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Note

Effect of phytoplankton containing paralytic shellfish and amnesic shellfish toxins on the culture of the king scallop *Pecten maximus* in Málaga (SE Spain)

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Abstract – The impact of toxic outbreaks of *Gymnodinium catenatum,* producer of paralytic shellfish poisoning toxins (PSP toxins) and *Pseudonitzschia* spp., producer of amnesic shellfish poisoning toxins (ASP toxins), was studied throughout the culture cycle of the king scallop *Pecten maximus* (length from 40 to 100 mm). Toxin concentration, in plankton and in the digestive gland of the scallops, other variables that describe the state of the cultured scallops (mortality, growth, length/weight ratio, gonadosomatic index) and the main environmental conditions (temperature and *in vivo* fluorescence) were determined from December 2001 to April 2003. Temperature ranged from 14 °C during the winter to 22 °C at the end of July. *In vivo* fluorescence ranged from not detectable to 25 relative units, with a maximum also recorded at the end of July. ASP toxin levels, in both plankton and organisms, were high during the first half of the sampling period (maximum 450 ng domoic acid L^{-1}, in plankton, and 175 $\mu g \cdot g^{-1}$ of scallop digestive gland) when the scallops were juvenile. PSP toxins attained larger concentrations in the second half of the sampling period (maximum of 1.8 nmol L^{-1} in plankton and 550 nmol g^{-1} of digestive gland, of gonyautoxins after hydrolysis). Several mortality events took place during the period studied, but none of them coincided neither with an ASP or PSP outbreak nor with long exposure of the scallops to these toxins. One of the recorded mortalities occurred simultaneously with a high concentration of PSP in the scallops, but also with an important spawning. Additionally, it was not coincidental with the bloom of toxic plankton, thus, post-spawning stress seemed to be the main responsible for the mortality. No appreciable effect of ASP or PSP toxins on growth or reproductive state of the cultured scallops was found. Therefore, neither ASP nor PSP toxins appeared to represent a direct threat to the culture of *Pecten maximus* in that case.

Key words: Toxins / ASP / PSP / Domoic acid / GTX / King scallop / Mortality / Growth / Gonadosomatic index / *Gymnodinium catenatum* / *Pseudonitzschia* spp. / Mediterreanean Sea

1 Introduction

Toxic and harmful phytoplankton outbreaks are frequent processes in the highly productive areas where aquaculture activities are usually carried out (Hallegraeff 1993; Anderson et al. 2002). High mortalities of fish due to toxic algal blooms have been recorded in many places and on a great number of occasions. In the case of bivalve molluscs, although the direct effects are usually less dramatic than on fish, these blooms produce severe losses mainly because of the accumulation of toxins which affect humans, but also owing to direct effects on survival, growth, organoleptic characteristics, and others (Shumway 1990; Burkholder 1998; Blanco-Pérez 2001; Landsberg 2002; Wikfors 2005; Leverone et al. 2006; Wang et al. 2006).

The king scallop *Pecten maximus* is a species suitable for aquaculture because it can be obtained easily, it grows fast and it has a high demand and economic value, especially in Europe. The main known problem for the culture of this species is that it can accumulate toxins, especially domoic acid, for a long time. Notwithstanding, toxic phytoplankton might be also a risk for the survival of the cultured populations. Two of the most frequent toxicities recorded in the Iberian Península, PSP and ASP -produced, mainly by *Gymnodinium catenatum*, and by several species of *Pseudonitzschia*, respectively- at least theoretically, might affect the survival or development of *Pecten maximus* because of three concurrent factors: a) they

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affect important physiological processes in mammals and, at least some bivalves, have receptors to which they can bind; b) some problems in shellfish populations have been observed coinciding in time with outbreaks of phytoplankton producing these kinds of toxins; and c) some effects have been experimentally demonstrated on bivalve species.

Domoic acid, the most important ASP toxin, in humans, act on the central nervous system by blocking some of the glutamate receptors of the neurons (mainly Kainate-sensitive AMPA-type glutamate receptors). Although, to our knowledge, the direct effect of these toxins on pectinids has not been tested, the freshwater gastropod *Lymnaea stagnalis*, has been shown to have a glutamate receptor -with an identical sequence to that of the mammalian kainate-sensitive glutamate receptor- which is involved in feeding responses (Hutton et al. 1991). Moreover, in a recent study, the razor clam *Siliqua patula* was also found to have receptors that were supposedly involved in the accumulation of domoic acid over a long period of time (Trainer and Bill 2004). Additionally, some organisms producing ASP toxins have been suggested to have a transient effect on the clearance rate of *Placopecten magellanicus* (Douglas et al. 1997), to produce some alterations in the hemolymph of *Crassostrea gigas*, largely owing to shell closure for some time after the exposure to toxic phytoplankton (Jones et al. 1995b). These observations suggest, therefore, that ASP producing organisms might have an adverse effect on growth or survival of cultured *Pecten maximus.*

PSP toxins are mainly characterised by their ability to block the sodium channel – which is especially important in nervous cells of most organisms – modifying in this way the potential of action of the nerves but they target multiple receptors (Llevellyn 2006). The level of the effect on shellfish, probably depends on the sensitivity of their nerves (Twarog and Yamaguchi 1975; Bricelj et al. 2005). The sensitivity of *Pecten maximus* has not been estudied but it has been shown that one of the two pectinids studied is sensitive (Twarog and Yamaguchi 1975). Apart from this unequivocal effect of the toxins, some bivalves are killed by the exposure to PSP producing organisms, or mortalities have been recorded coinciding with outbreaks of some of those species. High mortality rates were recorded, in *Choromytilus meridionalis* and *Donax serra,* exposed to *Alexandrium catenella* in South Africa (De Villiers 1975; Horstman 1981), experimental exposure to *Alexandrium tamarense* produced mortalities of *Crassostrea virginica*, *Ostrea edulis* (Lesser and Shumway 1993), *Mytilus edulis* (Shumway and Cucci 1987). *Alexandrium taylori* was associated with *Crassostrea gigas* larvae mortality (Matsuyama et al. 2001) and *A. minutum* killed juvenile specimens (3 cm) of *Mytilus edulis*, *M. galloprovincialis* and hybrids of these two species (Blanco and Fuentes 2002). *Pecten maximus* in Galicia has suffered mortality in several occasions coinciding with outbreaks of *Gymnodinium catenatum*, a dinoflagellate that produces PSP toxins (Román and Maneiro, and Molares, comm. pers), as well as other pectinid, *Lyropecten nodosus,* in Venezuela (Lodeiros et al. 1998). Other scallops, as *Placopecten magellanicus*, generally react to PSP producing organisms by triggering a strong swimming behaviour, by closing the valves, and by increasing mucus production (Shumway and Cucci 1987) or, as *Chlamys farreri*, suffer larval mortality and inability to hatch eggs (Fu et al. 2000).

In the framework of a more general study of PSP and ASP toxin accumulation and their effects on the culture of the king scallop, we have studied the concentration of ASP and PSP toxins in plankton and in the digestive gland of the scallops, the mortality and growth rate, the reproductive state, and several environmental variables, throughout the culture cycle of the king scallop in Fuengirola, Málaga (Spain) – an area in which *Gymnodinium catenatum* (PSP producer) and *Pseudonitzschia* spp. (ASP producers) bloom frequently (Mamán et al. 2004; Fernández et al. 2004) – in order to determine whether or not the toxic outbreaks may have an important effect on the development of *Pecten maximus* subjected to the usual culture conditions. The two ways of quantifying the exposure to toxins – in plankton and in digestive gland – are complementary, as toxins in plankton can be used as index of the toxicity of food and therefore of the acute toxicity, while the toxin concentration in the scallop digestive gland can be used as an index of the possibility of chronic toxicity.

Material and methods

Biological material

Seawater samples were taken every week from January 2002 to April 2003, with a Niskin oceanographic bottle at a depth of 10 m, in the area in which scallops were maintained, Fuengirola, Málaga (SE Spain). Two 500 ml aliquots, one for PSP and one for ASP toxins, were filtered through Watman GF/C glass fiber filters that were stored frozen at −20 °C until extraction and analysis.

Scallops were obtained by natural settlement on artificial collectors. They were grown in trays suspended from a long-line at a depth of 10 m and at an initial density of 24 individuals·per tray and 16 individuals·per tray since June 2002. Every week 12 individuals were taken at random from the trays, and their length, width and total weight of soft tissues were recorded. Each scallop was dissected into six organs or groups of organs -digestive gland, adductor muscle, kidneys, foot, gills+mantle and gonad- and each of these fractions was weighed. In order to reduce the inter-sample variance, the organs corresponding to groups of three scallops were pooled into four batches. The resulting batches were homogenized and the toxins extracted and analysed.

Mortality was determined at least once a month. In order to have an index of the reproductive state of the scallops the gonadosomatic index was computed as the ratio gonad wet weight/soft tissues wet weight.

Toxin extraction and analysis

To quantify the concentration of ASP toxin in plankton the filters corresponding to one of the aliquots, were extracted and then analyzed by the method of Pocklington et al. (1990), using a Beckman high-performance liquid chromatography (HPLC) system, with a Jasco FP1520 fluorescence detector, a Supelco LC-PAH chromatographic column

and domoic acid reference solution (DACS-1C) from the NRC, Canada. The lowest quantification level of the technique is 15 pg domoic acid ml^{-1}.

To quantify PSP toxins in plankton, the method developed by (Franco and Fernández-Vila 1993) was used. Not all toxins were quantified, as the known PSP toxin producer in the area is *Gymnodinium catenatum* (Mamán et al. 2004; Fernández et al. 2004), and this species has a toxic profile characterized by the predominance of sulfo- toxins (Franco and Fernández-Vila 1993; Bravo et al. 1998; Ordás et al. 2004) which cannot be directly quantified but that yield gonyautoxins (GTX toxins) after hydrolysis. The concentration of gonyautoxins after hydrolysis was therefore used as an index of the concentration of the total PSP toxins. The chromatographic equipment used was the same as with ASP toxins. Reference solution PSP 1C from the NRC, Canada was used to identity and quantify the toxins. The lowest quantification level of this technique is of 0.55 nmol ml^{-1} for GTX1, 4 and 5, and 0.1 nmol ml^{-1} for GTX2,3, and dcGTX2,3.

The extraction and analysis of ASP toxins from scallop samples were carried out by the method of Wright and Quilliam (1995), using a Beckman HPLC system, with a 168 PDA-UV detector, with an Agilent Technologies LiChrospher RP-18 12.5×4.6, 5 μm, chromatographic column. Domoic acid reference solution (DACS-1C) provided by the NRC Canada was used to quantify the toxin contents. The lowest quantification level of the technique is 40 ng domoic acid g^{-1} of soft tissue

The extraction and analysis of PSP toxins from scallop samples were carried out by the same method used to quantify these toxins from seawater.

Environmental conditions

Temperature and fluorescence were monitored continuously throughout the culture period by means of an Aanderaa RCM-9 current meter with a temperature sensor and an ECO Wetlabs fluorometer at the same depth at which the scallops were placed.

Results

Environmental conditions

Water temperature ranged from 14 to 22 °C, during the period studied. Temperature was between 14 and 15 °C during winter, increasing in the spring to 16 °C, reaching the maximum in August and maintaining over 16 °C until middle autumn.

In vivo fluorescence was also characterised by a summer maximum but some smaller maxima were also found in spring and autumn (Fig. 1F).

PSP and ASP toxins in plankton and scallops

Toxins in plankton

ASP and PSP toxins were frequently found in plankton from the location where the cultures were placed. Numerous peaks of concentration of the two toxin groups were recorded. PSP was found to have a greater incidence during the latter third of the sampling period, while ASP was much more prevalent during the first half (Fig. 1E). The maximum concentrations of the two types of toxins detected in plankton where moderately high (ASP) and high (PSP). About 1800 pmol of GTX toxins L^{-1} and 400 ng of domoic acid L^{-1} were found during the study.

Toxins in scallop digestive gland

The amount of toxins, both PSP and ASP, accumulated in the digestive gland of the scallops followed the same trend than the observed in plankton (Fig. 1E) but did not match it exactly. In the case of PSP toxins three main discordances were found: a) detection of PSP toxins in plankton were not paralleled by proportional increases in the digestive gland of the scallops; b) maximum concentrations in the scallop were recorded one or two weeks after the maximum in plankton; and c) toxin remains in the scallops well after the toxins have disappeared from the water (because of the retention of toxins by the scallops) (Fig. 1D).

Both, the first and the third kind of discordances were also found for ASP toxins. Notwithstanding, in the case of this group of toxins, due to the extremely strong retention capability that the digestive gland has, the third discordance was the most important one (Fig. 1D).

The maximum recorded concentrations were, as the ones in plankton, moderately high in the case of ASP toxins, with 175 μg·domoic acid g^{-1} of scallop digestive gland (nearly ten times the maximum allowed level), and high in the case of PSP toxins, with 550 nmol g^{-1} of digestive gland, of gonyautoxins after hydrolysis.

Growth, gonadal development and mortality

Growth

Scallop grew from an initial weight of *ca.* 12 g of soft tissues and a length of 40 mm at the end of January 2002, up to *ca.*110 g and 95 mm one year after (January 2003). Since then, a slight decreasing trend of the weight was recorded up to the end of the experiment in April 2003 (Fig. 1B). In both curves, during July and most of August 2002, and during November and part of December, the scallop population stop growing and even had a negative growth (slight in the first period). These two stops or decreases occurred simultaneously with a decrease of the shell length.

Gonadosomatic index

The gonadosomatic index showed a clear seasonality, following an approximately asymmetric sinusoidal curve, reaching a maximum during the spring of 2002, decreasing during summer and autumn and increasing quickly after the middle of December, to reach an absolute maximum in middle February. In 2002, the scallop were not mature but during 2003 several spawning events took place.

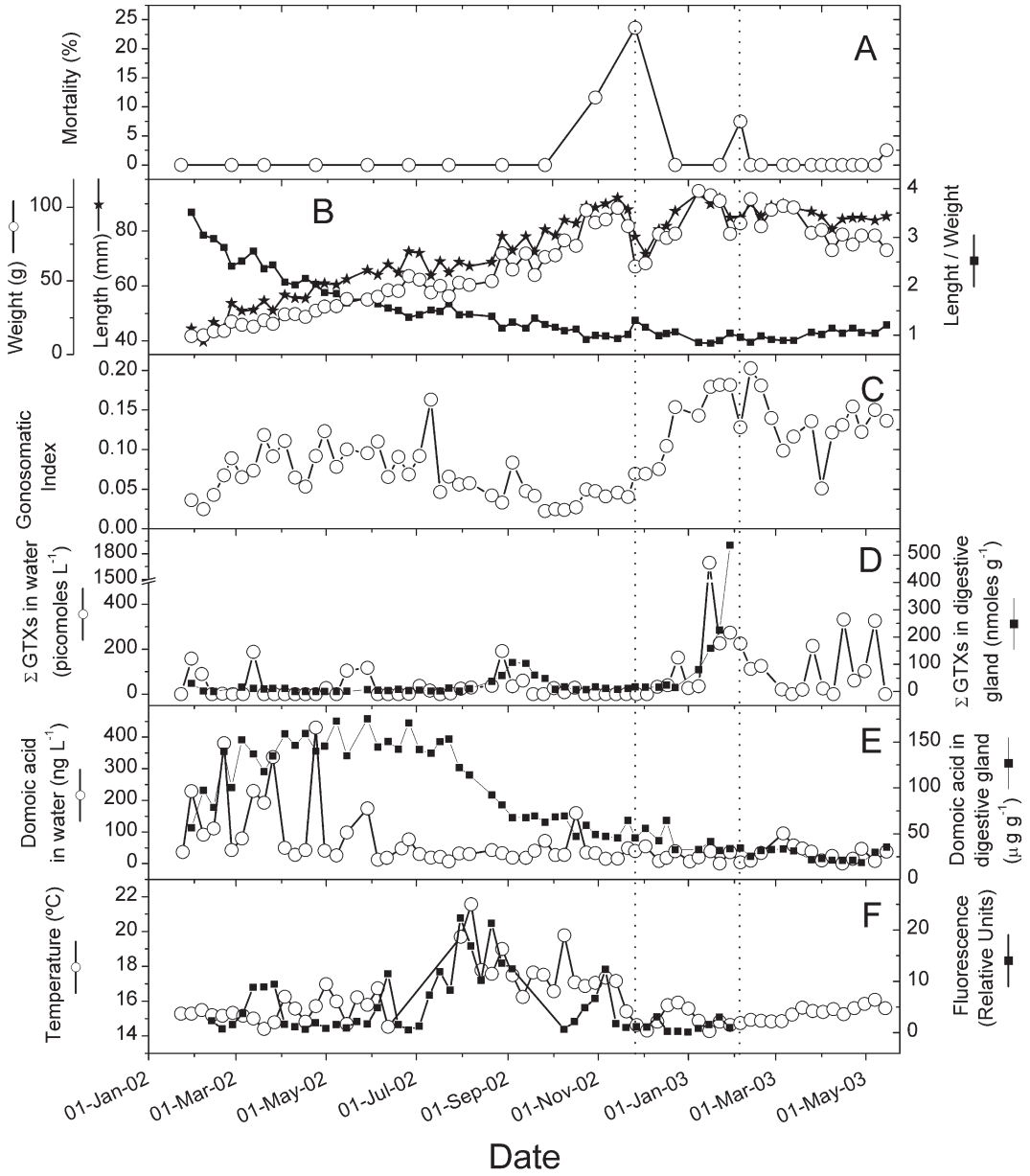

Fig. 1. Main variables describing the scallop culture and the environmental characteristics during the study: (A) mortality, (B) weight, length and length/weight ratio, (C) gonadosomatic index of the cultured scallops, (D) PSP toxin concentration in plankton and scallop digestive gland, (E) ASP toxin concentration in plankton and scallop digestive gland, and (F) *in vivo* fluorescence and water temperature.

Length/weight ratio

The length/weight ratio decreased throughout the study from 3.5 to nearly 1 after September 2002, with a final slight increase due to the small loss of weight at the end of the experiment. Only two very slight anomalies of the ratio, with higher values than expected, were detected. During those events, both length and weight decreased.

Mortality

Accumulated mortality throughout the study was less than 40%. Mortality took place mostly in two events, the first one in October-November (with an accumulated mortality in the two sampling dates of *ca.* 35%) and the second one in February (*ca.* 5%). In both mortalities, larger scallops seem to have been more affected than smaller ones as the average shell length of the population decreased.

Relationship of toxin concentration with the other variables

ASP concentration does not seem to be related to any of the parameters of the scallop populations measured in this study. Even though, the largest concentration of these kind of toxins was found when the scallops were still small in size,

no mortality and no apparent retardation of growth were detected during the recorded outbreaks. The length/weight ratio was not affected either. A rough coincidence between the largest drop in domoic acid concentration in the digestive gland of the scallops and an increase in the gonadosomatic index was found but it is not possible, with the actual knowledge, to establish a cause/effect relationship.

What occurs with PSP toxins is practically the same. The highest mortality rate detected during the study did not coincide in time with any PSP outbreak (Fig. 1D) and it started when PSP toxins had almost completely disappeared from the digestive gland of the scallops (Fig. 1D). The second mortality peak – much smaller than the first one – coincided with high concentration of PSP toxins in the scallops but it took place 20 days after the maximum concentration in plankton. Additionally, it was also coincidental with a steep decrease of the gonadosomatic index, which indicates that spawning took place at that time.

Growth and gonadosomatic index seem also not to be affected by this group of toxins.

Discussion

The king scallop *Pecten maximus* do not seem to be affected by ASP outbreaks, even though the scallops were still juvenile when the main outbreaks of this group of toxins took place, and small organisms are usually more sensitive to toxins than larger ones (Shumway 1990; Blanco-Pérez 2001; Landsberg 2002).

No mortality, no apparent retardation of growth and no uncoupling between shell and tissue growth (as described by the presence of anomalies in the length/weight ratio) were detected neither during the outbreaks nor after long time of toxin accumulation in the scallops. Even when reduced or negative growth was recorded in two occasions, these events were coincidental with negative shell growth, which cannot take place unless mortality of the largest scallops take place, or because of sampling error. The first explanation cannot apply to the first event as no mortality at all was detected. Therefore, for *Pecten maximus*, our data do not support the possibility that domoic acid producers might reduce the filtration or ingestion rate as suggested (among other alternatives) for *Placopecten magellanicus* (Douglas et al. 1997), and by the observation that a diatom that produces these toxins induces valve closures in the Pacific oyster *Crassostrea gigas* (Jones et al. 1995a,b).

A rough coincidence between the drop in domoic acid concentration in the digestive gland of the scallops and an increase in the gonadosomatic index was found, but it is unlikely that domoic acid would play a role in gonad development. It is far more likely that the loss of domoic acid from the digestive gland would be affected by the physiological processes that take place at this stage of the reproductive cycle. The increase of the gonadosomatic index during the second year appears to be the product of this seasonality combined with the size/age of the scallops at that time (the normal reproductive cycle (Pazos et al. 1996, 1997, 2003; Cano 2000; Campos et al. 2006)), rather than due to a decrease in the domoic acid concentration in the scallops.

Our results suggest that PSP toxins do not have either any relevant effect on the development of the cultures of the king scallop. Although one of the detected mortality events occurred when the scallops had high toxin concentrations, it was also concurrent with an important spawning event, and therefore it seems to be the usual effect of the post-spawning stress more than an effect of the toxins. These results are consistent with those obtained by means of short-term experiments with *Gymnodinium catenatum* (Acosta and Blanco 2004) and *Alexandrium tamarense* (Lassus et al. 1992).

Notwithstanding, against our findings on PSP toxin effects are, apart from the already cited evidences of adverse effects, of both producers and toxins on other bivalve molluscs, mortality of natural populations of *Pecten maximus* has been observed in Galicia during *Gymnodinium catenatum* blooms (unpubl. obs. Román and Maneiro and Molares, for the 1998 and the 2005 blooms, respectively). Neither bloom was unispecific and the DSP toxin producer *Dinophysis acuta* was also present in both of them. As the toxin profile of the *Gymnodinium catenatum* that bloomed in Málaga is very similar to that of the Galician one (Ordás et al. 2004), there are three possibilities to explain those mortalities together with the lack of effect that we have found:

- The toxin levels attained during our experiment were not high enough to produce detectable effects. There are no data of scallop toxicity in the two Galician outbreaks, but mussels of the area reached, in both cases, toxicities that could be five times higher than those that could be expected from the toxin concentrations attained in Málaga (data are not directly comparable as toxicities in Galicia were obtained by means of mouse bioassay and those in Málaga, has to be computed basing on the known toxin profile and the toxin quantification by HPLC).
- The toxin does not kill the scallops but have other effects that decrease their survival in natural conditions. It was observed by scuba divers that, in the 2005 outbreak in Galicia, the scallops show an unusually slow reaction to the presence of depredators (mainly starfish and crabs) and, even though this situation could cause mortality in natural conditions, it is unlikely to have that effect on cultured organisms, which are mostly protected from predators.
- Other toxins, as those of the DSP group that are produced by *Dinophysis acuta*, could be more active on scallops than PSP toxins. Recently, Suzuki et al. (2005) have found that injection of DSP toxins can kill a large percentage of the Japanese scallop *Patinopecten yessoensis*, which suggests that, in the two Galician outbreaks, these toxins more than the PSP ones, could be responsible for the mortality. The levels of DSP toxins (okadaic acid and DTX2) in some samples of the area, analysed by HPLC-MS (unpubl. obs.), were in excess of 2 μg g^{-1} of mussel soft tissue (ca. 20 μg mussel^{-1}), higher that the levels injected by Suzuki et al. (2005) that were 6 μg of okadaic acid per scallop.

In conclusion, it seems therefore that neither ASP toxins nor PSP toxins – at the levels reached in this study – have any significant effects on scallop culture, and that their incidence is limited to making the scallops unsuitable for human consumption.

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