Preliminary investigation on the microbiological quality of edible marine gastropods of the Adriatic Sea, Italy

Patrizia Serratore, Emanuele Zavatta, Giorgia Bignami, Luna Lorito

Department of Veterinary Medical Science, University of Bologna, Italy

Abstract

According to the European Legislation, marine gastropods placed unprocessed on the market must comply with the same requirements established for live bivalve molluscs but, being considered not filter-feeding and unable to concentrate fecal contaminants, they may be harvested outside the classified areas. Despite this statement, little scientific information is available on the microbiological quality of these animals. The aim of the present study was to investigate 28 batches of edible snails of the Adriatic Sea, namely *Nassarius mutabilis* and *Bolinus brandaris*, with respect to i) smell and viability, by a method here reported; ii) the bacterial component of the whole body referred to *E. coli*, *Vibrio* spp., *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. alginolyticus*. A total of 21 batches of *N. mutabilis* and 7 batches of *B. brandaris* were analyzed. Batches of both species retrieved from the primary production were all largely composed of viable animals, had saltwater/neutral smell, and showed mean value of *Vibrio* spp. of 5,34 and 5,79 $\log_{10}$ UFC g^{-1} in *N. mutabilis* and *B. brandaris* respectively. 47% of the batches of *N. mutabilis* retrieved from the market, were largely composed of dead animals, had acrid/nasty smell, and showed mean value of *Vibrio* spp. of 6,53 $\log_{10}$ UFC g^{-1}. *E. coli*, *V. vulnificus* and *V. cholerae* were never detected, but all samples were positive for *V. alginolyticus*. One sample of *B. brandaris* was positive for *V. parahaemolyticus* genotyped by PCR at the specie level (*Tox*R+) and positive for the thermostable direct hemolysin gene (*tdh*+).

Introduction

Marine gastropods represent a common source of seafood all over the world. Most of the edible species are catched, but some high-value species, as abalone (*Haliotis* spp.), one of the most expensive of any seafood item worldwide, are farmed reaching over 95% of the total production (FAO, 2018). Fishing of *Nassarius mutabilis* is by far the most important activity carried out by artisanal fisheries using basket traps in the central and northern Adriatic Sea, yielding from 2000 to 3000 Tonnes of landings each year (Polidori *et al*., 2015).

Historically, *Bolinus brandaris* has been a valuable species since the time of the Roman Empire, when it was caught for extracting the purple dye (Spanier and Karmon, 1987), so it is also known as "purple dye murex". Nowadays, *B. brandaris* is frequently caught as bycatch by bottom trawlers, but also by artisanal fishing, particularly in southern Europe, including Italy, being considered also as a candidate species for molluscan aquaculture (Vasconcelos *et al*., 2012).

According to the European Legislation, marine gastropods placed "fresh" (unprocessed) on the market must comply with the same requirements laid down for live bivalve molluscs (European Commission, 2004a), but they may be harvested from non-classified production areas, being considered not filter-feeding, and therefore unable to concentrate fecal contaminants (European Commission, 2010) as *E. coli*, quantitatively utilized for the classification of the production areas and the suitability for direct human consumption (European Commission, 2004b; European Commission, 2005). For the same reason, the purification process is considered unworkable for these animals (European Commission, 2004a).

Despite these provisions, it must be recognized that the scientific knowledge about the microbiological quality of marine gastropods is poor, and substantially lacking for the edible marine species of the Italian seas. Moreover, the overview report undertaken by the European Commission's Food and Veterinary Office (European Commission, 2015), pointed out that the Official Controls on live gastropods harvested from non-classified production areas are not yet fully implemented by the Member States, being absent or inappropriate. Lastly, a standardized method for the assessment of the viability of these animals placed on the market is not currently available, considering that the provision of European Commission (2004a) is based on bivalve molluscs characteristics, to whom marine gastropods are assimilated.

Marine gastropods are not filter feeding, but most of them are carnivorous, with a degree of predatory activity that varies from actively seeking prey, to grazing on sessile invertebrates, to scavenging (Modica and Holford, 2010).

Among gastropods, marine snails are considered the source of a variety of toxic compounds, particularly neurotoxins, mainly utilized for predation (West *et al*., 1996), but it is also well documented that carnivorous species can accumulate toxins through predation on contaminated bivalve molluscs. In fact, Paralytic Shellfish Toxins (PST) and Tetrodotoxin (TTX), and/or related compounds, have been detected in several species of gastropods, including members of the family *Nassaridae* (Rodiguez *et al*. 2008; Luo *et al*., 2012; Silva *et al*., 2012; Turner *et al*., 2014; Asakawa, 2017). On the other hand, since the last century, it has been reported that TTX producing bacteria, identified as *V. alginolyticus* and *V. parahaemolyticus*, have been isolated from the gastropod *Niotha clathrata* (*Nassarius conoidalis*), whose tissues resulted highly contaminated by bacteria, predominantly *Vibrio* spp., which resulted 6-8 $\log_{10}$ CFU g^{-1} in the muscle, and 6-9 $\log_{10}$ CFU g^{-1} in the digestive gland (Cheng *et al*., 1995).

Vibrio spp. represents a major genus of bacteria inhabiting the marine ecosystem, and within the taxon, serious pathogenic species for both human and marine animals are included (Fukui *et al*., 2010). The four species most frequently isolated in clinical microbiology laboratories are *V. cholerae*, *V parahaemolyticus*, *V. vulnificus* and *V. algi-*

Correspondence: Patrizia Serratore, Department of Veterinary Medical Science - UOS Cesenatico Viale A. Vespucci 2, 47042 Cesenatico (FC), Italy.
Tel.: +39.0547.338948 - Fax: +39 0547 338941.
E-mail: patrizia.serratore@unibo.it

Key words: Marine gastropods, *E. coli, Vibrio* spp., *V. alginolyticus, V. parahaemolyticus*.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no potential conflict of interest.

Funding: The study was funded by the Department of Veterinary Medical Science, University of Bologna, utilizing economies resulting from other research activities.

Received for publication: 12 July 2018.
Revision received: 13 February 2019.
Accepted for publication: 13 February 2019.

Italian Journal of Food Safety 2019; 8:7691
doi:10.4081/ijfs.2019.7691

nolyticus (Mustafa *et al*., 2013). *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* are the most important species in terms of number, distribution, and severity of infections (Boyd, 2007), and are responsible for a dramatic increase of seafood-borne infections worldwide (Bonnin-Jusserand *et al*., 2019). Notwithstanding the highest densities of vibrios in the environment are frequently found in warm waters ranging from 20°C to 30°C, infections of *V. parahaemolyticus* and *V. vulnificus* in humans have recently occurred even in cold high latitude areas where such infection had never been experienced, and these cases are reported to be a result of global warming trends in the ocean (Fukui *et al*., 2010).

In any case, a multiyear retrospective study conducted on the bivalve *Ruditapes philippinarum* in the North west Adriatic Sea, did not reveal a significant correlation between seawater temperature and *Vibrio* spp. load, resulted 4.71±0.10 and 4.70±0.29 $\log10^{g-1}$ in the warmer months (April-October) and in the cooler month (November-March) respectively, although the prevalence of *V. parahaemolyticus* and *V. vulnificus* resulted significantly higher in the warmer months (Serratore *et al*., 2016).

Infection by *V. cholerae* and *V. parahaemolyticus* typically results in gastrointestinal disease, while *V. vulnificus* can cause more severe illness, including septicemia and death, particularly in individuals with predisposing conditions (Jones *et al*., 2014). *V. alginolyticus* is considered the most frequent species living freely in water and sediments (Harriague *et al*., 2008), being able to survive even under conditions of nutrient stress while maintaining its virulence (Ben Kahla-Nakbi *et al*., 2007), and it has been found to cause serious seafood-poisoning or fatal extra-intestinal infections in humans (Fu *et al*., 2016).

The aim of the present study was to investigate the microbiological quality of two edible snails of the Adriatic Sea, namely *Nassarius mutabilis* and *Bolinus brandaris*, with respect to *E. coli*, as safety criterion (European Commission, 2005), *Vibrio* spp., as the most representative autochthonous bacterial component of the marine environment, and the potentially pathogenic species *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. alginolyticus*. Moreover, the applicability of a simple method to evidence the viability of a batch is presented.

Materials and Methods

A total of 21 batches of *N. mutabilis*, 17 at retail and 4 from primary production, and 7 batches of *B. brandaris*, all from primary production, were considered. Samples of *N. mutabilis* were collected from October to May, that is the fishing season of the species (Polidori *et al*., 2015), and samples of *B. brandaris* from May to June, when *N. mutabilis* samples were not available on the market. The catch area was the FAO zone 37.2.1 (Adriatic Sea), along the coast from Ravenna to Rimini.

Each batch was firstly analyzed for smell and viability. The smell was expressed based on five descriptors: saltwater (very fresh), neutral (freshness), slightly acrid (initial loss of fresh) acrid (loos of freshness), nasty (spoiled). To verify the viability, the whole batch was distributed on a large platter and then dusted by table salt to evidence any sign of reactivity, and only reactive animals were considered viable.

This approach was adopted because any attempt to verify gastropods viability by foot puncture or foot traction, as commonly indicated, resulted time consuming and unsatisfactory compared with the proposed method. For the bacteriological analyses, the sample units (SU) of *N. mutabilis* were constituted of about 20 individuals, and the SU of *B. brandaris* of about 10 individuals. Each SU was carefully rinsed off with sterilized seawater and shells were cut aseptically, to remove the whole body, representing the edible part of these animals. Animals bodies were blended with salt solution NaCl 3%, to obtain the first dilution 10^{-1} and from this the additional ones.

The abundance of *Vibrio* spp. was checked on thiosulfate-citrate-bile salts-sucrose (TCBS) agar (OXOID) NaCl 3% by the spread plate method, and incubation at 20 °C for 3–5 days as reported elsewhere (Passalacqua *et al*., 2016). The results were expressed as Colony Forming Units (CFU) g^{-1}.

The isolation of *V. parahaemolyticus*, *V. cholerae*, *V. vulnificus* and *V. alginolyticus* was performed on CHROMagar™ Vibrio (CAV) (PBI) incubated at 37°C for 24 h. The colorless colonies were tentatively scored as *V. alginolyticus*. The mauve and the green blue to turquoise blue colonies were tentatively scored as *V. parahaemolyticus* and *V. cholerae/V. vulnificus* respectively. The number of suspected colonies tested for each sample was from 1 to 5 (if available), as is the normal practice. Selected colonies were purified on Tryptone-Soya-Agar (TSA) (OXOID) NaCl 3%, and then tested to confirm the typical traits of the genus as reported elsewhere: Gram negative straight or curved rods, oxidase positive, able to reduce nitrate, dextrose fermenting and sensitive to the vibriostatic O/129 (150 µg) (Passalacqua *et al*., 2016). Growth on TCBS and modified cellobiose-polymyxin B-colistin Agar (m-CPC), according to the FDA Bacteriological Analytical Manual (BAM, 1998), and appearance of colonies were also checked. Additional biochemical tests were performed: dextrose fermentation and lactose utilization on Kligler Iron Agar (KIA) (OXOID); lysine and ornithine decarboxylase and arginine dihydrolase on Decarboxylase Broth, Moeller (DIFCO). The phenotypical traits utilized to characterize the suspected colonies are reported in Table 1.

Suspected *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* strains were also genotyped by Polymerase Chain Reaction (PCR) as reported elsewhere (Passalacqua *et al*., 2016), utilizing specific primers targeting *tox*RP, *tdh* and *trh* genes for *V. parahaemolyticus*; *tox*RC, *hlya*, *tcpI*, *tcp*A, *ctx*A, *ctx*B, *stn*/*sto* for *V. cholerae*; *vvh*A, *hsp*, *vcg*C, *vcg*E, CPS operon allele 1, CPS operon allele 2, 16s-rRNA type A gene, 16s-rRNA type B gene for *V. vulnificus*.

E. coli enumeration was performed according to the ISO 16649-2:2001 method.

Results

The sensory evaluation of the batches of *N. mutabilis* and *B. brandaris* was successfully achieved. The five descriptors of smell resulted adequate, and the dusting by table salt to evidence reactivity easily applicable to any batch. The viability of the batches was scored as follows: mortality less than 10% (++++), mortality less than 20% (+++); mortality 20-40% (++); mortality >40% (+); mortality 100% (-). The results of the sensory evaluation coupled with the bacteriological findings are reported in Table 2. All the batches from the primary production (*N. mutabilis* N=4 and *B. brandaris* N=7), and some batches of *N. mutabilis* at retail (9 out of 17), had salty or neutral smell, except sample 1323 having slightly acrid smell, and were composed of high reactive animals (viability from ++++ to +++) when dusted by table salt: extroversion of the foot and active movements (*N. mutabilis*), or abundant foam production (*B. brandaris*). The *Vibrio* spp. load of each sample, expressed as Colony Forming Units (CFU) g^{-1} have been log-transformed prior to calculate the mean value, that resulted 5,34 $\log_{10}$ (SD=±1,21) in *Nassarius* batches, and 5,79 $\log_{10}$ (SD=±0,98) in *B. brandaris* batches. Among the batches of *N. mutabilis* at retail, the 47% (8 out of 17) had acrid or nasty smell and poor or no reaction when dusted by table salt (viability from ++ to -). This was the case of samples 1324, 1327, 1328, 1332, 1333, 1379, 1406, 1407, with mean value of *Vibrio* spp. of 6,53 $\log_{10}$ CFU

g^{-1} (SD=±0,79).

Considering the bacteriological results of each batch acquired at retail and the time from the day of packaging and the day of laboratory analyses, controversial aspects were evidenced. Batches 1332, 1333 and 1379, acquired the sixth day from packaging, had nasty smell and were completely composed of dead animals, showing the highest counts of *Vibrio* spp. resulting 7.10, 7.29 and 7.69 $\log_{10}$ CFU g^{-1} respectively. On the other hand, batches 1327 and 1328, acquired the same day of packaging, had acrid smell and only low viability (++) as batch 1324 acquired one day from packaging, and batch 1407, also acquired one day from packaging, had nasty smell and very low viability (+).

All batches resulted negative for *E. coli*, *V. vulnificus* and *V. cholerae*, but positive for *V. alginolyticus*. One batch of *B. brandaris* was also positive for *V. parahaemolyticus*, genotyped and confirmed to the specie level (*Tox*R+), and possessing the gene marker hemolysin (*tdh*+), commonly recognized as an indicator of pathogenicity (Jones *et al*., 2014).

Table 1. Phenotypical traits utilized to characterize the suspected colonies grown on CHROMagar™ Vibrio: *V. alginolyticus* colorless, *V. parahaemolyticus* mauve, *V. cholerae*/*V. vulnificus* green blue to turquoise blue. Expected results.

	TCBS Agar	m-CPC Agar	Gram-roads	Oxidase	Nitrate reduction	O129/150 µg S/R O129/10 µg S/R	SIM motility/indole/H_2S	A/L/O	KIA
VA	Y	ng	+	+	+	SR	+/+/-	-/+/+ or -	GF+/L-/no H_2S/no gas
VC	Y	P/G	+	+	+	SS	+/+/-	-/+/+ or -	GF+/L-/no H_2S/no gas
VV	G/Y	Y/W/ng	+	+	+	SS	+/+/-	-/+/+ or -	GF+/L+ or L-/no H2S/no gas
VP	G	ng	+	+	+	SR	+/+/-	-/+/+ or -	GF+/L-/no H_2S/no gas

VA, *V. alginolyticus*; VP, *V. parahaemolyticus*; VC/VV, *V. cholerae*/*V. vulnificus*; Y, yellow; G, green; W, white; P, purple; ng, no growth; GF, glucose fermentation; L, lactose utilization; SIM, Sulfide Indole Motility medium; A, arginine dihydrolase; L, lysine decarboxylase; O, ornithine decarboxylase; KIA, Kligler Iron Agar.

Table 2. Smell, viability, *Vibrio* spp. and *E. coli* enumeration in *N. mutabilis* e *B. brandaris* and origin of the batches.

Sample Number	Species	Origin	Smell/ Viability	*Vibrio* spp. $\log_{10}$ CFU g^{-1}	Positivity for VA/VC/VP/VV	*E. coli*
1321	*N. mutabilis*	C	S/++++	3.20	VA+	nd
1323	*N. mutabilis*	C	SA/+++	5.95	VA+	nd
1324	*N. mutabilis*	C	A/++	6.25	VA+	nd
1327	*N. mutabilis*	C	A/++	6.41	VA+	nd
1328	*N. mutabilis*	C	A/++	5.88	VA+	nd
1332	*N. mutabilis*	C	NA/-	7.10	VA+	nd
1333	*N. mutabilis*	C	NA/-	7.29	VA+	nd
1337	*N. mutabilis*	C	S/++++	6.31	VA+	nd
1338	*N. mutabilis*	C	S/++++	6.23	VA+	nd
1339	*N. mutabilis*	C	S/++++	5.95	VA+	nd
1340	*N. mutabilis*	C	S/++++	6.09	VA+	nd
1378	*N. mutabilis*	C	N/++++	6.00	VA+	nd
1379	*N. mutabilis*	C	NA/-	7.69	VA+	nd
1382	*N. mutabilis*	C	S/++++	5.20	VA+	nd
1384	*N. mutabilis*	C	N/+++	6.42	VA+	nd
1386	*N. mutabilis*	C	S/++++	3.45	VA+	nd
1389	*N. mutabilis*	PP	N/++++	5.92	VA+	nd
1406	*N. mutabilis*	C	A/++	6.32	VA+	nd
1407	*N. mutabilis*	C	NA/+	5.26	VA+	nd
1408	*N. mutabilis*	PP	S/++++	5.81	VA+	nd
1409	*B. brandaris*	PP	S/++++	5.67	VA+	nd
1414	*N. mutabilis*	PP	S/++++	5.43	VA+	nd
1415	*B. brandaris*	PP	S/++++	3.54	VA+	nd
1416	*B. brandaris*	PP	S/++++	5.54	VA+	nd
1417	*B. brandaris*	PP	S/++++	3.86	VA+	nd
1418	*B. brandaris*	PP	S/++++	6.06	VA+	nd
1419	*B. brandaris*	PP	S/++++	6.96	VA+	nd
1420	*B. brandaris*	PP	S/++++	6.51	VA+/VP+	nd

C, commerce; PP, primary production; S, saltwater; NE, neutral; SA, slightly acrid; A, acrid; NA, nasty. ++++, mortality less than 10%; +++, mortality less than 20%; ++, mortality 20-40%; +, mortality >40%; -, mortality 100%. VA, *V. alginolyticus*; VC, *V. cholerae*; VP, *V. parahaemolyticus*; VV, *V. vulnificus;* nd, not detected.

Discussion

According to European Legislation (European Commission, 2004a) live gastropods, together with live echinoderms and live tunicates intended for human consumption, must comply with the requirements laid down for live bivalve molluscs, so they must "have organoleptic characteristics associated with freshness and viability, including shells free of dirt, an adequate response to percussion and normal amounts of intravalvular liquid". This provision, clearly based on bivalve molluscs characteristics, is obviously unsuitable for marine gastropods, and therefore a standardized method to verify their viability is lacking for the Competent Authorities and for the Food Operators.

According to European Legislation (European Commission, 2004a), a batch of gastropods may be placed on the market by a Dispatch Center after repackaging of a batch received from another Dispatch Center and so on, moving the product further and further away from the day and place of landing. In this way, the date of packaging is not indicative of the time between landing and placing on the market of the product at retail. Consequently, it is not surprising that 47% of the batches at retail considered in this study, had acrid or nasty smell and were largely or completely composed of dead animals after 6 days from packaging.

In the present study, the bacteriological component of *N. mutabilis* and *B. brandaris* tissues was checked without evisceration, considering that the edible part of these animals is the whole body, as opposed to the Argentina legislative requirements, establishing that the commercialization of uneviscerated snail is not allowed (Turner *et al*., 2014).

Despite it is well documented in literature the ability of marine gastropods to accumulate toxins through predation (Rodiguez *et al*. 2008; Luo *et al*., 2012; Silva *et al*., 2012; Turner *et al*., 2014; Asakawa, 2017), the possibility to harbor high concentration of bacteria in their tissues, including potentially pathogenic species, is poorly investigated, and to our knowledge, it is actually unrecognized as safety issue.

Only few reports on this matter have been published, and among them the investigation on disease outbreaks of *Abalone*, reporting bacterial contamination of tissues also in healthy animals (Shi *et al*., 2017), and the study of Cheng *et al*. (1995) reporting an high a contamination of *N. clathrata* muscle by *Vibrio* spp. (6-8 $\log_{10}$ CFU g^{-1}), coupled with positivity for *V. alginolyticus* and *V. parahaemolyticus* producing TTX.

Accordingly, our study evidenced high *Vibrio* spp. value in gastropods tissues, exceeding 5 $\log_{10}$ CFU g^{-1} also in viable batches, and positivity for *V. alginolyticus* (100% of the batches) and *V. parahaemolyticus* (1 out of 28, corresponding to the 3,6% of the batches).

Interestingly, it should be noted that the abundance of *Vibrio* spp. here reported is higher than the abundance registered on the bivalve *Ruditapes philippinarum* belonging to the same sea-area, shoving a mean value of *Vibrio* spp. less than 5 $\log_{10}$ CFU g^{-1} (Serratore *et al*. 2016), but with a positivity for pathogenic *V. parahaemolyticus* of 6,7%. Our data seem to confirm that marine gastropods may accumulate in their tissues bacteria present in their feed, just like bivalves do by their filter-feeding from seawater, and this represents a food safety issue actually underestimated.

Conclusions

To our knowledge, this is the first study on the bacterial component associated to edible marine gastropods of the Adriatic Sea, namely *N. mutabilis* and *B. brandaris*, together with the evaluation of sensory characteristics of the batches both from primary production and commerce.

Even if this may be considered only a preliminary investigation, two specific findings are of concern: 1) the concrete possibility to evaluate viability and freshness of the gastropods batches to avoid the placing on the market of products with poor or no signs of viability, and therefore not conforming with the European legislative requirements (European Commission, 2004a); 2) the high concentration of bacteria as *Vibrio* spp. in the whole body, that is the edible part, and the positivity for pathogenic bacteria as *V. alginolyticus* and *V. parahaemolyticus*.

The failure to evidence *E. coli*, seems to justify the provision of the European Commission (2010), laying down that marine gastropods may be harvested from non-classified production areas, but only hypothesizing an extremely low concentration of *E. coli* in the areas of harvesting, or a different kinship of *E. coli* towards gastropods tissues with respect to *Vibrio* spp.

To confirm our achievements, more data are needed, and obviously other gastropods species should be tested. Finally, regarding food safety, the study suggests the need to investigate the biogenic amine content of edible gastropods, because the catabolic activity of a high bacterial load makes not far-fetched the production of these natural anti-nutritional factors in their tissues.

References

Asakawa M, 2017. Marine biotoxins: occurrence, toxicity, and detection methods. In: Materials Science and Engineering. Proceeding of the International Conference on Food Science and Engineering 2016. IOP Publishing 193. doi:10.1088/1757-899X/193/1/012001

BAM, 1998. Media M98: Modified Cellobiose-Polymyxin B-Colistin (m-CPC) Agar: In Bacteriological Analytical Manual, Food and Drug Administration, 8th Edition, Revision A, 1998. Silver Spring, MD, US. Available from: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064011.htm

Ben Kahla-Nakbi A, Besbesa A, Chaieba K, Rouabhiab M, Bakhroufa A, 2007. Survival of Vibrio alginolyticus in seawater and retention of virulence of its starved cells. Mar Environ Res 46:469-78.

Bonnin-Jusserand M, Copin S, Le Bris C, Brauge T, Gay M, Brisabois A, Grard T, Midelet-Bourdin G, 2017. Vibrio species involved in seafood-borne outbreaks (Vibrio cholerae, V. parahaemolyticus and V. vulnificus): Review of microbiological versus recent molecular detection methods in seafood products. Crit Rev Food Sci Nutr 59:597-610.

Boyd EF, 2007. The biology of vibrios. In: The biology of vibrios. Lancet Infect Dis. Thompson FL, Austin B, Swings J eds., ASM Press 7:384.

Chan R, Kalaitzis JA, Neilan BA, 2014. Tetrodotoxin. In: Manual of Security Sensitive Microbes and Toxins. Liu D, ed. CRC Press, Boca Raton FL US. pp. 559-70.

Cheng CA, Hwang DF, Tsai YH, Chen HC, Jeng SS, Noguchi T, Ohwada K, Hasimoto K, 1995. Microflora and tetrodotoxin-producing bacteria in a gastropod, Niotha clathrata. Food Chem Toxicol 33:929-34.

European Commission, 2004a. Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for the hygiene of foodstuffs, 853/2004/CE. In: Official Journal, L 139/55, 30/04/2004.

European Commission, 2004b. Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption, 854/2004/EC. In: Official Journal,

L 139/206, 30/04/2004.
European Commission, 2005. Commission Regulation of 15 November 2005 on microbiological criteria for foodstuffs, 2073/2005/EC. In: Official Journal of the European Union, L 338/1, 22/12/2005.
European Commission, 2010. Commission Regulation of 24 June 2010 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin. In: Official Journal of the European Union, L 159/18, 25/6/2010.
European Commission, 2015. Overview report bivalve molluscs. DG Health and Food Safety. Luxembourg Publications Office of the European Union, 2015. doi:10.2772/64443.
FAO, 2018. Analysis and information on world fish trade. Globefish - The Food and Agriculture Organization of the United Nations. Available from: http://www.fao.org/in-action/globefish/market-reports/resource-detail/en/c/902588/.
Fu K, Li J, Wang Y, Liu J, Yan H, Shi L, Zhou L, 2016. An innovative method for rapid identification and detection of Vibrio alginolyticus in different infection models. Front Microbiol 7:651.
Fukui Y, Saitoh S-I, Sawabe T, 2010. Environmental determinants correlated to Vibrio harveyi-mediated death of marine gastropods. Environ Microbiol 12:124-33.
Harriague A, Covazzi Brino MD, Zampini M, Albertelli G, Pruzzo C, Misic C, 2008. Vibrios in association with sedimentary crustaceans in three beaches of the northern Adriatic Sea (Italy). Marine Poll Bull 56:574-79.
ISO, 2001. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide. International Standardization Organization ed., Geneva, Switzerland.
Jones JL, Lüdeke CHM, Bowers JC, DeRosia-Banick K, Carey DH, Hastbacke W, 2014. Abundance of Vibrio cholerae, V. vulnificus, and V. parahaemolyticus in oysters (Crassostrea virginica) and clams (Mercenaria mercenaria) from Long Island Sound. Appl Environ Microbiol 80:7667-72.
Luo X, Yu R-C, Wang X-J. Zhou M-J, 2012. Toxin composition and toxicity dynamics of marine gastropod Nassarius spp. collected from Lianyungang, China. Food Addit Contam 29:117-27.
Modica MV, Holford M, 2010. The Neogastropoda: Evolutionary Innovations of Predatory Marine Snails with Remarkable Pharmacological Potential. In: Evolutionary Biology. Concepts, Molecular and Morphological Evolution. Pontarotti P, ed. Springer-Verlag, Berlin, Heidelberg. pp 249-70.
Mustapha S, Mustapha EM, Nozha C, 2013. Vibrio Alginolyticus: an emerging pathogen of foodborne diseases. Int J Sci Technol 3:302-9.
Passalacqua PL, Zavatta E, Bignami G, Serraino A, Serratore P, 2016. Occurrence of Vibrio parahaemolyticus, Vibrio cholerae and Vibrio vulnificus in the clam Ruditapes philippinarum (Adams & Reeve, 1850) from Emilia Romagna and Sardinia, Italy. Ital J Food Safety 5:5709.
Polidori P, Grati F, Bolognini L, Domenichetti F, Scarcella G, Fabi G, 2015. Towards a better management of Nassarius mutabilis (Linnaeus, 1758): biometric and biological integrative study. Acta Adriat 56:233-44.
Rodriguez P, Alfonso A, Vale C, Alfonso C, Vale P, Tellez A, Botana LM, 2008. First toxicity report of tetrodotoxin and 5,6,11-trideoxyTTX in the trumpet shell Charonia lampas lampas in Europe. Anal Chem 80:5622-9.
Serratore P, Ostanello F, Passalacqua PL, Zavatta E, Bignami G, Serraino A, Giacometti F, 2016. First multi-year retrospective study on Vibrio parahaemolyticus, and Vibrio vulnificus prevalence in Ruditapes philippinarum harvested in Sacca di Goro, Italy. Ital J Food Safety 5: 6161.
Silva M, Azevedo J, Rodriguez P, Alfonso A, Botana LM, Vasconcelos V, 2012. New gastropod vectors and tetrodotoxin potential expansion in temperate waters of the Atlantic Ocean. Mar Drugs 10:712-26.
Spanier E, Karmon N, 1987. Muricid snails and the ancient dye industries. In: The royal purple and the biblical blue: argaman and tekhelet. Spanier E ed. Keter Publishing House, Jerusalem, pp 179-92.
Liu-yang Shi, Shuang Liang, Xuan Luo, Cai-huan Ke & Jing Zhao, 2017. Microbial community of Pacific abalone (Haliotis discus hannai) juveniles during a disease outbreak in South China. Aquacult Res 48:1080-88.
Turner AD, Tarnovius S, Goya AB, 2014. Paralytic shellfish toxins in the marine gastropods Zidona dufresnei and Adelomelon beckii from Argentina: toxicity and toxin profiles. J Shellfish Res 33:519-30.
Vasconcelos P, Moura P, Barroso CM, Gaspar MB, 2012. Reproductive cycle of Bolinus brandaris (Gastropoda: Muricidae) in the Ria Formosa lagoon (southern Portugal). Aquatic Biol 16:69-83.
West DJ, Andrews EB, Bowman D, McVean AR, Thorndyke MC, 1996. Toxins from some poisonous and venomous marine snails. Comp Biochem Physiol 113:l-10.