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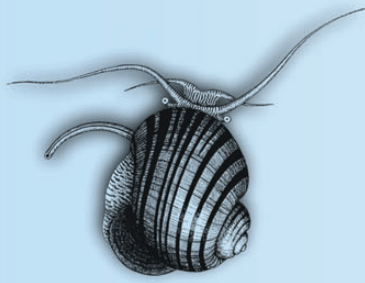
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## RESEARCH NOTE

### Responses of the common periwinkle *Littorina littorea* to exposure to the toxic dinoflagellate *Alexandrium minutum*

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Periwinkles are among the most abundant snails on the coasts of western and northern Europe (Sommer, 1999). The common periwinkle, *Littorina littorea*, inhabits rocky shores in the high and middle intertidal zones, rock pools and mud-bottom habitats in estuaries (Moore, 1937). This herbivorous species has a mixed diet (Davies & Beckwith, 1999) including benthic macroalgae and a wide variety of benthic microalgal species (Norton *et al.*, 1990).

Toxic dinoflagellates of the genus *Alexandrium* occur worldwide, with blooms recorded in Europe, North America, South America, Africa, Southeast Asia, Australia and New Zealand (Anderson *et al.*, 2012). Several species of *Alexandrium* produce phycotoxins, such as saxitoxin (STX) and its many analogues, which are responsible for paralytic shellfish poisoning (PSP) in organisms at higher trophic levels. The life cycle of *A. minutum* is comprised of a planktonic vegetative cell phase that undergoes asexual reproduction by binary fission and a benthic phase in the form of resting cysts produced through sexual reproduction (Anderson, 1998). When vegetative cells of *Alexandrium* species are exposed to unfavorable conditions, such as mechanical shock or sudden changes in environmental conditions, they can form pellicle or temporary cysts, which sink to the bottom and enter the benthic food web. When conditions become favourable again, the pellicle cysts quickly re-establish a motile existence, without an obligate dormancy period (Bravo *et al.*, 2010). The toxin content of *A. minutum* pellicle cysts has not yet been described. However, vegetative cells germinated from pellicle cysts exhibit only a slight decrease in the total toxin content (11%), indicating that *A. minutum* pellicle cysts remain toxic (Laabir *et al.*, 2007). In addition, pellicle cysts of another *Alexandrium* species (*A. fundyense*) retain toxicity even after encystment (Persson *et al.*, 2012). Vegetative cells of *A. minutum*, mainly found in sheltered marine ecosystems and estuaries, usually occur in low concentrations. However, blooms can sometimes reach extremely high densities, as in Brittany, France (over  $4 \times 10^7$  cells  $l^{-1}$ ; Chapelle *et al.*, 2013) and off the Catalan coast (up to  $10^6$  cells  $l^{-1}$ ; Garc s *et al.*, 2004). Large numbers of cysts can be produced by these massive proliferations of vegetative cells (Garc s *et al.*, 2004); up to  $2.4 \times 10^4$  cysts per gram of wet sediment were detected after the occurrence of an *A. minutum* bloom on the coast of Brittany (Erard-Le Denn *et al.*, 1993).

PSP toxins occur mainly in the digestive glands of predatory marine gastropods that feed on contaminated clams, mussels and

other filter-feeders in the natural environment (reviewed by Deeds *et al.*, 2008). Similarly, Ito *et al.* (2004) and Bricelj *et al.* (2012) found brevetoxins in predatory gastropods. However, little is known about PSP contamination in marine herbivorous and deposit-feeding snails, which could feed directly on toxic cells and cysts, and transfer biotoxins to organisms at higher trophic levels. Contamination in a herbivorous snail was reported by Shumway (1995), who found PSP toxins in *Littorina sitkana*.

The present study assessed the potential role of toxic pellicle cysts of *A. minutum* as a source of food and, consequently, a source of PSP toxin for the herbivorous gastropod *L. littorea*. We examined feeding rates and toxin accumulation in snails after exposure to different densities of pellicle cysts of two species of dinoflagellates, the toxic *A. minutum* and the nontoxic *Heterocapsa triquetra*. Toxin accumulation was also monitored in *L. littorea* during the 2013 summer bloom of *A. minutum* in the Bay of Brest. Toxin content in snails was monitored long after the bloom, in order to evaluate the potential role of cysts as a source of PSP toxins for benthic grazers even after the end of the bloom. To our knowledge, this is the first study evaluating the relationship between a toxic marine microalga and the benthic snail *L. littorea*. It is important to highlight that this study has public health significance, since this species is one of the main shellfish consumed in France and it is harvested on the coast of Brittany, where annual *A. minutum* blooms occur.

The dinoflagellates *A. minutum* and *H. triquetra* were grown using filtered sea water (0.2  $\mu m$ ) supplemented with L1 enrichment medium and cultures were maintained at 16  $^{\circ}C$  and 100  $\mu mol$  photon  $m^{-2} s^{-1}$  with a dark/light cycle of 12:12 h. Dinoflagellates were maintained in the exponential growth phase (approx. 12 d of culture). At this phase, the strain of *A. minutum* produced 7.38 ( $\pm 0.02$ ,  $n = 3$ ) pg STX per vegetative cell and 7.12 ( $\pm 0.02$ ,  $n = 3$ ) pg STX per pellicle cyst, as measured by the ELISA Saxitoxin Microtiter Plate Kit (Abraxis). Cultures yielded more than 90% encystment and were centrifuged at  $500 \times g$  for 10 min to obtain pellicle cysts. Cysts of both species were measured to assess cell biovolume (Hillebrand *et al.*, 1999) and carbon content was calculated per the equation expressed by Menden-Deuer & Lessard (2000). Counts of cysts were performed by flow cytometry (FACScalibur, BD Biosciences) using the fluorescent dye SYBR-Green I (Molecular Probes, Life Technologies) diluted in 10% DMSO at 1/1,000 of the commercial solution.

Periwinkles (<10 mm) were collected in April 2013 in the Bay of Brest (Brittany, France) and snails were evaluated for toxin content to guarantee no previous contamination (same method as presented below). A day before the experiment, snails were acclimated to the experimental conditions and fed nontoxic pellicle cysts. Each snail was incubated at a specific density of cysts in flasks of 75 cm<sup>2</sup> bottom area (150 ml) at 16 °C with a dark/light cycle of 12:12 h. Five replicates of each cyst density were obtained, and control flasks (without snails) for both algae were run in triplicate to determine any possible growth or reduction in algal density without grazing. Snails were exposed to pellicle cysts of either toxic (*A. minutum*) or nontoxic (*H. triquetra*) dinoflagellates at six cyst densities: 2,000, 3,000, 6,600, 8,000, 11,000 and 17,800 cysts cm<sup>-2</sup>, respectively, 0.8, 1.2, 2.64, 3.2, 4.4 and 7.12 µg C cm<sup>-2</sup>. In each replicate, 500 µl of each dilution was sampled for cell count and feeding rate determination prior to adding the snail and after 24 h. Samples were incubated with SYBR-Green I (1% of final volume) in the dark at 20 °C for 30 min before flow-cytometry analyses.

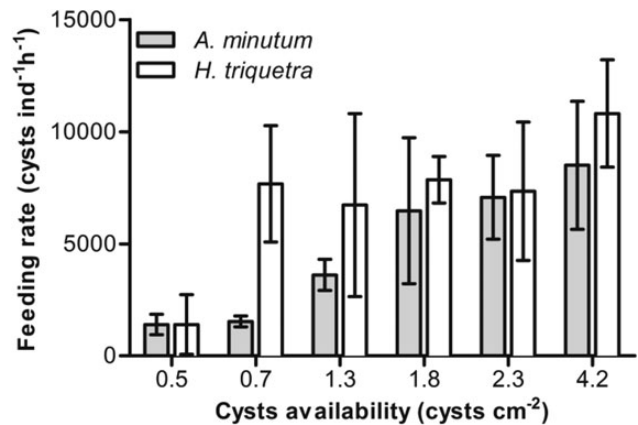
Feeding rates (µgC ind<sup>-1</sup> h<sup>-1</sup>) were determined as the ratio of the natural log (ln) of the initial and final cyst densities over 24 h of incubation, corrected by control densities (Ohman, Theilacker & Kaupp, 1991). Thus, each replicate yielded an independent measurement of the ingestion rate.

Snails were removed from individual flasks and dissected for digestive gland preparation and toxin analysis after 48 h of incubation. Analysis of PSP toxins was performed by immuno-assay with an ELISA Saxitoxin Microtiter Plate Kit (Abraxis), which recognizes STX and other PSP toxins at concentrations from 0.02 to 0.4 ng ml<sup>-1</sup>. Toxin was extracted from digestive glands in 0.1 M HCl, 1 µl of acid to 1 mg of tissue, at 4 °C according to the manufacturer's instructions. After homogenization (2,218 × g, 3 periods of 20 s), samples were boiled (104 °C) for 5 min and centrifuged (3,500 × g) for 10 min at 8 °C. Supernatants were stored at 4 °C until analysis.

During the 2013 summer (June-August), algal densities were monitored in the Bay of Brest, where a massive bloom of *A. minutum* had occurred the previous year (Chapelle *et al.*, 2013). The maximum *A. minutum* density was observed on 22 July, about 3 × 10<sup>5</sup> cells l<sup>-1</sup>. Subsequently, vegetative cells density decreased to <10<sup>4</sup> cells l<sup>-1</sup> on 5 August and <10<sup>3</sup> cells l<sup>-1</sup> on 12 August (Chapelle *et al.*, 2013). Nine snails were collected prior to the bloom (July 4), and again 2 d after the highest density of *A. minutum* (24 July), 26 d after the peak of the bloom (August 19), and 42 d after the peak of the bloom (4 September). Snails were dissected for evaluation of the toxin content in their digestive glands according to the methods described above. Toxin analysis was performed using a pool of digestive gland extracts from three individuals; three pools were analysed through random sampling.

Differences in feeding rates between densities and treatments (toxic and nontoxic) were tested by two-way analysis of variance (ANOVA). Negative values of grazing, without biological meaning, were removed from analysis. Differences in toxin content in gastropods following the course of the natural bloom (before, during and after the bloom) were tested by one-way ANOVA, and a *post hoc* Tukey test. Data were tested for normality and homogeneity of variance (Levene test,  $P > 0.05$ ). If assumptions were not met, a square-root transformation was applied. Statistical analyses were performed using Statistica v. 8.0 (StatSoft). Feeding rates were plotted against toxin accumulation and a linear regression was fitted using the Sigma Plot v. 10.0 (Systat).

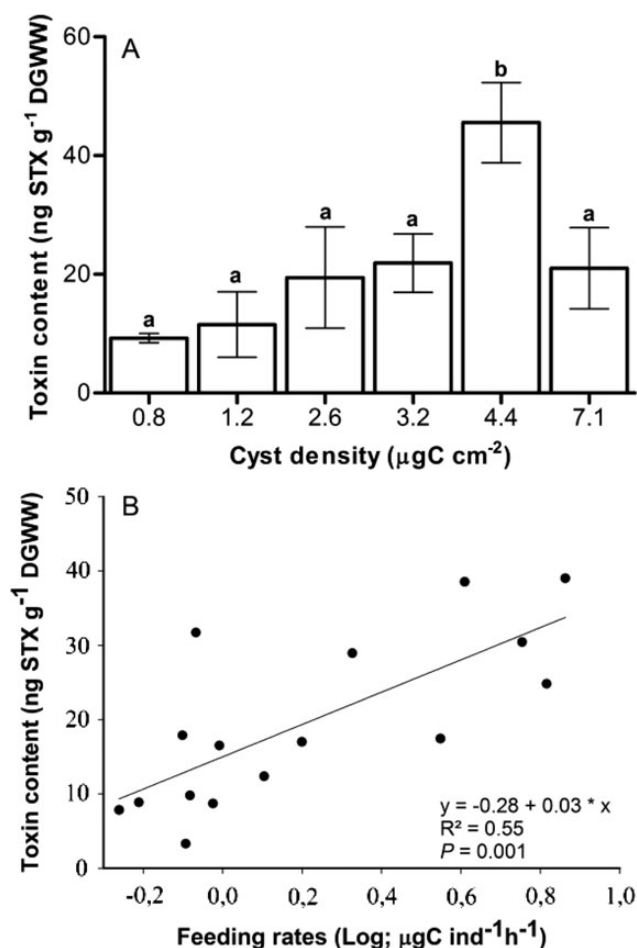
*Littorina littorea* fed on pellicle cysts of both *A. minutum* and *H. triquetra* (Fig. 1). There was a significant difference in feeding rates between toxic and nontoxic treatments (ANOVA,  $F_{(1,39)} = 4.28$ ,  $P = 0.04$ ,  $n = 51$ ). No significant differences in grazing rates were found among cyst densities (ANOVA,  $F_{(5,39)} = 2.28$ ,  $P = 0.06$ ,  $n = 51$ ), or in the interaction between cyst densities



**Figure 1.** Feeding rates of *Littorina littorea* exposed to pellicle cysts of the toxic *Alexandrium minutum* and nontoxic *Heterocapsa triquetra* at different densities of cysts (µg C cm<sup>-2</sup>). Values are given as mean ± SE,  $n = 5$ . Feeding rates of *L. littorea* differed significantly between toxic and nontoxic treatments (ANOVA,  $F_{(1,39)} = 4.28$ ,  $P = 0.04$ ).

and treatments (ANOVA,  $F_{(5,39)} = 1.75$ ,  $P = 0.14$ ,  $n = 51$ ). Despite the significant difference in grazing rates between treatments, toxic exposure does not seem to negatively affect feeding rates of *L. littorea*. Grazing on pellicle cysts of *A. minutum* by *L. littorea* provides evidence that cysts may be used as a food item in the natural environment, mainly when cysts are more available, such as during and after blooms (Erard-Le Denn *et al.*, 1993; Garcés *et al.*, 2004). Even though this study evaluated *L. littorea* grazing on pellicle cysts, one can infer that snails may also feed on resting cysts as they fall to the bottom. *Littorina littorea* is a generalist herbivore, presumed to be unselective (Norton *et al.*, 1990), feeding on macroalgae (Chlorophyta, Phaeophyta and Rhodophyta) and microalgae. In their natural environment, snails could feed directly on *A. minutum* cysts deposited in the sediment, as seen in the present study, or by grazing on macroalgae covered by toxic vegetative cells or cysts.

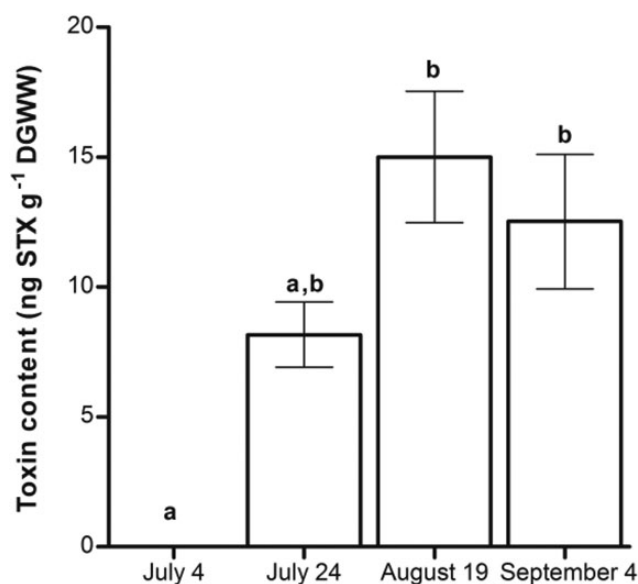
Paralytic shellfish-poisoning toxins accumulated in the digestive glands of *L. littorea* exposed to pellicle cysts of *A. minutum* (Fig. 2A); no toxin accumulation was observed in snails fed the nontoxic dinoflagellate *H. triquetra*. The toxin content in digestive glands of *L. littorea* was significantly different (ANOVA,  $F_{(5,10)} = 7.39$ ,  $P = 0.004$ ,  $n = 16$ ) among cyst concentrations; snails exposed to a specific density of toxic cysts (4.4 µg C cm<sup>-2</sup>) accumulated more toxins than snails exposed to the other densities (Tukey test,  $P < 0.02$ ). Toxin accumulation in snails indicates that cysts of *A. minutum* were completely or partially digested by *L. littorea*. The consumption and digestion of resting cysts of *Alexandrium* have been previously observed in the mudsnail *Ilyanassa obsoleta* and in the oyster *Crassostrea virginica* that digested cysts of *A. fundyense* (Persson *et al.*, 2006; Persson *et al.*, 2008). A significant positive linear correlation was found between toxin content and grazing rate of snails fed on toxic cysts when data were log-transformed ( $R^2 = 0.55$ ,  $F_{(1,14)} = 17.11$ ,  $P < 0.001$ ; Fig. 2B), with grazing explaining 55% of the variance in toxin content in snails. The residual variance in toxin content might be related to inter-individual differences in digestibility and/or toxin assimilation and accumulation. Inter-individual variability in sensitivity to toxins and toxin accumulation rates has been demonstrated in bivalve species (Bricelj & Shumway, 1998; Haberkorn *et al.*, 2011) and these variations can result from genetic differences (Bricelj *et al.*, 2005). Inter-individual variations in toxin sensitivity could also lead to differences in grazing and behavioural responses between toxic and nontoxic treatments. In the present study, after exposure to toxic cysts, some snails seemed to be affected



**Figure 2.** **A.** Toxin content (ng STX  $\text{g}^{-1}$  wet weight) in digestive glands (DG) of *Littorina littorea* after laboratory exposure at different densities of *Alexandrium minutum* cysts ( $\mu\text{g C cm}^{-2}$ ). Values are given as mean  $\pm$  SE,  $n = 5$ . Different letters indicate significant difference ( $P < 0.05$ ). **B.** Positive linear relationship between feeding rates (log-transformed;  $\times$ ) and toxin accumulation in digestive glands ( $y$ ) of *L. littorea*.

behaviourally by remaining inert (data not shown), turned upside-down and with the operculum only partially opened or completely closed. This behaviour might be related to the amount of toxic cysts consumed, and/or individual susceptibility to toxin exposure. Some differences in behavioural responses of individuals were observed between populations of molluscs previously exposed to toxic cells and naive populations such as in *Mya arenaria* (Bricelj *et al.*, 1996; MacQuerre & Bricelj, 2008) or within a single population of molluscs such as in *C. gigas* (Haberhorn *et al.*, 2011). Differences in the behaviour of snails exposed to toxic cells in natural environments may modify the susceptibility of contaminated individuals to predation, which could have important ecological consequences for accumulation of toxins and food-web transference.

Several studies have reported accumulation of PSP toxins in marine gastropods following blooms of *Alexandrium* species (reviewed by Deeds *et al.*, 2008). Many of the gastropods listed by Deeds *et al.* (2008) are carnivores, preying on filter-feeding molluscs that were already contaminated by PSP toxins. In the present study, toxins were also detected in the digestive glands of snails following natural exposure during the peak of the bloom (24 June) and even more than 1 month after the end of the bloom (4 September; Fig. 3). Toxin content in snails exposed to the natural *A. minutum* bloom reached a maximum of  $15 \pm \text{SE} =$



**Figure 3.** Toxin content (ng STX  $\text{g}^{-1}$  wet weight) in digestive glands (DG) of *Littorina littorea* on 4 July 4 (before bloom of *Alexandrium minutum*), July 24 (peak of bloom), August 19 (26 d after peak of bloom) and September 4 (42 d after peak of bloom). Values are given as mean  $\pm$  SE,  $n = 3$  pools of 3 individuals. Different letters indicate significant difference ( $P < 0.05$ ).

4.4 ng STX  $\text{g}^{-1}$  DGWW ( $n = 3$  pools of 3 individuals) 26 d after the peak of the bloom (19 August). Toxin content in snails collected before, during and after the bloom were significantly different (ANOVA,  $F_{(3,8)} = 11.79$ ,  $P = 0.003$ ,  $n = 9$  pools of 3 individuals). The toxin content in snails collected before the bloom was significantly different from toxin content in snails collected after the bloom on 19 August (Tukey test,  $P = 0.003$ ) and 4 September (Tukey test,  $P = 0.007$ ). Snails collected during and after the bloom showed no significant difference in toxin content (Tukey test,  $P > 0.05$ ). In contrast to the findings of Prakash, Medcof & Tennant (1971), who did not find PSP toxins in *L. littorea* exposed to *Gonyaulax tamarensis* in the natural environment, *L. littorea* may be contaminated by harmful blooms of *A. minutum*. In the present study, toxin accumulation by the snails collected during and after the bloom suggests that this benthic species feed on cysts of *A. minutum* in natural environments. As the bloom ends, cyst sedimentation potentially increases cyst availability to benthic grazers, which could explain the persistence of toxicity in benthic grazers long after the bloom ends. As the pellicle and resting cysts of *A. minutum* contain similar amounts of toxins found in vegetative cells (Persson *et al.*, 2012; present study), this study emphasizes the importance of cysts as a source of PSP contamination for benthic grazers.

*Littorina littorea* accumulated  $24.4 \pm \text{SE} = 5.3$  ng STX  $\text{g}^{-1}$  DGWW ( $n = 30$ ) in experimental exposure, whereas toxin content in snails exposed to the natural bloom was  $11 \pm \text{SE} = 1.33$  ng STX  $\text{g}^{-1}$  DGWW ( $n = 9$  pools of 3 individuals). Differences between laboratory and field results were expected as the snails were collected in the intertidal zone and therefore less often exposed to the bloom. In addition, even though a high (but variable) number of cysts probably reach the sediment during and after the natural bloom, several other food sources are available to the snails. *Littorina littorea* could represent an alternative pathway for toxin transfer to higher trophic levels other than via suspension-feeders. Snails are eaten by several carnivorous predators such as the crabs *Carcinus maenas* and *Cancer borealis* (Perez *et al.*, 2009), thus transferring toxins to

higher levels of the food chain even several weeks after the bloom. The present study confirms that gastropods can act as nontraditional vectors of saxitoxins to human consumers, as proposed in the reviews of Shumway (1995) and Deeds *et al.* (2008). Snails are able to bioaccumulate high concentrations of STXs and are known to be the cause of some human fatalities as a result of toxin exposure (reviewed by Prakash *et al.*, 1971; Deeds *et al.*, 2008).

Long-term studies assessing the physiological responses of *L. littorea* to cysts of *A. minutum* and other toxic species are needed to understand the impacts of harmful algae on health status of this gastropod and other benthic grazers. This study is the first evaluation of ingestion rates of the snail *L. littorea* consuming toxic microalgal cysts and the first to assess toxin content in this snail after both experimental and natural exposure to *A. minutum*. The present results confirm that benthic snails could be a source of PSP contamination at the base of the food chain, even long after a bloom. Moreover, this study highlights the importance of monitoring deposit-feeders and benthic grazers, in addition to suspension-feeders, during and after harmful algal blooms.

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#### REFERENCES

- ANDERSON, D.M. 1998. Physiology and bloom dynamics of toxic *Alexandrium* species, with emphasis on life cycle transitions. In: *Physiological ecology of harmful algal blooms* (D.M. Anderson, A.D. Cembella & G.M. Hallegraeff, eds), pp. 29–48. Springer, Berlin.
- ANDERSON, D.M., ALPERMANN, T.J., CEBELLA, A.D., COLLOS, Y., MASSERET, E. & MONTRESOR, M. 2012. The globally distributed genus *Alexandrium*: multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae*, **14**: 10–35.
- BRAVO, I., FIGUEROA, R.I., GARCÉS, E. & MASSANET, S.F.A. 2010. The intricacies of dinoflagellate pellicle cysts: the example of *Alexandrium minutum* cysts from a bloom recurrent area (Bay of Baiona, NW Spain). *Deep Sea Research Part II*, **57**: 166–174.
- BRICELJ, M.V., CEBELLA, A.D., LABY, D., SHUMWAY, S.E. & CUCCI, T.L. 1996. Comparative physiological and behavioral responses to PSP toxins in two bivalve mollusks, the soft clam, *Mya arenaria*, and surfclam, *Spisula solidissima*. In: *Harmful and toxic algal blooms* (T. Yasamoto, Y. Oshima & Y. Fukuyo, eds), pp. 405–408. IOC-UNESCO, Paris.
- BRICELJ, V.M., CONNELL, L., KONOKI, K., MACQUARRIE, S.P., SCHEUER, T., CATTERALL, W.A. & TRAINER, V.L. 2005. Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. *Nature*, **434**: 763–767.
- BRICELJ, V.M., HAUBOIS, A.G., SENGCO, M.R., PIERCE, R.H., CULTER, J.K. & ANDERSON, D.M. 2012. Trophic transfer of brevetoxins to the benthic macrofaunal community during a bloom of the harmful dinoflagellate *Karenia brevis* in Sarasota Bay, Florida. *Harmful Algae*, **16**: 27–34.
- BRICELJ, V.M. & SHUMWAY, S.E. 1998. Paralytic shellfish toxins in bivalve molluscs: occurrence, transfer kinetics and biotransformation. *Reviews in Fisheries Science*, **6**: 315–383.
- CHAPELLE, A., LE BEC, C., LE GAC, M., LABRY, C., AMZIL, Z., GUILLOU, L., DREANNO, C. & PINEAU, L. 2013. *Etude sur la prolifération de la micro algue Alexandrium minutum en rade de Brest: projet Daoulex rapport d'avancement*, Vol. 1. IFREMER, Plouzané.
- DAVIES, M.S. & BECKWITH, P. 1999. Role of mucus trails and trail-following in the behaviour and nutrition of the periwinkle *Littorina littorea*. *Marine Ecology Progress Series*, **179**: 247–257.
- DEEDS, J., LANDSBERG, J., ETHERIDGE, S., PITCHER, G. & LONGAN, S. 2008. Non-traditional vectors for paralytic shellfish poisoning. *Marine Drugs*, **6**: 308–348.
- ERARD-LE DENN, E., DESBRUYERES, E. & OLU, K. 1993. *Alexandrium minutum*: resting cyst distribution in the sediments collected along the Brittany coast, France. In: *Toxic phytoplankton blooms in the sea* (T.J. Smayda & Y. Shimizu, eds), pp. 109–114. Elsevier, Amsterdam.
- GARCÉS, E., BRAVO, I., VILA, M., FIGUEROA, R.I., MASÓ, M. & SAMPEDRO, N. 2004. Relationship between vegetative cells and cyst production during *Alexandrium minutum* bloom in Arenys de Mar harbour (NW Mediterranean). *Journal of Plankton Research*, **26**: 637–645.
- HABERKORN, H., TRAN, D., MASSABUAU, J.C., CIRET, P., SAVAR, V. & SOUDANT, P. 2011. Relationship between valve activity, microalgae concentration in the water and toxin accumulation in the digestive gland of the Pacific oyster *Crassostrea gigas* exposed to *Alexandrium minutum*. *Marine Pollution Bulletin*, **62**: 1191–1197.
- HILLEBRAND, H., DÜRSELEN, C.D., KIRSCHTEL, D., POLLINGER, U. & ZOHARY, T. 1999. Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, **35**: 403–424.
- ITO, K., ASAKAWA, M., BEPPU, R., TAKAYAMA, H. & MIYAZAWA, K. 2004. PSP-toxicification of the carnivorous gastropod *Rapana venosa* inhabiting the estuary of Nicho River, Hiroshima Bay, Hiroshima Prefecture, Japan. *Marine Pollution Bulletin*, **48**: 1116–1121.
- LAABIR, M., AMZIL, Z., LASSUS, P., MASSERET, E., TAPILATU, Y., DE VARGAS, R. & GRZEBYK, D. 2007. Viability, growth and toxicity of *Alexandrium catenella* and *Alexandrium minutum* (Dinophyceae) following ingestion and gut passage in the oyster *Crassostrea gigas*. *Aquatic Living Resources*, **20**: 51–57.
- MACQUERRIE, S.P. & BRICELJ, V.M. 2008. Behavioral and physiological responses to PSP toxins in *Mya arenaria* populations in relation to previous exposure to red tides. *Marine Ecology Progress Series*, **366**: 59–74.
- MENDEN-DEUER, S. & LESSARD, E.J. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography*, **45**: 569–579.
- MOORE, H.B. 1937. The biology of *Littorina littorea*. Part I. growth of the shell and tissues, spawning, length of life and mortality. *Journal of Marine Biological Association of the United Kingdom*, **21**: 721–742.
- NORTON, T.A., HAWKINS, S.J., MANLEY, N.L., WILLIAMS, G.A. & WATSON, D.C. 1990. Scraping a living: a review of littorinid grazing. *Hydrobiologia*, **193**: 117–138.
- OHMAN, M.D., THEILACKER, G.H. & KAUPP, S.E. 1991. Immunochemical detection of predation on ciliate protists by larvae of the northern anchovy (*Engraulis mordax*). *Biological Bulletin*, **181**: 500–504.
- PEREZ, K.O., CARLSON, R.L., SHULMAN, M.J. & ELLIS, J.C. 2009. Why are intertidal snails rare in the subtidal? Predation, growth and the vertical distribution of *Littorina littorea* (L.) in the Gulf of Maine. *Journal of Experimental Marine Biology and Ecology*, **369**: 79–86.
- PERSSON, A., SMITH, B.C., ALIX, J.H., SENFT-BATOH, C. & WIKFORS, G.H. 2012. Toxin content differs between life stages of *Alexandrium fundyense* (Dinophyceae). *Harmful Algae*, **19**: 101–107.
- PERSSON, A., SMITH, B.C., DIXON, M.S. & WIKFORS, G.H. 2008. The eastern mudsnail, *Ilyanassa obsoleta*, actively forages for, consumes, and digests cysts of the dinoflagellate, *Scrippsiella lachrymosa*. *Malacologia*, **50**: 341–345.
- PERSSON, A., SMITH, B.C., WIKFORS, G.H. & QUILLIAM, M. 2006. Grazing on toxic *Alexandrium fundyense* resting cysts and vegetative cells by the eastern oyster (*Crassostrea virginica*). *Harmful Algae*, **5**: 678–684.
- PRAKASH, A., MEDCOF, J.C. & TENNANT, A.D. 1971. Paralytic shellfish poisoning in eastern Canada. *Fisheries Research Board of Canada*, **177**: 1–87.
- SHUMWAY, S.E. 1995. Phycotoxin-related shellfish poisoning: bivalve molluscs are not the only vectors. *Reviews in Fisheries Science*, **3**: 1–31.
- SOMMER, U. 1999. The susceptibility of benthic microalgae to periwinkle (*Littorina littorea*, Gastropoda) grazing in laboratory experiments. *Aquatic Botany*, **63**: 11–21.