that took place in Vilanova harbour (Mediterranean Sea, Spain) in March 2009 as explained in Garcés and Hoppenrath (2010). The parasite culture is propagated by transferring aliquots of mature sporangium (20–25) every 6-7 days into uninfected host stock culture

of exponentially growing *A. minutum* strain P4 in sterile polystyrene Petri dishes (Iwaki, Japan, 16-mm diameter). The cultures were incubated at 20+/- 1°C in a 12:12 light:dark cycle of white fluorescent light at 90 µmol photons m^2 s^1.

Life cycle stages under light microscopy and spectral confocal microscopy

To identify infection stages and the parasite life cycle, we inoculated 20-25 mature sporangia of *Parvilucifera sinerae* ICMB852 to healthy *Alexandrium minutum* P4 culture growing exponentially. Infection process was followed twice per day (at hours 2 and 8 after dark period of the light cycle) over 4 days, until all host cells remain infected and zoospores were formed inside mature sporangia ready to start a second round of infection. Samples were fixed in formaldehyde (1% final concentration) and photographed using a Leica–Leitz DMIRB inverted microscope (Leica Microsystems GmbH, Wetzlar, Germany) and the ProgRes CapturePro image analysis software (JENOPTIK Laser, Optik, Systeme GmbH). Moreover, samples from the infection process were collected to be examined under spectral confocal microscopy in order to describe stages that are undetectable under light microscopy. Those samples were fixed adding 1 volume of formaldehyde 37% for 9 volumes of sample and incubated for 2 hours at 4ºC in the dark. A subsample (2ml) from the fixed sample was filtered onto 8 µm pore size polycarbonate filters (25 mm diameter) with a gentle vacuum of 150 mbar at room temperature. Cellulose acetate support filters were employed to favour homogeneous distribution of cells. The samples were subsequently stored at -80 °C until further processing. Filters were cut in pieces and application of fluorescent *in situ* hybridization coupled with tyramide signal amplification (FISH-TSA) was carried out using the temperatures and conditions as described in Not et al. (2002). Filters were previously dipped in agarose (0.1% (wt/vol)), incubated with HCl 0.01 M (to inactivate endogenous peroxidases) for 10 minutes at room temperature and washed with Milli-Q water. For hybridization using Parvi-2R probe (Johansson et al., 2006) 40% formamide was used and after this step samples were equilibrated in PBS buffer. The signal amplification was done using a tyramide labeled with Alexa 488 as in Pernthaler et al. (2004).Finally, filter sections were mounted in a

mixture that contained 4 parts Citifluor and 1 part Vecta Shield containing 4'-6'-diamidino-2-phelylindole (DAPI) (final concentration 1µg ml^{-1}). Cells were visualised with a Leica TCS-SP confocal laser scanning microscope (CLSM), mounted on a Leica DCM IRB epifluorescence microscope equipped with a 50 W mercury lamp, PL APO 60×/1.4 na oil objective and appropriate filter sets for FITC and Cy3 (Leica Microsystems, Germany).

Parasite prevalence and time-course of infection

Parasite prevalence was determined as a function of inoculums size in sterile vials containing 10mL of host cells at 10^3 mL^{-1} and inoculum size of parasite were adjusted to give zoospores:host ratios of 1:1, 2:1, 5:1, 10:1, 20:1, 30:1, 40:1 and 80:1. Those vials were incubated during 5 days under growth conditions described above. This incubation time was required for the formation of mature sporangia but was shorter than the time needed for a second whole round of infection took place. Samples were collected twice per day (at hours 2 and 8 after dark period of the light cycle) and preserved with formaldehyde (1% final concentration) and examined by inverted light microscopy (Leica–Leitz DMIRB). Parasite prevalence was calculated as a percentage of infected cells (dark mature sporangia) and determined by scoring at least 300 hosts cells per sample as infected or uninfected in a Sedgwick-Rafter chamber. Data were fitted to a single two parameter exponential rise to maximum. The equation for the curve fit was $y = a(1 - e^{-bx})$, where a is the maximum infection level (I_{max}) and b is α/I_{max}. Alpha (α) represents the slope of the initial linear portion of the fitted curve and reflects the potential of zoospores to infect host cells. Alpha was estimated as I_{max}*b.

Time-course of infection was conducted in 10mL uninfected host cultures at initial density $10^3 \cdot mL^{-1}$ inoculating recently formed zoospores to give a zoospore:host ratio 2:1. Culture conditions, incubation time and sampling time were the same as described above for parasite prevalence. Every stage of *P. sinerae* life cycle (described in Fig. 1 and 2) were quantified throughout the time necessary to complete parasite cycle. Temporal differences in the maximum

occurrence of the different infection stages were used to estimate parasite intracellular development time.

Specificity of P. sinerae to Alexandrium genus

The susceptibility of 199 strains belonging *Alexandrium* genera to parasitoid infection was tested using the strain of *P. sinerae* ICMB852. 13 different species were tested: *Alexandrium affine*, *A. andersonii, A. margalefi, A. minutum, A. ostenfeldi, A. peruvianum, A. tamutum, A. taylori* and four different groups of *A. tamarense* complex from the culture collection of the Centro Oceanográfico (CCVIEO) in Vigo, Spain, and the culture collection of the Institut Ciències del Mar, Barcelona, Spain (Table A1 in Annex). Culture were maintained in 50-mL polystyrene tissue culture flasks filled with 20 mL of L1 medium (Guillard 1995) without silica at 31 of salinity. Cultures were grown at same conditions described above. Recently formed mature sporangia of *P. sinerae* (day 6 post-infection of the host at 20ºC) were added to exponentially growing cells of each microalgae strain. The infections were carried out in sterile polystyrene Petri dishes (Iwaki, Japan, 22mm diameter) at a parasitoid:host ratio of 10:1 in a total volume of 3 mL. Parasitoid:host cultures were followed daily under an inverted light microscope, initially to monitor infection of the cells and later to follow the development of infection process. The detection of the various stages of infection until formation of the mature sporangium unequivocally confirmed strain infection. In case of negative result, a second round of *P. sinerae* parasitoid inoculation was started adding mature sporangia in the same Petri dish.

Cross infections

Hosts.- 9 Alexandrium minutum clonal strains from the culture collection of Centro Oceanográfico de Vigo (Spain) isolated in different years and from different locations of the Mediterranean sea (Table A2 annex) were used. Cultures were maintained in 450-mL polystyrene tissue culture flasks filled with L1 medium (Guillard 1995) without silica. Cultures were grown at 15±1 ºC with a

photoperiod of 12:12 h (light:dark) cycle. Illumination was provided by fluorescent tubes with a photon irradiance of about 45 µmol photons m^{-2} s^{1}.

Parasites.-10 *Parvilucifera sinerae* clonal strains were isolated from different geographical locations of the Catalan coast in different years (Table A3 in Annex). The parasitoids cultures were propagated by transferring aliquots of mature sporangium (1 ml) every 6–7 days into an uninfected host stock culture of *A. minutum* strain P4 (growing exponentially) in sterile polystyrene Petri dishes (Iwaki, Japan, 16-mm diameter) at same culture conditions described for the hosts maintenance. When most sporangia were mature, cultures were stored at 4ºC and darkness to synchronize the maturation of the rest of parasites.

Every host strain was infected with every parasite strain in four replicates carried out for each of these 90 combinations and four replicated host cultures were left uninfected to serve as controls. Five recently formed mature sporangia were added to infect host cultures at initial concentration of $5 \cdot 10^{3}$ cells·mL^{-1}in a total volume of 20 mL, giving a final zoospores:host ratio 1:60 (similar to the ratio in nature). These five sporangia were previously isolated in sterile polystyrene Petri dishes and stored at 4ºC until the inoculation time in order to maintain zoospores inactive. The infections were running during 3 weeks at 15±1ºC with a photoperiod of 12:12 h (light:dark) cycle and 45 µmol photons m^{-2} s^{1} of irradiance. Samples were taken every two days, completing a total of 7 sampling dates. One mL of each flask was fixed with formaldehyde (1% final concentration) and stored at 4ºC in dark. Outcome of infection was estimated based on infectivity defined as the proportion of bottles of a given host-parasite combination where sporangia were observed (on day 16). Following infection was determined under light microscopy using a Leica–Leitz DMIRB inverted microscope (Leica Microsystems GmbH, Wetzlar, Germany).

RESULTS

Life cycle

We were able to follow the evolution of *P. sinerae* infection in *A. minutum* and the various stages of infection. Theoretical scheme of the infection process of dinoflagellates with *Parvilucifera sinerae* is presented in Fig. 1.

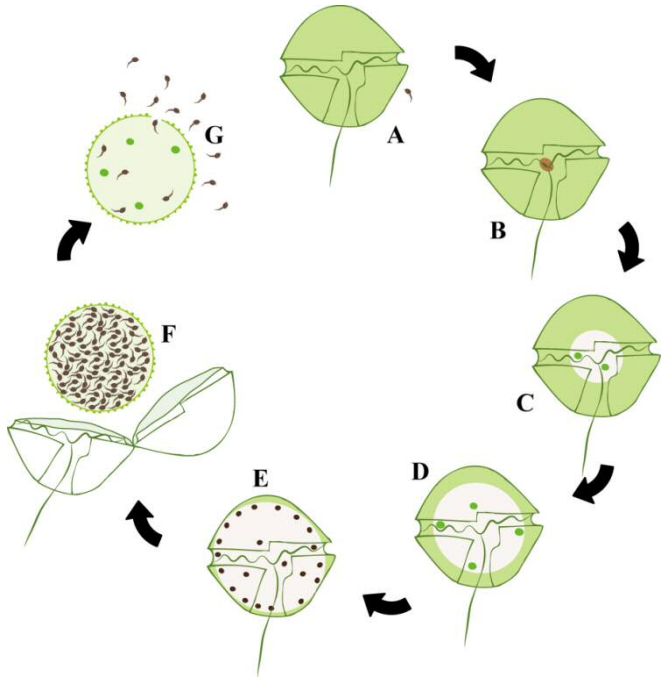

Figure 1. Schematic view of several stages of the life cycle *Parvilucifera sinerae* infecting *Alexandrium minutum* as a host cell. The infection stages and the transition between them (arrows) are based on observations on light and confocal microscopy of this study and those observations in Garcés and Hoppenrath (2010). **A**. Healthy cell of *Alexandrium minutum*. **B.** One parasite zoospore penetrates inside host cell. **C**. Early stage of infection, parasitoid's round body, containing vacuole-like structures (stage 1) **D.** Late-stage infection of the parasitoid with spherical shape (stage 2) which is a transparent immature sporangium. **E**. Stage filled with flagellated cells (zoospores), beginning from peripheral sites (stage 3). **F**. Mature sporangium containing approximately 200 zoospores occupying whole sporangia body and **G**. Empty sporangium.

Transmitted light microscopic examination of living cells of *Alexandrium minutum* allowed identification of stages of the infection in comparison to healthy cells (Fig. 2A). Infections were initiated by the invasion of host cells by one small infective flagellate cell called zoospores (confocal microscopy image) (Fig.2B). Note that small flagellate cells had green fluorescence due to Parvi2R

probe. Early stages of infection were recognizable based on the presence of a round body in the host (stage 1, Fig. 2C). This early stage of infection is a process where the parasitoid grows until his body occupy most part of the host cytoplasm and it is understood as the stage that the parasitoid destroy the host cytoplasm. A later stage is recognizable when this round body detaches from the theca of the cell (in case of the thecated hosts), appearing clearly spherical (Fig. 2D). The parasitoid sometimes causes the breakage of the host cell wall. This was called immature sporangium between stage 2 and 3. This immature sporangium appears transparent, containing some peripheral lipid vacuole-like structures. The immature sporangium within in a few hours start to produce hundreds of flagellate cells (zoospores) from peripheral areas to the inside (Fig.2E, F), each flagellate is able to infect a new host. Note that confocal microscopy shows the appearance of the zoospores from peripheral area to inside with their nucleus in blue (DAPI). The late stage of infection, called the mature sporangium, is very dark and spherical sporangia that contains 200 zoospores approximately (Fig. 2G). In a second round of infection, after the release of the zoospores (Fig.2H), the sporangium appears empty (Fig. 2F).

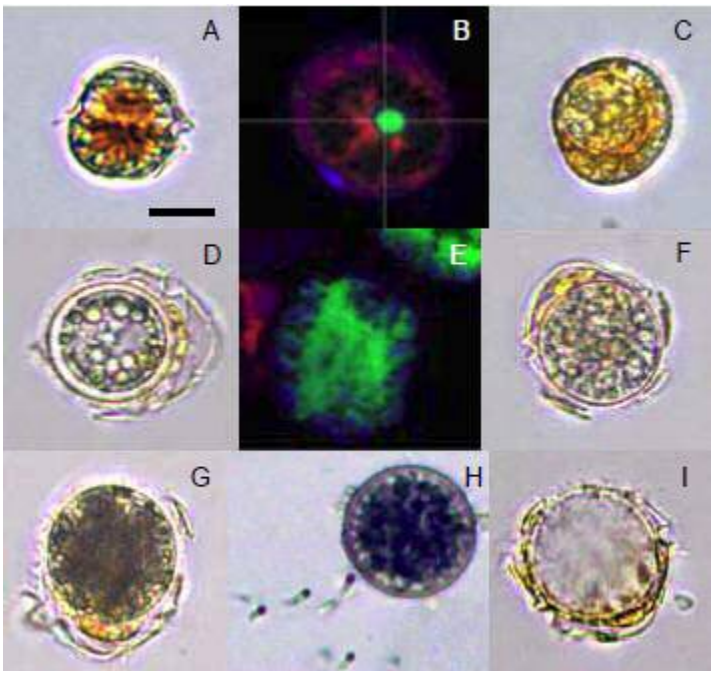

Figure 2. Infection process of *Alexandrium minutum* strain P4 with *Parvilucifera sinerae* in optical (A, C, D, F, G-I) and confocal (B and E) microscopy. **A**. Healthy cell of *Alexandrium minutum*. **B.** A zoospore inside host cell beginning the infection

process. **C.** Early stage of infection, parasitoid's round body inside an entire dinoflagellate (stage 1). **D**. Spherical late-stage infection of the parasitoid with spherical shape (stage 2) which is a transparent immature sporangium, containing vacuole-like structures. **E**. Growing of zoospores from peripheral areas to inside under confocal microscopy, flagellate nucleus in blue (DAPI). **F.** Stages filled with flagellated cells (zoospores) (stage 3). **G**. Mature sporangium with some parts of dinoflagellate theca **H.** Zoospores release. **I.** Empty sporangium with lipid vacuole-like structure.
Scale bar = 10 µm.

Parasite prevalence and time-course of infection

Parasite prevalence showed an exponential increase to a maximum relative to inoculums size (Fig. 3). Estimates for maximum infection level (Imax) and initial slope of the fitted curve (α) were Imax=82.63%±2.68, α=16.71 (r^2=0.98079). Zoospores of *P. sinerae* shows a parasite prevalence rising near maximum levels by a zoospores:host ratio of 20:1. Inoculations above 20:1 ratio showed more than 80% infection of host cells, but failed to reach 100% of infection levels even at zoospores:host ratio 80:1.

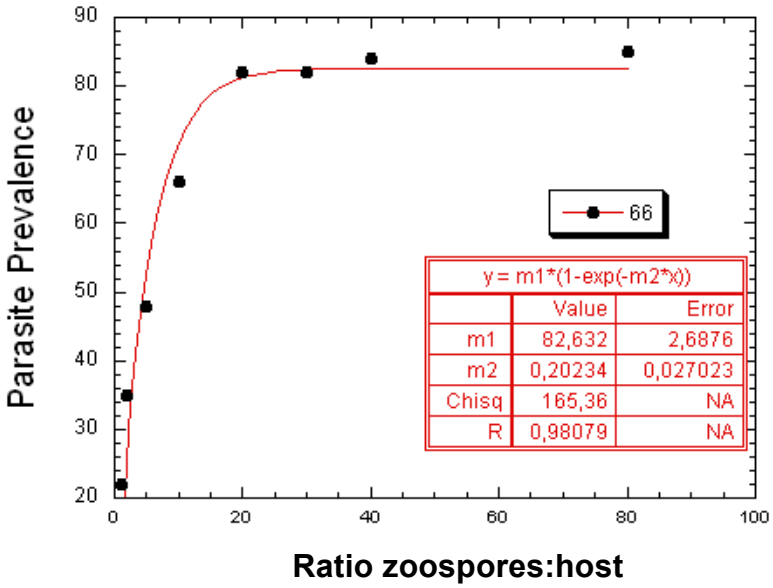

Figure 3. *Parvilucifera sinerae* (ICMB852) prevalence infecting *A. minutum* (P4) as a function of inoculum size. Host density was maintained at 10^3 cells·mL^{-1}, with parasite density varied to yield zoospores:host ratios of 1:1, 2:1, 5:1, 10:1, 20:1, 30:1, 40:1 and 80:1.

Time for the whole formation of the mature sporangium (from stage 1 to 3) at 20 ºC is presented in figure 4. In *Alexandrium minutum* under a parasite:host

ratio 2:1, the whole infection cycle lasted 4 days at the above described culture conditions. The first infection stage (immature E1) and stage 2 (immature E2) were observed in 24 hours after adding parasitoid to the healthy culture. This situation evolved after two days, were almost 40 % of the *A. minutum* cells are infected and quickly in 6 hours more than 80 % are infected. At time 72, very few healthy cells are present and the 80% of the cells remained as immature sporangium during 6 hours more evolving from immature 1 to immature 2. After 90 hours, zoospores began to be formed inside the sporangium (stage immature E3), and mature sporangia were observed. At this time, void sporangia are detected because the releasing of flagellates occurred at this period. After 102 hours the majority of the sporangia are void due to a second round of infection.

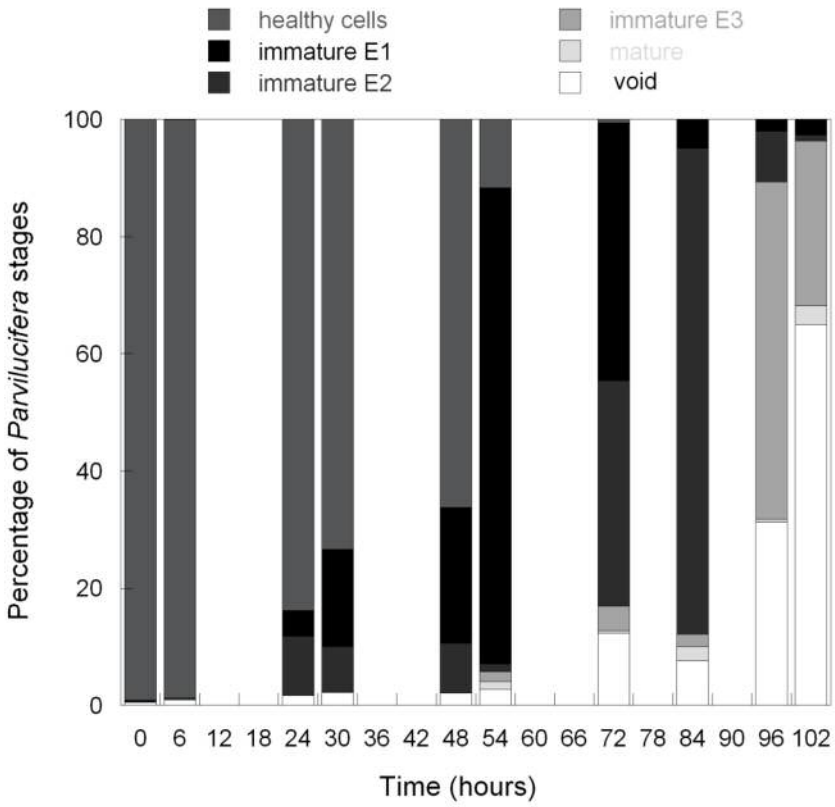

Figure 4. Time-course of *Parvilucifera sinerae* infecting *Alexandrium minutum* inoculated at zoospores:host ratio of 2:1. Bars show cumulative percentage of different stages abundance with color regions represents healthy (uninfected) cells, immature 1, 2, and 3 stages, mature sporangium and empty sporangium as described in Fig. 1.

Specificity of P. sinerae

Specificity results of *P. sinerae* strain (ICMB852) to its capacity to infect different species of the dinoflagellate genus *Alexandrium* are shown in table 1. Of the total of 199 strains analyzed, including 13 different species of this genus, 90% of the strains were sensible to *P. sinerae* and only 10% were resistant to the infection. Intra-species variability was observed, as in strains of *Alexandrium minutum, A. andersonii, A. tamutum and A. tamarense* complex (Group III). While *A. andersonii* presented the lowest percentage of infected strains, which was 40%, the other infected species that had intra-species variability showed more than 80% of strains infected.

Table 1. Strains of the genus *Alexandrium* infected by *Parvilucifera sinerae.* In the case of an infected strain, if > 3 strains were tested, the percentage of infected strains is shown; n =number of tested strains; - no infection detected. The *A. tamarense* complex formed a monophyletic clade subdivided into five groups, Groups I, II, III, IV, and V following Scholin and Anderson (1994), John et al. (2003) and Lilly et al. (2005).

Genus	Species	Infected	Infected strains (%)	n
Alexandrium	*affine*	yes		2
	andersonii	yes	40	5
	tamarense complex (Group I)	yes	100	7
	tamarense complex (Group II)	yes	100	12
	tamarense complex (Group III)	yes	85	13
	tamarense complex (Group IV)	yes	100	56
	margalefi	yes	100	4
	minutum	yes	94	86
	ostenfeldii	yes	100	6
	peruvianum	yes		1
	tamutum	yes	83	6
	taylori	no		1

Cross infection

Infectivity for the 90 host-parasite combinations is shown in figure 5. Considering host genotypes, host 1(H1) followed by host 3 (H3) were those with greater resistance to most parasite strains infectivity. Host 7 was the most sensible to all parasite strains, reaching all of them to 100% infection level on it. Other hosts present intermediate levels of infection, varying the percentage of successful infection depending on the infecting parasite. P10 strain was the most virulent strain reaching 100% successful infections almost in all host

strains, except for the host 1 which was not infected. These differences on infectivity are clearly dependent to genotype-by-genotype interactions.

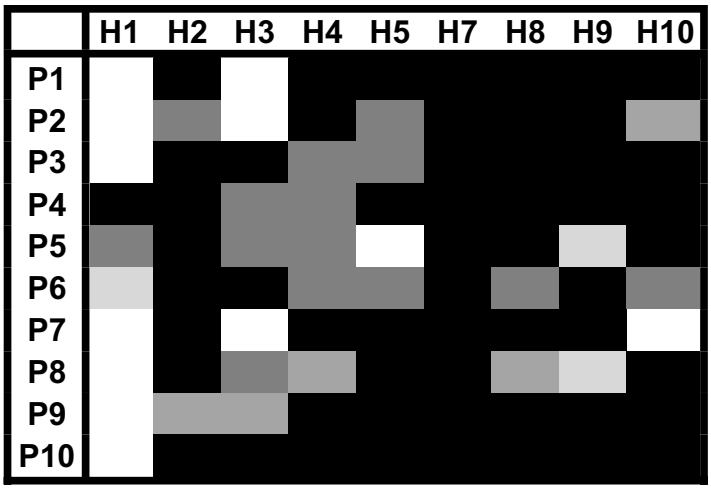

Figure 5. Proportion successful infections in different host-parasite combinations. Colors indicate: white 0%, light grey 25%, medium grey 50%, dark grey 75%, and black 100%.

DISCUSSION

Understanding biological and physiological traits of the interaction between *Parvilucifera sinerae* and its host is of primary importance to better understand the ecology of this parasite-host model, as the potential use of this species for HABs control. In the present study, biological and behavioral traits of *P. sinerae* life cycle are described and also intra/inter species variability in the specificity of this parasitoid in the *Alexandrium* genus. These results highlight the complexity of the infection patterns, which rely on genotype-by-genotype interactions between parasite and host.

Life cycle and infection process

After penetrating the host, *P. sinerae* grows and divides to yield about 200 flagellated cells in *A. minutum*, thereby transforming the degraded host cell into a mature sporangium. In sporangium maturation, formation and division of flagellate cells starts from the peripheral area to inside, becoming in a spherical

dark sporangia full occupied by zoospores which leave the spherical sporangium throughout one or several opercula to infect new hosts in a second round of infection. The present study reports for the first time the development time needed for each stage in *Parvilucifera sinerae* as well as zoospores growing patterns.

The three *Parvilucifera* species described until now, share generally traits in their life cycles; they infect and complete their life cycle in one specimen producing host dead, they have two completely different morphological stages, as free-living flagellate outside the host which is the infective stage, and a non-motile spherical sporangia. *P. infectans* and *P. sinerae* share more life cycle traits than *P. prorocentri*. For example, during zoospores formation, *P. prorocentri* develops a prominent germ tube that allows zoospores release (Hoppenrath and Leander 2009) while in *P. infectans* are produced by the same mechanism explained above for *P. sinerae* (Norén et al., 1999). These differences are supported by the recent phylogenetic analysis done by Hoppenrath and Leander (2009), which conclude that taxonomic classification of *P. prorocentri* needs to be revised.

Generation time of *P. sinerae* infecting *A. minutum* culture lasted 4 days, which indicate a higher growth rate than the host species (Stolte and Garcés 2006), thus confers to parasite an evolutionary advantage to maintain local adaptation. In comparison to other *Parvilucifera* species, *P. infectans* generation time ranges from 1-2 days in *A. fundyense* to 1- 6 days in *Dinophysis* (Norén et al., 1999). Time for mature sporangia development has been demonstrated recently that depends on sporangium size, which in turn depends on cell width of the specie parasited (Garcés et al., submitted). Furthermore, *P. sinerae* life-history traits are difficult to compare with those described in other studies for other *Parvilucifera* species due to the differences in the procedures and in experimental design, e.g. the parasite:host ratio used in each infection or the hosts used. In fact, in this study has been demonstrated that parasite prevalence of infected cells depend on the parasite:host ratio. Higher *P. sinerae* zoospores inoculation showed parasite prevalence almost reaching 90% of *A. minutum* infected cells. However, *Parvilucifera* zoospores never reach 100% infection level, remaining between 10-20% cells uninfected, suggesting that

some specimens of *A. minutum* are resistant to infection. The source of this apparent resistance is unknown, but may be related to cell cycle events, genetic changes or cell states (e.g. unhealthy cells or in a suboptimal metabolic rate) that render *A. minutum* unattractive to zoospores or capable of avoiding parasite infection. No data exist of prevalence as a function of inoculum size in other species belonging *Parvilucifera* genus. Zoospores of *P. sinerae* show a medium level of virulence in comparison to dinospores of *Amoebophrya*, that depending on the host specie, the ratio needed to reach 100% of infection levels is variable, being the most aggressive on *Akashiwo sanguinea*, where maximum level of infection were achieved with a dinospore:host inoculations above 10:1 (Coats and Park 2002). *P. sinerae* prevalence increases exponentially as a function of inoculum size, which is explained because at high ratios the encounter probability with host cells is enhanced and therefore, also the probability of infection success. This result suggests, accordingly to Delgado (1999), that infective zoospores have a short life span and need to found quickly an appropriate host to infect in order to survive.

The high number of zoospores produced from an individual infection, together with the exponential increment of parasite prevalence as a function of zoospores:host ratio, indicate a high reproductive asexual rate of *P. sinerae*. Thus, this means that with only one successful infection, the parasite has already the capacity to infect a high number of host cells. In marine environments, outbreaks of this parasitoid coincided with seasonal peaks of *Alexandrium minutum* (Figueroa et al., 2008), a similar pattern reported by Johansson et al. (2006) for the spatial distribution of *P. infectans* in Swedish coast. Thus, the high reproduction rate of the genus *Parvilucifera* seems to be adapted to fit the outbreaks of its host. Although the factors that cause phytoplankton blooms termination are not clear, our results suggest that *P. sinerae* could be one relevant factor involved in the end of dinoflagellate blooms.

Host-parasite interactions: Specificity of P. sinerae

Parasites have the capacity to exert selective pressures on its hosts, whose strength in turn, depends on the specific nature of the interaction determining the dynamic, diversity and evolution of host populations. As a consequence, these specific pressures could lead to changes in the structure of microalgae communities. *P. sinerae* is a generalist parasitoid of dinoflagellates (Garcés et al., submitted) that shows high specificity for the *Alexandrium* genus, as demonstrated also in the present study, where 90% of *Alexandrium* strains became infected despite of being tested several species from very different geographical origins. In addition, some intra-specific variability was found supporting the previous study of Figueroa et al. (2008). These observations provide evidence for the parasitoid's ability to differentially impact potential hosts belonging to the *Alexandrium* genus, thus potentially driving changes in microalgal community composition and microalgae succession in natural communities. In this experiment, we consider as resistant strains those in which no mature sporangia were observed, although this experimental design was unable to demonstrate total resistance to parasitoid because exposure of the host strain to the tested parasitoid strain does not mean that the same results would be achieved if using other *P. sinerae* strains. Importantly, it must be noted that successful infection under laboratory conditions does not mean that infections would not occur under other conditions either in the laboratory or in the field.

Resistance studies of host microalgae to their parasites usually focus on the response of a host line against a single parasite genotype (Garcés et al., submitted; Figueroa et al., 2008) but in the natural environment it may be expected that microalgae-parasite relationship are based, as in many other host-parasite systems, on host-parasite genotype interactions (Lively and Dybdahl 2000; Lambrechts et al., 2005; de Roode and Altizer 2009). However, such interactions have been not studied in marine protists. *P. sinerae* differences in specificity at intra-species level leaded us to study the infectivity of *P. sinerae* in *A. minutum* using a multiple genotypes crossing experiment. In that experiment, we demonstrate that host resistance and parasite virulence

depend on genotype-specific interactions, from both the parasite and the host. Hosts can provide a variable backdrop for parasite evolution, such that selection of virulence will be complicated by variation among host genotypes. Our result is consistent with a recent work showing that parasite virulence can depend on host properties (Salvaudon et al., 2007) and indicates that predictions regarding virulence evolution may need to account for genotype-specific interactions. In a local adapted parasite-host system characterized by G x G interactions, this genotype specific interactions could generate frequency-dependent selection, with parasites evolving optimal virulence in the most common host genotypes, showing intermediate levels of virulence, hence by natural selection. In the crossing experiment, including parasite and host isolates from different but geographically close populations, we found, accordingly to Gandon et al. (2002), a high genetic variation for both parasite virulence and host resistance, with one parasite isolate (P10) really aggressive to almost all host lines, one host isolate (H1) resistant to almost all parasite lines, and one host isolate (H7) very susceptible to all parasites. But, what are the costs to be resistant to almost all parasitoid genotypes? What are the costs for the parasitoid to be highly virulent?

A further study of the resistance and susceptibility trades-offs needs to be done in order to answer these questions. The costs to be resistant to parasitoid genotypes involve life cycle traits of the host as sexual and asexual reproduction, more specifically, effects on host sexuality rates, in resting cyst production and in growth rate. The cost also may involve how the different host genotypes affect exploitation and transmission of *P. sinerae* genotypes, but all these processes remain unknown. One question that emerges in terms of local adaptation in this host-parasite system is how is the genetic diversity within an *Alexandrium* bloom, affected by the parasite infection, or in turn, how variable is the genetic diversity of the parasite within the blooms. These questions need to be solved to better understand the genotype-specific interactions in a bloom and to know if parasites and traits of infection are locally adapted in such system. A further study of *P. sinerae* and its host interactions in natural populations is needed to better understand how this system works at a bloom level, which is essential to know the ecological and evolution impacts of this

parasitoid to its host natural population, therefore determining if is possible the use of this parasite in controlling HAB events caused by dinoflagellates.

CONCLUSIONS

P. sinerae is an intracellular parasite with a rapid generation time and a high reproduction rate, traits conferring it advantages to adaptation of its host. This parasite is able to infect a wide host range, especially dinoflagellates belonging *Alexandrium* genus. However, intra-species variability exists in this complex system. Some specific strains within the same specie seem to be resistant to parasitic infection of *P. sinerae*, the causes of this variability being unknown. The factors that confer this capability to avoid the infection need to be further studied, but host-parasite dynamics in this model system indicate that resistance is regulated by genotype-specific interactions. A further research on biological and behavior traits affected by genotype-specific interactions in this model may help to understand the ecological and evolutionary implications of *P. sinerae* in its host natural populations.

AKNOWLEDGEMENTS

This study was supported by the Spanish funded project PARAL (CTM2009-08399) and project CCVIEO. I wish to thank Esther Garcés (ICM, Barcelona) and Rosa Figueroa (Lund's University and IEO) for experimental designs, for advises given and for supervising the project. I wish thank Isabel Bravo (IEO, Vigo) and Isabel Ramilo (IEO, Vigo) for the laboratory cultures. I wish thank also Lars Råberg for his help in experimental design and sampling during my stay at the University of Lund, Sweden.

REFERENCES

Agosta, SJ., Janz, N., Brooks, DR. 2010. How specialists can be generalists: resolving the “parasite paradox” and implications for emerging infectious disease. *Zoologia* 27:151–62.

Anderson, R. M., and R. M. May. 1979. Population biology of infectious diseases. I. *Nature* 280:361–367.

Boots, M. and Haraguchi, Y. 1999. The evolution of costly resistance in host-parasite systems. *Am. Nat*. 153, 359-370.

Brussaard CPD. 2004. Viral control of phytoplankton populations – a review. *J Eukaryot Microbiol* 51:125–138.

Buckling, A. and Rainey, P. 2002. The role of parasites in sympatric and allopatric host diversification. *Nature* 420: 496–9.

Bull, J.J. 1994. Virulence. *Evolution* 48:1423-1437.

Carius, H. J., T. J. Little, and D. Ebert. 2001. Genetic variation in a hostparasite association: Potential for coevolution and frequency-dependent selection. *Evolution* 55:1136–1145.

Casabianca S., Penna A., Pecchiolo E., Jordi A., Basterretxea G. and Vernesi C. 2011. Population genetic structure and connectivity of the harmful dinoflagellate Alexandrium minutum in the Mediterranean sea. *Proc. R. Soc*, 279: 129-138.

Chambouvet, A., Morin, P., Marie, D. and Guillou, L. 2008. Control of toxic marine dinoflagellate blooms by serial parasitic killers. *Science* 322:1254-57.

Coats, D. W. and Park, M. G. 2002. Parasitism of photosynthetic dinoflagellates by three strains of *Amoebophrya* (dinophyta): parasite survival, infectivity, generation time, and host specificity. *J Phycol* 38:520-28.

Delgado, M. 1999. A new “diablillo parasite” in the toxic dinoflagellate *Alexandrium catenella* as a possibility to control harmful algal blooms. *Harmful Algae News,* 19:1–3.

de Roode JC., and Altizer S. 2009. Host-parasite genetic interactions and virulence transmission relationships in natural populations of monarch butterflies. *Evolution* 64: 502–514.

Elbrächter M. and Schnepf E. 1998. Parasites of harmful algae. In: Anderson DM, Cembella.

Figueroa, R. I., Garces, E., Massana, R. and Camp, J. 2008. Description, host-specificity, and strain selectivity of the dinoflagellate parasite *Parvilucifera sinerae* sp nov (Perkinsozoa). *Protist* 159:563-78.

Figueroa, R.I., Garcés E., Camp J. 2010. Reproductive plasticity and local adaption in the host-parasite system formed by the toxic *Alexadrium minutum* and the dinoflagellate parasite *Parvilucifera sinerae. Harmful Algae* 10: 56-63.

Frank, S.A. 1996. Models of parasite Virulence. *Q. Rev. Biol*. 71, 37-78.

Gandon, S., Van Baalen, M., Jansen, VAA. 2002. The evolution of parasite virulence, superinfection, and host resistance. *Am. Nat.* 159:658–69

Gandon, S. 2004. Evolution of multihost parasites. *Evolution*, 58, 455–469.

Garcés, E. and Hoppenrath, M. 2010. Ultraestructure of the intracellular parasite *Parvilucifera sinerae* (Alveolata, Myzozoa) infecting the marine toxic planktonic dinoflagellate *Alexandrium minutum* (Dinophyceae). *Harmful algae* 10: 64-70.

Garcés E., Alacid E., Bravo I., Figueroa R., Fraga S. (submitted) *Parvilucifera sinerae* (Alveolata, myzozoa) is a generalist parasitoid of dinoflagellates Protist.

Guillard RRL.1995. Culture Methods. In Manual on Harmful Marine microalgae IOC Manuals and Guides (ed) G.M. Hallegraeff, D.M. Anderson, and A.D. Cembella. UNESCO, pp 551.

Hackett JD., Andersson DM., Erdener DL. and Bhattacharya D. 2004. Dinoflagellates: a remarkable evolutionary experiment. *Americal Journal of Botany* 91 (10): 1523-1534).

Hallegraeff,G. M., Marshall, J. A., Valentine, J. and Hardiman, S. 1998.
Short cyst-dormancy period of an Australian isolate of the toxic dinoflagellate *Alexandrium catenella*. *Mar. Freshw. Res.*, 49, 415–420.

Hallegraeff GM (eds). 2004. Physiological ecology of harmful algal blooms. NATO ASI Series 41. Springer, Berlin Heidelberg New York, pp 351–369.

Hoppenrath M, Leander BS. 2009. Molecular Phylogeny of *Parvilucifera prorocentri* (Alveolata, Myzozoa): Insights into Perkinsid character evolution. *J Eukaryot Microbiol* 56: 251-256.

Ibelings BW, De Bruin A, Kagami M, Rijkeboer M, Brehm M, van Donk E. 2004. Host parasite interactions between freshwater phytoplankton and chytrid fungi (Chytridiomycota). *J Phycol* 40:437–453.

Johansson, M., Eiler, A., Tranvik, L. and Bertilsson, S. 2006. Distribution of the dinoflagellate parasite *Parvilucifera infectans* (Perkinsozoa) along the Swedish coast. *Aquat. Microb. Ecol.* 43:289-302.

John U, Cembella A, Hummert C, Elbrächter M, Groben R, Medlin L. 2003. Discrimination of the toxigenic dinoflagellates *Alexandrium tamarense* and *A. ostenfeldii* in co-occurring natural populations from Scottish coastal waters. *Eur J Phycol* 38: 25-40

Kaltz, O., and J. Shykoff. 1998. Local adaptation in host-parasite systems. *Heredity* 81:361–370.

Lambrechts, L., J. Halbert, P. Durand, L. C. Gouagna, and J. C. Koella. 2005. Host genotype by parasite genotype interactions underlying the resistance of anopheline mosquitos to Plasmodium falciparum. *Malaria Journal* 4:3, doi:10.1186/1475-2875-4-3.

Lambrechts, l., Fellous, S. and Koella, J. 2006. Coevolutionary interactions between host and parasite genotypes. *Trends Parasitol*. 22, 12–16.

Leander, B. S. and Hoppenrath, M. 2008. Ultrastructure of a novel tube-forming, intracellular parasite of dinoflagellates: Parvilucifera prorocentri sp nov (Alveolata, Myzozoa). *Eur. J. Protistol.* 44:55-70.

Lilly E.L., Kulis, D.M., Gentien, P., Anderson, D.M., 2002. Paralytic shellfish poisoning toxins in France linked to a human introduced strain of Alexandrium catenella from the western Pacific: evidence from DNA and toxin analysis. *J. Plankton Res*. 24 (5), 443–452.

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

Little, T. J., and D. Ebert. 2000. Associations between parasitism and host genotype in natural populations of Daphnia (Crustacea:Cladocera). *J. Anim. Ecol.* 68:134–149.

Lively, C. M., and M. F. Dybdahl. 2000. Parasite adaptation to locally common host genotypes*. Nature* 405:679–681.

Norén, F., Moestrup, O. & Rehnstam-Holm, A. S. 1999. *Parvilucifera infectans* Noren et Moestrup gen. et sp nov (Perkinsozoa phylum nov.): a parasitic flagellate capable of killing toxic microalgae. *Eur. J. Protistol.* 35:233-54.

Not F, Simon N, Biegala IC, Vaulot D. 2002. Application of fluorescent *in situ* hybridization coupled with tyramide signal amplification (FISH-TSA) to assess eukaryotic picoplankton composition. *Aquat Microb Ecol* 28: 157-166.

Park, M. G., Yih, W. and Coats, D. W. 2004. Parasites and Phytoplankton, with Special Emphasis on Dinoflagellate Infections. *The Journal of Eukaryotic Microbiology* 51:145–55.

Pernthaler, J., F, Glöckner, O., Schönhuber, W. & Amann., R. 2004. Fluorescence in situ hybridization (FISH) with rRNAtargeted oligonucleotide probes. *In*: Paul, J. H. [Ed.] *Marine Microbiology.* Academic Press, London, pp. 207-26.

Price, P.W. 1980. Evolutionary biology of parasites. Princeton, Princeton University Press.

Regoes, R. R., M. Nowak, *and* S. Bonhoeffer 2000. Evolution of virulence in a heterogeneous host population. *Evolution.* 54*:* 64 – 71*.*

Restif, O. and Koella, J.C. 2003. Shared control of epidemiological traits in a coevolutionary model of host-parasite interactions. *Am. Nat.* 161, 827-836.

Salvaudon, L. Héraudet, V. Shykoff J.A. 2007. Genotype-specific interactions and the trade-off between host and parasite fitness. BMC *Evol. Biol*. 7, 189.

Scholin CA. and Anderson DM. 1994. Identification of species and strain-specific genetic markers for globally distributed *Alexandrium* (Dinophyceae). I. RFLP analysis of SSU rRNA genes. *J Phycol* 30: 744-754.

Stolte W. and Garcés E. 2006. Ecological aspects of harmful algal in situ population growth rates. In Ecology of Harmful Algae (ed) E. Graneli, and J.T. Turner. Springer-Verlag Berlin, Berlin, pp 139-152.

Taylor, D. L. 1968. In situ studies on the cytochemistry and ultrastructure of a symbiotic marine dinoflagellate. *Journal of themarine biological Association of United Kingdom* 48:349-66.

Toth, G., Nore´n, F., Selander, E. & Pavia, H. 2001. Parasite-induced morphological defense in a dinoflagellate through water-borne chemical cues. Abstract 189. Phycologia, 40(Suppl.):59.

Van Dolah FM. 2000. Marine Algal Toxins: Origins, Health Effects, and Their Increased Occurrence. *Environmental Health Perspectives* 108 (1): 133-141.

Vila M., Giacobbe MG., 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.

Zingone, A. and Enevoldsen, HO. 2000. The diversity of harmful algal blooms: a challenge for science and management. *Ocean & Coastal Management* 43: 725-748.

ANNEX

Table A1. Host strains of *Alexandrium* species tested for susceptibility *P. Sinerae* infection and their origin.

Genus	Specie	Strain	Origin
Alexandrium	*affine*	PA3V	Ría de Vigo, Galicia, Spain
	affine	PA8V	La Linea de la Concepción, Spain
	andersonii	ICMB222	Alfacs, Delta l'Ebre, Catalunya, Spain
	andersonii	SZN12	Napoli, Italy
	andersonii	VGO664	Elefsis Bay, Saronikos Gulf, Greece
	andersonii	CCMP1718	Town Cove, Eastman, MA, USA
	andersonii	clon CCMP1597-9A2	Town Cove, Eastham, MA, USA
	catenella (Group I)	AL10	Monterey Bay, CA, USA
	catenella (Group I)	AL52	Pacifica Pier, CA, USA
	catenella (Group I)	AL78	Morro Bay, CA, USA
	catenella (Group I)	ACQ06	Quellón, X Región, Chile
	catenella (Group I)	ACSD01	Bahía Sto. Domingo, XI Región, Chile
	fundyense (Group I)	CCMP1719	Portsmouth, New Hampshire, USA
	cf. *tamarense* (Group I)	MDQ1096	Mar del Plata, Argentina
	cf. t*amarense* (Group II)	CNRATAA1	Mar Piccolo di Taranto, Ionian Sea, Italy
	cf. t*amarense* (Group II)	VGO654	Paguera, Mallorca, Spain
	cf. t*amarense* (Group II)	OLFA-B5	Tunis
	cf. t*amarense* (Group II)	VGO1042	Alfacs, Delta l'Ebre, Catalunya, Spain
	cf. t*amarense* (Group II)	BT 30	Bay of Tunis, Tunis
	cf. t*amarense* (Group II)	BT 31	Bay of Tunis, Tunis
	cf. t*amarense* (Group II)	BT 32	Bay of Tunis, Tunis
	cf. t*amarense* (Group II)	BT33	Bay of Tunis, Tunis
	cf. t*amarense* (Group II)	BT34	Bay of Tunis, Tunis
	cf. t*amarense* (Group II)	BT36	Bay of Tunis, Tunis
	cf. t*amarense* (Group II)	BT 37	Bay of Tunis, Tunis
	cf. *kutnerae* (Group II)	VGO714	Port Vilanova, Catalunya, Spain
	tamarense (Group III)	CCAP1119/1	Tamar Estuary, United Kingdom
	tamarense (Group III)	PE1V	Ría de Vigo, Galicia, Spain
	tamarense (Group III)	VGO926	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	VGO927	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	VGO928	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	VGO1082	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	VGO1083	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	VGO1084	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	VGO1085	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	VGO1086	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	VGO1087	Praia de Carnota, Galicia, Spain
	tamarense (Group III)	SA1	Fangar, Delta l'Ebre, Catalunya, Spain

sp. (Group III)	VGO1078	Porto de Baiona, Galicia, Spain
cf. *catenella* (Group IV)	AC1C	Port de Barcelona, Catalunya, Spain
cf. *catenella* (Group IV)	AC2C	Port de Barcelona, Catalunya, Spain
cf. *catenella* (Group IV)	C6	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	C7	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	AC6T	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO561	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO562	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO563	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO564	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO565	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO566	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO567	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO570	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO571	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO573	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO574	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO583	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO584	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO585	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO587	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO588	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO589	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO710	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO593	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO594	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO595	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO596	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO598	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO599	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO600	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO601	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO603	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO604	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO606	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO607	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO608	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	VGO609	Port de Tarragona, Catalunya, Spain
cf. *catenella* (Group IV)	AT01	Etang de Thau, France
cf. *catenella* (Group IV)	VGO675	Etang de Thau, France
cf. *catenella* (Group IV)	VGO676	Etang de Thau, France
cf. *catenella* (Group IV)	AT02	Etang de Thau, France
cf. *catenella* (Group IV)	VGO673	Etang de Thau, France
cf. *catenella* (Group IV)	VGO814	Etang de Thau, France
cf. *catenella* (Group IV)	VGO815	Etang de Thau, France

cf. *catenella* (Group IV)	VGO816	Etang de Thau, France
cf. *catenella* (Group IV)	VGO817	Etang de Thau, France
cf. *catenella* (Group IV)	VGO818	Etang de Thau, France
cf. *catenella* (Group IV)	VGO819	Etang de Thau, France
cf. *catenella* (Group IV)	BZ3	Lac de Bizerte, Tunis
cf. *catenella* (Group IV)	BZ7	Lac de Bizerte, Tunis
cf. *catenella* (Group IV)	BZ9	Lac de Bizerte, Tunis
cf. *catenella* (Group IV)	BZ8	Lac de Bizerte, Tunis
cf. *catenella* (Group IV)	BZ10	Lac de Bizerte, Tunis
cf. *catenella* (Group IV)	BZ11	Lac de Bizerte, Tunis
cf. *catenella* (Group IV)	BZ14	Lac de Bizerte, Tunis
cf. *tamarense* (Group IV)	CCMP1493	Bahia de Da Yia, China
margalefi	VGO 763	Port Vilanova, Catalunya, Spain
margalefi	VGO 661	Alfacs, Delta l'Ebre, Catalunya, Spain
margalefi	VGO 794	Port Palamós, Catalunya, Spain
margalefi	661-A10	Alfacs, Delta l'Ebre, Catalunya, Spain
minutum	VGO650	Port Saint Hubert, Brittany, France
minutum	VGO651	Port Saint Hubert, Brittany, France
minutum	VGO 651(5)	Port Saint Hubert, Brittany, France
minutum	VGO652	Port Saint Hubert, Brittany, France
minutum	VGO653	Port Saint Hubert, Brittany, France
minutum	VGO657	Port Saint Hubert, Brittany, France
minutum	AL5V	Ponte de Toralla, Ría de Vigo, Spain
minutum	AMP13	Port de Palma, Mallorca, Spain
minutum	VGO577	La Fosca, Catalunya, Spain
minutum	VGO663	Sardinia, Italy
minutum	VGO707	Alfacs, Delta l'Ebre, Catalunya, Spain
minutum	VGO712	Port Vilanova, Catalunya, Spain
minutum	VGO713	Port Vilanova, Catalunya, Spain
minutum	VGO716	Port Vilanova, Catalunya, Spain
minutum	VGO717	Port Vilanova, Catalunya, Spain
minutum	VGO718	Port Vilanova, Catalunya, Spain
minutum	VGO719	Port Vilanova, Catalunya, Spain
minutum	VGO720	Port Vilanova, Catalunya, Spain
minutum	VGO721	Port Vilanova, Catalunya, Spain
minutum	VGO722	Cambrils, Catalunya, Spain
minutum	VGO723	Cambrils, Catalunya, Spain
minutum	VGO746	Saronikos Gulf, Greece
minutum	VGO756	Alfacs, Delta l'Ebre, Catalunya, Spain
minutum	VGO874	Boughrara, Tunis
minutum	VGO929	Boughrara, Tunis
minutum	VGO930	Boughrara, Tunis
minutum	VGO942	Mar Adriático, Italy
minutum	VGO 650(4)	Port Saint Hubert, Brittany, France
minutum	AL1V	Ría de Vigo, Galicia, Spain

minutum	AL2V	Ría de Vigo, Galicia, Spain
minutum	AL3V	Ría de Vigo, Galicia, Spain
minutum	AL4V	Ponte de Toralla, Ría de Vigo, Spain
minutum	AL6V	Lorbé, Galicia, Spain
minutum	AL7V	Lorbé, Galicia, Spain
minutum	AMAD01	Port River, SA. Australia
minutum	AMAD06	Port River, SA, Australia
minutum	AMAD21	Jervois Bridge, Port River, SA. Australia
minutum	AL8C	Arenys, Catalunya, Spain
minutum	AL9C	Arenys, Catalunya, Spain
minutum	AL10C	Estartit, Catalunya, Spain
minutum	AL12C	Estartit, Catalunya, Spain
minutum	AL13C	Estartit, Catalunya, Spain
minutum	Palmira 1	Palmira, Mallorca, Spain
minutum	Palmira 2	Palmira, Mallorca, Spain
minutum	Palmira 3	Palmira, Mallorca, Spain
minutum	Palmira 4	Palmira, Mallorca, Spain
minutum	Palmira 5	Palmira, Mallorca, Spain
minutum	Min1	Arenys, Catalunya, Spain
minutum	Min2	Arenys, Catalunya, Spain
minutum	Min3	Arenys, Catalunya, Spain
minutum	Min4	Arenys, Catalunya, Spain
minutum	Min5	Arenys, Catalunya, Spain
minutum	Min6	Arenys, Catalunya, Spain
minutum	Min7	Arenys, Catalunya, Spain
minutum	Min8	Arenys, Catalunya, Spain
minutum	Min9	Arenys, Catalunya, Spain
minutum	Min10	Arenys, Catalunya, Spain
minutum	Min11	Arenys, Catalunya, Spain
minutum	Min16	Arenys, Catalunya, Spain
minutum	Min17	Arenys, Catalunya, Spain
minutum	Min18	Arenys, Catalunya, Spain
minutum	Min19	Arenys, Catalunya, Spain
minutum	Min21	Arenys, Catalunya, Spain
minutum	Min22	Arenys, Catalunya, Spain
minutum	Min23	Arenys, Catalunya, Spain
minutum	VGO1074	Porto de Baiona, Galicia, Spain
minutum	VGO1075	Porto de Baiona, Galicia, Spain
minutum	VGO1076	Porto de Baiona, Galicia, Spain
minutum	VGO1079	Porto de Baiona, Galicia, Spain
minutum	AMP4	Port de Palma, Mallorca, Spain
minutum	AMP10	Port de Palma, Mallorca, Spain
minutum	VGO1089	Porto de Baiona, Galicia, Spain
minutum	VGO1090	Porto de Baiona, Galicia, Spain
minutum	VGO1091	Porto de Baiona, Galicia, Spain

minutum	Clon Startit A10	Estartit, Catalunya, Spain
minutum	Clon Startit A7	Estartit, Catalunya, Spain
minutum	18A	Lagoa d'Óbidos, Portugal
minutum	A.MIN	
minutum	AMITA	Mar Adriático, Italy
minutum	CCFWC417	Florida, USA
minutum	P4	Port de Palma, Mallorca, Spain
minutum	P4 Clon C6(8)	Port de Palma, Mallorca, Spain
minutum	GH min 04	Denmark
minutum	VGO1080	Porto de Baiona, Galicia, Spain
minutum	VGO1081	Porto de Baiona, Galicia, Spain
minutum	VGO1088	Porto de Baiona, Galicia, Spain
ostenfeldii	FAL50	Falmouth, United Kingdom
ostenfeldii	FAL50 9.06.11 301	Falmouth, United Kingdom
ostenfeldii	AOTV-B4	Tvärminne, Baltic Sea, Finland
ostenfeldii	AOTV-A1	Tvärminne, Baltic Sea, Finland
ostenfeldii	AOTV-A4	Tvärminne, Baltic Sea, Finland
ostenfeldii	AOTV-B3	Tvärminne, Baltic Sea, Finland
peruvianum	VGO956	Palamós, Catalunya, Spain
tamutum	SZN029	Golfo de Nápoles, Italy
tamutum	VGO615	Alfacs, Delta l'Ebre, Catalunya, Spain
tamutum	VGO616	Alfacs, Delta l'Ebre, Catalunya, Spain
tamutum	VGO617	Alfacs, Delta l'Ebre, Catalunya, Spain
tamutum	E6Q2 Sibling 12x10	
tamutum	A8	
taylori	VGO703	Alfacs, Delta l'Ebre, Catalunya, Spain

Table A2. Key, name, isolation year, year cloned and origin of host strains used in cross infection experiment.

Host Key	Strain	Isolation year	Year cloned	Origin
H1	AMP13	1995	2012	Palma de Mallorca (Med, Spain)
H2	VGO577	2002	2012	Girona (Med, Spain)
H3	VGO663	2003	2012	Sardinia (Italy)
H4	VGO707	2003	2012	Delta de l'Ebre (Med, Spain)
H5	VGO713	2003	2012	Vilanova (Med, Spain)
H7	VGO722	2003	2012	Cambrils (Med, Spain)
H8	VGO874	2003	2012	Tunis
H9	VGO942	2003	2012	Italy
H10	AL10C	2002	2012	Estartit (Med, Spain)

Table A3. Key, name, isolation year and origin of parasite strains used in the cross infection experiment.

Parasite Key	Strain	Isolation year	Origin
P1	EST21.5.F6	2011	Estartit (Med, Spain)
P2	A15.2.2012	2012	Arenys(Med, Spain)
P3	OL3.6.D7	2011	Olímpic, Barcelona (Med, Spain)
P4	VIL7.5.D6	2011	Vilanova (Med, Spain)
P5	EST17.6.A10	2011	Estartit (Med, Spain)
P6	VIL09-01	2009	Vilanova (Med, Spain)
P7	T20.6.D12	2011	Tarragona (Med, Spain)
P8	CAMB6.6.C5	2011	Cambrils (Med, Spain)
P9	VIL7.5.D5	2011	Vilanova (Med, Spain)
P10	VIL23.6.III(2)	2011	Vilanova (Med, Spain)