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# **OPINION**

of the French Food Safety Agency on the reduction of the algal toxin risk in Pectinidae by the introduction of an evisceration procedure

THE DIRECTOR GENERAL

## 1. REVIEW OF THE REQUEST

On 21 January 2008 the French Food Safety Agency (Afssa) received a request from the French Directorate for Maritime Fishing and Aquaculture (DPMA) and the French Directorate General for Food (DGAI) for scientific and technical support on the control of the algal toxin risk in Pectinidae by the introduction of an evisceration procedure.

## 2. CONTEXT

Like all shellfish and especially filter-feeders, Pectinidae are sensitive to contamination by algal toxins (toxins produced by marine phytoplankton). In France, Pectinidae production essentially consists of harvest in natural beds mainly situated off the coast. Over the last few years, algal toxin contamination of shellfish has been observed, thanks to the monitoring of both production areas and marketed products.

Regulation (EC) No. 854/2004 of 29 April 2004 lays down specific rules for the organisation of official controls on products of animal origin intended for human consumption and therefore applies to the presence of algal toxins in shellfish.

Regulation (EC) No. 853/2004 of 29 April 2004 sets permitted limits for amnesic shellfish poison (ASP), paralytic shellfish poison (PSP) and lipophilic toxins (okadaic acid, dinophysistoxin, pectenotoxins, yessotoxins and azaspiracids) above which shellfish are considered unfit for human consumption. This regulation offers the possibility of placing on the market only the edible parts of the shellfish, which have been proven not to exceed the sanitary limits (whereas the analyses of the whole meat may exceed them).

Following recurrent episodes of contamination of bivalve mollusc beds in Scotland by ASP toxins, the Commission adopted Decision 2002/226/EC, authorising the sale in certain conditions of 2 species of great scallops (*Pecten maximus* and *Pecten jacobaeus*) when the algal toxins in the whole meat exceed the permitted limits. The products intended for consumption then consist of just the adductor muscles and/or the gonads. The parts where the toxins are concentrated must be removed (digestive gland – or hepatopancreas - mainly, beards and mantles). In these conditions the harvest permit is accompanied by very strict control measures:

- the level of domoic acid in the shellfish must be lower than 250 mg/kg in the whole meat and lower than 4.6 mg/kg in the parts intended for sale under the conditions of this decision;
- the channelling of the products must be strictly supervised by the authorities responsible for fishing and placing on the market;
- each batch of final product must be analysed in order to prove that it is fit for human consumption (the level must be less than 20 mg/kg).

This decision was based on scientific data and has not as yet been extended to either other species of Pectinidae or other algal toxins.

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FRANÇAISE

<sup>27-31,</sup> avenue du Général Leclerc 94701 Maisons-Alfort cedex Tel 01 49 77 13 50 Fax 01 49 77 26 13 www.afssa.fr

Beards: outer edges of the mantle of of Pectinidae ASP: amnesic shellfish poison; PSP: paralytic shellfish poison

#### 3. QUESTIONS ASKED

The Agency has been asked to examine the following questions:

**Question I:** Would it be possible to extend the system set up by Decision 2002/226/CE for 2 species of scallops contaminated by ASP toxins to the other families of toxins (lipophilic or PSP toxins), in order to place on the market products fit for consumption (eviscerated products), even if the product (whole meat) is contaminated in the production area? In particular, is it necessary to introduce a limit at harvest similar to the 4.6 mg/kg limit on domoic acid for ASP toxins in the parts intended for sale?

**Question II:** Would it be possible to extend these measures (lipophilic, ASP, PSP toxins) to all Pectinidae produced in France and in particular to variegated scallops ("pétoncles" in French) subject to the technical conditions allowing the routine removal of the hepatopancreas?

**Question III:** Can the freezing of shellfish harvested in the expectation of their treatment by evisceration generate an extra risk (transfer of toxins into the muscle, bursting of the hepatopancreas during freezing...)?

**Question IV:** Might it be possible to envisage, without causing any extra risk, storing shellfish before treatment in clean water, characterised as such by a certain content of toxic algae?

**Question V:** If an analysis (self-control or official control) is done on a batch placed on the market (produced in France or imported), what kind of sampling plan will ensure its conformity or nonconformity? Can the provisions that apply to the search for environmental contaminants (in particular Regulations (EC) No. 1883/2006 and 333/2007) be applied to the search for algal toxins in shellfish in general and in Pectinidae in particular? Can the same sampling plan be used in case of doubt on the conformity of a batch (for example, to decide its conformity when an analysis on a sub-batch has given a nonconforming result or, conversely, to decide the conformity of sub-batches making up a batch when an unfavourable result has been given for this batch)?

**Question VI:** If the batches of Pectinidae (whole meat) are nonconforming for lipophilic, ASP and PSP toxins, in what conditions will evisceration enable the risk to be eliminated (sampling plan) before the products are placed on the market?

#### 4. METHOD OF ASSESSMENT

The expert assessment was carried out by the competent departments of the French Food Safety Agency, namely:

- the Toxins, organic pollutants and pesticides unit (TOP) of the Laboratory for Study and Research on Quality of Food and on Food Processes (LERQAP);
- the Physical-chemical risk assessment unit (UERPC) of the Health and Nutritional Risk Assessment Department (DERNS);
- the Quantitative risk assessment and epidemiology in microbiology and animal health unit (AQR-MSA) of the DERNS.

The expertise was based on the scientific and regulatory information available in the literature (cf. bibliography) and on the data provided by the IFREMER<sup>2</sup>:

- data on the contamination by azaspiracids and/or okadaic acid of the types of scallops known as "pétoncles" and "coquilles Saint-Jacques" along the French coastline, obtained by REPHY<sup>1</sup>;
- bibliographic summary concerning the contamination of "coquille Saint-Jacques"-type scallops by okaidaic acid and dinophysistoxins.

<sup>&</sup>lt;sup>2</sup> IFREMER: French Research Institute for Exploitation of the Sea; REPHY: French phytoplankton and phycotoxins monitoring network, set up by the IFREMER.

## 5. QUESTION 1

Reminder of Question I: Would it be possible to extend the system set up by Decision 2002/226/CE for 2 species of great scallops contaminated by ASP toxins to the other families of toxins (lipophilic or PSP toxins), in order to place on the market products fit for consumption (eviscerated products), in spite of the contamination of the product (whole meat) in the production area? In particular, is it necessary to introduce a limit at harvest similar to the 4.6 mg/kg limit on domoic acid for ASP toxins in the parts intended for sale?

The system set up following Commission Decision 2002/226/EC of 15 March 2002 only concerns domoic acid and scallops belonging to the *Pecten maximus* and *Pecten jacobaeus* species. Afssa is therefore being asked to determine whether a similar system could be applied to lipophilic and PSP toxins for these 2 species only.

First of all, it is necessary to emphasise the small number of studies available on the distribution of lipophilic and PSP toxins in the 2 species of scallops concerned by the request.

#### Concerning lipophilic toxins

A few studies have revealed the presence of azaspiracids (AZAs) in *Pecten maximus* scallops (Brana Magdalena *et al.*, 2003a; Furey *et al.*, 2003). Brana Magdalena *et al.* (2003b) reported that the digestive gland of these scallops concentrates a large part of the AZAs (85%), but not all of it, as smaller concentrations of toxins are found in the other organs.

An experimental contamination study of bay scallops (*Argopecten irradians*) using cultures of *Prorocentrum lima* which produce lipophilic toxins (okadaic acid (OA), dinophysistoxin-1 (DTX-1)) found the following distribution: digestive gland (76 %), gonad (12 %), muscle (4 %), mantle (4 %), gills (4 %) (Bauder *et al.*, 1996).

A similar distribution of AZAs has been described in shellfishes other than the 2 types of scallops concerned by the request, namely mussels *Mytilus edulis* (Flanagan *et al.*, 2000; James *et al.*, 2002a, 2002b). Hess *et al.* (2005) also found that the concentration of AZAs is 5 times higher in the digestive gland than in the whole meat of mussels originating from Ireland and Norway.

## Concerning PSP toxins

An experimental study conducted by Lassus *et al.* (1996) showed that when *Pecten maximus* scallops are exposed to a toxinogenic culture of *Alexandrium tamarense*, the PSP toxins accumulate mainly in the digestive gland, then to a lesser degree in the gonads and the adductor muscle (respectively, 2,620, 148 and less than 50 µg eq STX/100 g).

In the other pectinidae species, *Patinopecten yessoensis* and *Placopecten magellanicus*, the highest toxin levels were also found in the digestive gland (Lassus *et al.*, 1996).

A synthetic review conducted by Shumway and Cembella (1993) on Pectinidae in the genus *Pecten* also concluded that the majority of the accumulation was in the digestive gland. They observe that the toxin level in the gonads is correlated with that in the digestive gland, but they do not find any silmilar correlation for the level in the adductor muscle.

After the end of the exposure period, depuration occurs, with a reduction in the toxin level in the digestive gland, the gonads and the adductor muscle, but an increase in the kidneys<sup>3</sup>, where the toxins may be transformed into more toxic compounds. The kidneys retain a high toxin level, even after 20 days. The authors emphasise that the kidneys are often present in scallops sold "eviscerated" on the market (Lassus *et al.*, 1996).

#### **Conclusion**

Concerning lipophilic and PSP toxins, the very limited data available on the species *Pecten maximus* seem to indicate a toxin distribution mainly in the digestive gland. Nevertheless, certain data on contamination by PSP toxins show that the kidneys may still be present after evisceration. And the kidneys retain a high toxin level, even 3 weeks after the end of the exposure period. No data is currently available concerning the species *Pecten jacobaeus*.

<sup>&</sup>lt;sup>3</sup> In Pectinidae, both kidneys are small in size and brownish, and form sacs flattened against the anterior part of the adductor muscle. The kidneys empty into the mantle cavity through wide slits.

Consequently, it is not possible at present to establish a reliable correlation between the toxin levels in the digestive gland and those in the eviscerated parts (gonads, muscle and kidney), which would make it possible to determine, as was the case for domoic acid, specific conditions of removal and treatment (evisceration) for these shellfish, if the regulatory limits for lipophilic or PSP toxins are exceeded, thus allowing a satisfactory level of safety to be guaranteed for the consumer.

The 4.6 mg/kg limit at harvest was specifically determined for domoic acid, on the basis of contamination data and is in no way transposable to lipophilic toxins or PSP toxins. Indeed, the latter have safety limits of 800  $\mu$ g eq STX/kg and 160  $\mu$ g eq OA/kg respectively, considerably lower than that of domoic acid (20 mg/kg).

As proposed by the DGAI and the DPMA, it would be desirable to take advantage of the occurrence of new algal toxin poisoning episodes to conduct experimental studies of this evisceration procedure in the 2 species of scallops concerned and to acquire a sufficient amount of data to validate limits at harvest that would guarantee consumer safety from lipophilic and PSP toxins.

#### 6. QUESTION 2

**Review of Question II:** Would it be possible to extend these measures (lipophilic, ASP, PSP toxins) to all Pectinidae produced in France and in particular to variegated scallops ("pétoncles" in French) subject to the technical conditions allowing the routine removal of the hepatopancreas?

The experimental data provided by the IFREMER concerning levels of lipophilic toxin (okadaic acid, dinophysistoxins and azaspiracids) in scallops (the species is not specified) collected off Roscoff in October and November 2006 show a preferential accumulation of these toxins in the digestive gland, with concentrations above the safe limit for azaspiracids. On the other hand, the toxin concentration in the whole meat, the muscle or the remaining meat is considerably lower than the regulatory limits.

On the basis only of these data, it does not therefore seem to be necessary to eviscerate scallops, as long as the regulatory limits for the whole meat are not exceeded.

It would be preferable to have more experimental data for lipophilic, PSP and ASP toxins in order to gain a more representative picture of the level of contamination of scallops and other Pectinidae along the French coast and to be able to decide whether it is advisable to introduce an evisceration procedure.

In order to target the collection of contamination data, the DGAI would need to provide, to begin with, a list of the species of scallops produced and sold in France under the name "pétoncles".

### 7. QUESTION 3

**Review of Question III:** Is the freezing of shellfish harvested in the expectation of their treatment by evisceration of a nature to cause extra risk (transfer of toxins into the muscle, bursting of the hepatopancreas during freezing...)?

Several aspects must be considered in order to provide an answer:

- the effects of freezing/thawing on the physical integrity of the shellfish matrix;
- the stability of the algal toxins on freezing;
- the effects of freezing on the free fatty acids and their impact on toxin detection.

Concerning the effects of freezing/thawing on the physical integrity of the shellfish matrix. The conditions of freezing affect the physical integrity of the matrix via the formation of ice crystals liable to shear the tissue. Indeed, when freezing is slow, the number of crystals formed is

relatively low and the latter enlarge progressively, to reach several millimetres. They can thus have a mechanical action on the matrix, shearing it. However, when freezing is rapid, more, but smaller crystals form, preserving the physical structure of the matrix better.

It is important to observe the cold chain as variations in temperature can lead to partial thawing of the ice crystals. The water thus released into the matrix will fix onto the remaining crystals, which will then increase in size, but not in number, aggravating the risk of altering the structure of the matrix.

The thawing kinetics are also important insofar as they are likely to accentuate any damage caused on freezing. Indeed, when thawing is slow, the water released after the crystals melt will be able to progressively take up the space it occupied in the tissues once again. On the other hand, if thawing is rapid, the water released does not have time to return to its original position and is exuded.

To our knowledge there are no bibliographic data concerning the impact of the alteration of shellfish structure during freezing on the migration of toxins from one tissue to another. However, it is conceivable that during freezing, the structure of tissues highly contaminated with toxins is altered and that consequently, the movement of water due to the melting of the crystals on thawing is liable to cause a migration of the toxins to neighbouring tissues. The extent of this phenomenon will depend on the contamination levels and probably on the nature of the toxins (fat-soluble or water-soluble) as well as their "degree" of fixation in the matrix (bioavailability). Work to study these phenomena should be encouraged so as to obtain experimental data.

Different studies have shown that the phenomenon of freezing is accompanied by dehydration of the surface of the tissues, a sublimation phenomenon which depends on the characteristics of the matrix, but also on the ambient conditions and which leads to mass loss in the matrix (Campaneone *et al.* 2001; Campaneone *et al.* 2005; Olguin *et al.* 2008).

Water loss due to storage conditions was also observed by Smith *et al.* (2006) but at higher temperatures (between 5 and 12°C). Indeed, these authors report that for increasing temperatures, the water loss in *Pecten maximus* scallops during storage is accompanied by an increase in the domoic acid level. We may therefore venture the hypothesis that during storage at negative temperatures, water loss resulting from sublimation could lead to an increase in domoic acid in *Pecten maximus* scallops.

Although this has not been tested for the other marine toxins, we can reasonably assume that the phenomenon will be similar; in any case it is worth checking.

It is important to point out that if the dehydration of the tissues leads to an increase in the toxin concentration (but not the quantity), this may also have other consequences. Indeed, Smith *et al.* (2006) report that due to the water loss, the domoic acid is <u>bound more strongly to the matrix</u> and becomes less extractable, a phenomenon also observed by the Canadian National Research Council for certified reference materials for domoic acids stored for "a long time" (no information on the exact storage time). In terms of the health impact, this raises the question of the bioavailability of the toxin in the organism after storage, aspects which are largely unknown for marine toxins.

#### Concerning the stability of toxins on freezing

The bibliographic data relating to the stability of toxins when freezing shellfish have often been obtained for other shellfish than Pectinidae; nonetheless, we can expect the trend to be a similar one.

- Domoic acid: for freezing temperatures of -15 and -40°C, Vale *et al.* (2002) observed a slight drop in the domoic acid level in a sample of *Venerupis pallustra* clam (of the order of 10%) after one month's storage. On the other hand, no variation in the concentration of this toxin was reported by McCarron *et al.* (2007) for a reference material prepared from mussels *Mytilus edulis* and stored at -20°C for 240 days.
- Lipophilic toxins: the study carried out on a reference material prepared from mussels *Mytilus edulis* and stored at -20°C confirmed the stability of okadaic acid, dinophysistoxin-2 and azaspiracids-1 to 3 over 240 days (McCarron *et al.*, 2007).
- **PSP toxins**: these toxins are known to be pH sensitive. Indrasena and Gill (2000) reported that if these toxins are more stable at pH 3 than at pH 7, this makes a difference especially for storage temperatures of +5 and +25°C. On the other hand, a study conducted on a

homogenate of naturally contaminated scallops stored at -35°C demonstrated the stability of the analyte / matrix pair over a period of 12 months.

Overall, in view of current knowledge it seems that marine toxins are relatively stable on freezing.

Concerning the effects of freezing on free fatty acids and their impact on toxin detection.

Fukushi et al. (2003) reported that the storage at -20 and -45°C for 30 days of Patinopecten yessoensis scallops is accompanied by an increase in the free fatty acid (FFA) content, due to enzymatic hydrolysis of the triglycerides. The FFA content is higher at -20° than at -45°C and increases with storage time.

Now, some studies have shown that FFAs are likely to interfere during the mouse bioassay used as a reference method for lipophilic toxins (Takagi *et al.*, 1984; Lawrence *et al.*, 1994; Suzuki *et al.*, 1996).

Although this variation in the FFA content in scallops has no impact in itself on human health, it can give false positives in the mouse bioassay and lead to the withdrawal of products that do not in fact constitute a health risk for the consumer.

#### 8. QUESTION 4

**Review of Question IV:** Might it be possible to envisage, without causing any extra risk, storing shellfish before treatment in clean water, characterised as such by a certain level of toxic algal bloom?

Work done by Novaczek *et al.* (1991) showed that when mussels *Mytilus edulis* are exposed to domoic acid dissolved in water or encapsulated in liposomes, the levels accumulated are not the same. In fact, only 1% of the dissolved fraction was found in the shellfish compared to 6% in the case of the liposomes. Furthermore, the distribution of the toxicity differs according to the form of the toxin (dissolved or encapsulated). In the case of the dissolved form, the toxin is concentrated in the gills and kidneys. The toxin administered in encapsulated form accumulates in the digestive gland and kidneys.

This study illustrates the fact that the main route of contamination of mussels *Mytilus edulis* by domoic acid is food (consumption of toxigenic microalgae). The contamination risk related to the presence of the toxin in dissolved form in the water is lower.

Similar results were found for certain lipophilic toxins in the BIOTOX project conducted under the 6th FPRD (data not yet published). In fact, an *in situ* study monitoring the contamination of mussels *Mytilus edulis* by okadaic acid and dinophysistoxin-2 on the West coast of Ireland showed that after the disappearance of the *Dinophysis* cells in the water, the accumulation of these toxins in the shellfish stopped whereas these toxins were still present in dissolved form, as was proven by the use of passive samplers.

It would seem, therefore, that in the case of the toxins in the okadaic acid group, the main source contamination is also food.

These data, although patchy, tend to show that the use of clean sea water could be an option worth considering for the storage of <u>live</u> shellfish. However, it should be pointed out that no definition is currently available, at either national or international level, to qualify what is meant by "clean" sea water.

In its opinion of 26 July 2007 relating to the introduction of hygiene rules for the use of clean sea water for handling fishing products, Afssa estimated that sea water should be subject to a treatment adapted to the quality of the resource, consisting of the following three stages:

- a particle and colloid retention stage to arrive at a turbidity lower than 0.5 NFU after treatment, guaranteeing the effectiveness of the treatment applied (e.g.: clarification);
- an adsorption stage intended to retain chemical contaminants (e.g.: activated carbon);
- a disinfection stage intended to eliminate microbiological contaminants (e.g.: UV);

these stages in fine allow the safety of fishery product consumers to be guaranteed.

Concerning the algal toxin risk, as there is no treatment available at the present time to effectively retain or eliminate marine toxins, Afssa has recommended suspending the pumping of sea water

if the REPHY alert thresholds are exceeded at the phytoplankton monitoring point representative of the pumping point.

Recently, a sea water pumping system aimed at recovering the toxins trapped on a resin has been developed by Rundberget *et al.* (2007). This is not really a system of purifying sea water since its aim is to collect toxins in order to prepare contaminated samples, but it would be interesting to use this work as a starting point to study the feasibility of using resin-based filters capable of trapping the toxins present in the water before supplying shellfish storage tanks.

#### 5. QUESTION 5

Review of Question V: If an analysis (self-control or official control) is done on a batch placed on the market (produced in France or imported), what kind of sampling plan will ensure its conformity or nonconformity? Can the provisions that apply to the search for environmental contaminants (in particular Regulations (EC) No. 1883/2006 and 333/2007) be applied to the search for algal toxins in shellfish in general and in Pectinidae in particular? Can the same sampling plan be used in case of doubt on the conformity of a batch (for example, to decide its conformity when an analysis on a sub-batch has given a nonconforming result or, conversely, to decide the conformity of sub-batches making up a batch when an unfavourable result has been given for this batch)?

It is necessary to distinguish between batches of shellfish produced in France and imported batches. In the first case, they will generally be fresh animals, whilst in the second case, the animals will most often be frozen whole or already eviscerated.

Concerning French-produced batches of shellfish, the general Pectinidae sampling procedure under the monitoring plans (apart from the special case of evisceration and the management of nonconforming areas) was dealt with in Afssa's note on 28 April 2008 in response to Request No. 2007-SA-0009, and therefore replies to the first two sub-questions of Question 5.

The third sub-question deals with the notion of the sub-batch. According to the DGAI, the notion of the sub-batch corresponds to the management of small batches of different origins transported together, in the same lorry, for example. As indicated in the note in response to Request No. 2007-SA-0009, the notion of the batch, as regards algal toxin risk, must concern:

- a single species of shellfish,
- a single origin,
- and animals taken from the sea at the same time.

If the sampler has prior knowledge of there being small batches from different origins, these will in fact be different batches, and there can be no definition of sub-batches, as in fact this is a mixture of populations with different risks.

Concerning batches of imported shellfish, a study was conducted by Fremy *et al.* (1993) on a cargo of pacific scallops (*Patinopecten yessoensis*) imported into France from Asia and consisting of muscles and gonads frozen in 1 kg bags. This study revealed great variation in PSP toxin contamination from one bag to another and from one animal to another inside the same bag. An analysis of the muscles and gonads taken separately revealed a significant difference in contamination between these two organs for some animals and no difference for other animals. The latter observation could be explained either by a transfer *post mortem* and before evisceration between the two organs or by the presence of kidneys which remained next to the muscles when animals were eviscerated.

The notion of intra-batch heterogeneity (same origin) and of the number of test portions to be taken for the same batch, or of the number of samples for one test portion, would require some research work in order to optimise the sampling plan. This question is also dealt with in the response to Request No. 2007-SA-0009.

## 10. QUESTION 6

**Review of Question VI:** If the batches of Pectinidae (whole meat) are nonconforming for lipophilic, ASP and PSP toxins, in what conditions will evisceration enable the risk to be eliminated (sampling plan) before the products are placed on the market?

The quantification of the excess risk requires the following information:

- 1. The level and distribution of the initial contamination in the nonconforming area concerned must be known.
- 2. The effectiveness of the evisceration technique must be quantitatively known in a specified context (initial contaminations levels, shellfish species, for example).
- 3. The effectiveness of human handling in the evisceration procedure (risk of human error) must be quantitatively known in a specified context (freezing, shellfish size, for example).

These quantitative data would enable the risk for these products to be assessed compared to non-eviscerated products and the quantitative justification of a sampling strategy adapted to this type of situation.

It should be recalled that according to Decision 2002/226/EC, "each batch of final product must be analysed by the establishment specifically authorised".

#### 11. CONCLUSIONS AND RECOMMENDATIONS

The preferential accumulation of lipophilic and PSP toxins in the digestive gland of *Pecten maximus* scallops allows us to envisage the <u>principle</u> of an evisceration procedure as is already the case for domoic acid (Commission Decision 2002/226/CE of 15 March 2002). On the other hand, there are no data available concerning the *Pecten jacobaeus* species, whether for lipophilic or PSP toxins. Consequently, it is not possible at the present time to determine conditions of removal and treatment (evisceration) for these 2 species of scallop. **Studies are required on the decontamination by evisceration of the species** *Pecten maximus* and *Pecten jacobaeus* **contaminated by these toxins, in order to be able to determine limits at harvest** which would guarantee the sanitary safety of consumers.

The extension of this approach to other Pectinidae and in particular to the variegated scallops sold as "pétoncles" in French requires, on the one hand, that the DGAI issue a list of species of Pectinidae produced and sold in France under the names "coquilles Saint-Jacques" and "pétoncles"; and on the other hand, that **studies on decontamination by evisceration** be carried out on these species.

The freezing/thawing of shellfish before evisceration is liable to lead to an extra risk related in particular to the alteration of the contaminated tissues, facilitating the cross contamination of other organs. Furthermore, if the ambient conditions are not stable, this can encourage the phenomenon of sublimation with water loss and therefore an increase in the toxin concentration. To avoid this, it is necessary to ensure that:

- freezing is rapid;
- the temperature is constant (no break in the cold chain);
- shellfish are packed individually to limit any risk of cross contamination and avoid the phenomenon of sublimation.

Consequently, it will be necessary to conduct studies intended to determine the effects of freezing and thawing on the algal toxin level and their impact in terms of toxin detection (effects on free fatty acids). In fact, as long as the reference method remains the mouse bioassay, the increase in the free fatty acid content during cold storage of shellfish is likely to give rise to false positives.

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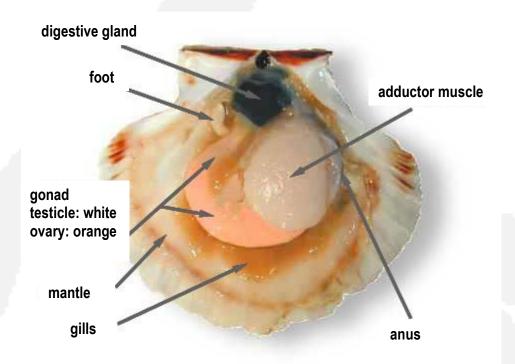
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## 13. KEY WORDS.

Marine biotoxins, algal toxins, Pectinidae, scallops, evisceration.

**Pascale BRIAND** 

ANNEX
ANATOMY OF THE INTERNAL PART OF THE SOFT BODY OF A HERMAPHRODITE SCALLOP



Source: http://www.finemaree.com/biologie.html