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41 Abstract

The capacity of coast recovering after interruption of pollution sources has been explored employing mussels as biomarkers. In an area polluted by sewage sludge in Puerto Madryn (Argentina), abnormally high cytogenetic records (micronuclei) had been detected in the mussel *Mytilus edulis*, even higher than those obtained in this and other mussel species (*Brachydontes rodriguezi*, *Aulacomya atra atra*, *Perumytilus purpuratus*) sampled from heavily polluted industrial areas, and much higher than those recorded in samples from unpolluted areas of the same region. Normal cytogenetic patterns were recovered in Puerto Madryn less than 1 year after cessation of sewage sludge discharges, without additional treatment of the affected area. This discovery opens the possibility of considering restored coastal areas for aquaculture purposes instead of endangering natural populations in virgin areas.

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NOTE

Recovery of Normal Cytogenetic Records in Mussels After Cessation of Pollutant Effluents in Puerto Madryn (Patagonia, Argentina)

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Abstract The capacity of coast recovering after interruption of pollution sources has been explored employing mussels as biomarkers. In an area polluted by sewage sludge in Puerto Madryn (Argentina), abnormally high cytogenetic records (micronuclei) had been detected in the mussel *Mytilus edulis*, even higher than those obtained in this and other mussel species (*Brachydontes rodriguezi*, *Aulacomya atra atra*, *Perumytilus purpuratus*) sampled from heavily polluted industrial areas, and much higher than those recorded in samples from unpolluted areas of the same region. Normal cytogenetic patterns were recovered in Puerto Madryn less than 1 year after cessation of sewage sludge discharges, without additional treatment of the affected area. This discovery opens the possibility of considering restored coastal areas for aquaculture purposes instead of endangering natural populations in virgin areas.

Keywords Ecosystem recovery · Micronucleus test · Mollusks · Monitoring · *Mytilidae*

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Introduction

Scientific analyses are critical to the ongoing effort to understand events and to improve guidelines for pollution control (Woodfield et al. 2003; Moss et al. 2005). Although recovery of marine ecosystems from pollution has tended to receive less attention than the study of new or continuing impacts, such studies are important to deal with chronic contamination (Hawkins et al. 2002). As pollution load decreases, responses would be expected at the molecular and cellular levels. These should be detectable by the growing battery of sensitive biomarkers (Depledge 1999). Among them, the micronuclei (MN) test has been applied in surveys for detection of mutagens in water ecosystems employing mussels as target species (Scarpato et al. 1990; Mersch and Beauvais 1997; Izquierdo et al. 2003).

Disposal of sewage sludge in the marine environment has been practiced globally and affects the seafloor and its biota even in apparently clean areas (Costello and Read 1994; Studholme et al. 1995). Puerto Madryn city, located in the protected area of Peninsula Valdes, is one of the most relevant touristic points in Patagonia, principally for its marine fauna. Evidence of contamination by urban effluents had been detected in the beach in the year 2000 employing mussels as bioindicators (Izquierdo et al. 2003). Those effluents were interrupted later due to the construction of a system for urban wastes disposal.

The aim of this study was to evaluate the capacity of environmental recovery after cessation of urban discharges in Puerto Madryn, for exploring the possibility of re-utilizing formerly polluted sites for purposes of mussel culture. To discard the general effects of diffuse pollution in the region as a source of variation in the results obtained, two additional sites were studied as controls: a positive control subjected to intense pollution by discharge of industrial effluents and a negative control sampled in a pristine area without human activity. The micronucleus test in all mussel species present in the selected areas was chosen as a tool to monitor environmental health. Other authors used jointly MN tests and evaluation of DNA strand breaks, allowing the detection of a recent exposure (Bolognesi et al. 2004). We have chosen the MN test based on its simplicity, ease, and low cost (Sanchez-Galan et al. 1998), for encouraging its future use in long-term monitoring in the area, where systematic pollution monitoring does not exist at the present.

Materials and Methods

Sampling Locations and Characterization

Site 1. Puerto Madryn beach. In the beach of Puerto Madryn (Patagonia, Argentina, 43° S 65° W), urban effluents (sewage outfall) had been discharged without treatment until September 2001, when they were interrupted. Further cleaning of the affected area was not carried out.

Site 2. Positive control. A neighboring beach with industrial effluents (fish-processing enterprise discharges) was chosen as a positive control site with conspicuous pollution.

Site 3. Negative control. Punta Este beach, 14 km east from Puerto Madryn, was chosen as an unpolluted negative control. This point can be considered completely clean because it is a virgin beach without urban, industrial, or other wastes.

The three locations considered are shown in a map (Fig. 1). Although there is no public information available about water quality in the two control sites 2 and 3, heavy pollution (site 2) and high water quality, respectively, are evident. Data on some physical–chemical parameters monitored in site 1 before and after interruption of sludge discharge are shown in Table 1. They were provided by the Municipality of Puerto Madryn and corresponded to official records of water quality obtained five times during the 15 months previous to the interruption of discharge and one time 15 months after.

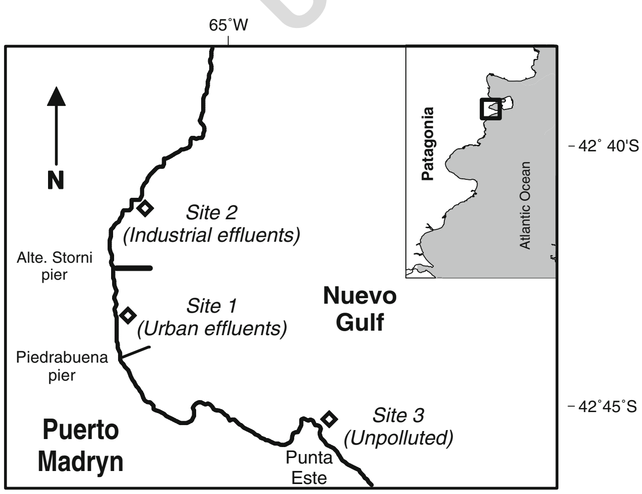

Fig. 1 Map showing the study area and the three sampling locations (Patagonia, Argentina)

Target Species and Sampling Schedule

Mussel individuals (sample size, 12 individuals per species and sampling point) of similar sizes (major axis 3.4± 1.2 cm) were sampled from each site. Gamete production was not observed in any case. *Mytilus edulis* was the only species found in site 1. In site 2 and site 3, four *Mytilidae* species were present: *Brachydontes rodriguezi*, *Aulacomya atra atra*, *Perumytilus purpuratus*, and *M. edulis.* All mussels sampled were immediately transported to the laboratory alive in marine water, to avoid desiccation and anoxia conditions, then processed for slide preparations.

In site 1, samples were obtained just before the interruption of the sewage discharge (time 0, September 2001) and 6 (time 1) and 12 (time 2) months after the interruption.

Tissue Sampling and Slide Preparation

We analyzed MN in the subpopulation of cells prevailing in gill tissue, following Scarpato et al. (1990). A portion of gill was removed with tweezers and dragged along a slide in a single layer of well-spread cells, then allowed to dry for a few minutes. Two slides per animal were prepared.

Staining procedure followed Ayllon and Garcia-Vazquez (2000). Briefly, slides were sequentially stained with May–Grünwald for 2 min; May–Grünwald/distilled water 1:1 for 3 min; and Giemsa/distilled water 1:6 for 10 min; then rinsed with distilled water, allowed to dry, and mounted with Eukitt.

For each animal, 1,000 main gill cells (500 per slide whenever possible) were scored under ×1,000 magnification to determine the frequency of micronucleated cells. Coded slides were randomly sorted and scored by a single observer.

Statistical Analysis

Micronuclei frequencies were expressed per 1,000 cells (per mill). ANOVA tests were employed to compare MN frequencies between sampling sites and species. Statistical analyses were carried out with the SPSS 8.0 program (SPSS Inc.) for PC computers.

Table 1 Physico-chemical characteristics of sea water from site 1 (before and after, 16 months prior, and consecutive to the cessation of sewage discharge, respectively)

Parameter	Site 1 (before)	Site 1 (after)
pH	6.75 (1.25)	8.8
Conductivity ($\mu\Omega$ cm^{-1})	1,406 (433)	958
Dissolved oxygen (mg l^{-1})	4.8 (4.7)	9
BOD (mg l^{-1})	1,12.5 (27.5)	78
DRP (mg l^{-1})	7.7 (0.0)	4.43

Average (standard deviation) of five and one records before and after discharge interruption, respectively

BOD biochemical oxygen demand, *DRP* dissolved reactive phosphorus

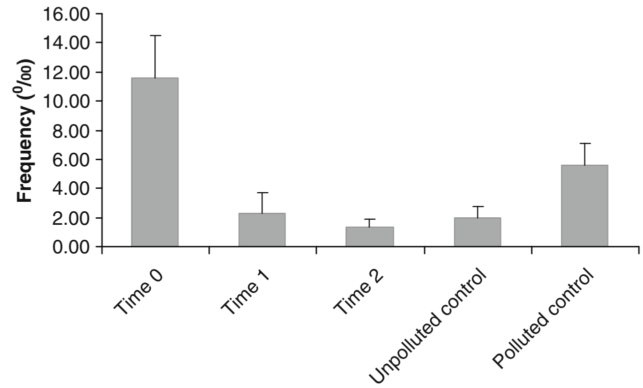

Fig. 3 *Mytilus edulis*. Mean micronuclei frequency (per mill) and standard deviation before (time 0) and after 6 (time 1) and 12 (time 2) months of the urban effluent interruption (September 2001), and in the unpolluted and polluted control areas (sites 3 and 2, respectively)

Results

Micronuclei frequencies for the different species in the polluted and unpolluted controls (sites 2 and 3, respectively) are shown in Fig. 2. Micronuclei averages ranged from 1.5 (*P. purpuratus*, site 3) to 7.3 (*A. atra atra*, site 2). Micronuclei frequencies were significantly higher for individuals exposed to industrial effluents (site 2) than for unpolluted controls (site 3) for all four species considered (P<0.001 in all cases). The four Mytilidae species were similarly sensitive to in situ pollution. Between species, significant differences were not found for MN frequency in the polluted (site 2) and in the unpolluted (site 3) stations (P=0.065 and 0.3974, respectively). The micronucleus test was sensitive enough for detecting pollution.

With respect to the urban sewage sludge, it was associated with noticeable nuclear damage in mussels. MN frequencies found for *M. edulis* sampled in site 1 before and after the interruption of urban effluents discharges (times 0 to 2) are shown in Fig. 3, together with the average MN frequencies found in the control polluted and unpolluted sites (sites 2 and 3, respectively). The mean frequency of micronuclei scored in site 1 when the area was still subjected to pollutant discharges was 11.58$^0/_{00}$, as previously reported by Izquierdo et al. (2003) in the same area. This value was even higher than the average record obtained for this species near the heavily polluted industrial effluent in site 2. MN frequency significantly declined in the urban area to 2.29$^0/_{00}$ and 1.38$^0/_{00}$ at 6 and 12 months, respectively, after discharge interruption (P<0.01). The MN average obtained for the *M. edulis* samples after 12 months from the cessation of the discharges was significantly lower (P<0.001) than that obtained for the polluted control (site 2), and not significantly different in MN frequencies of *M. edulis* sampled from the unpolluted site 3 (P=0.085). Although it is not possible to evaluate the *M. edulis* MN base level in the area due to the lack of records previous to the beginning of the effluents, as MN frequency was similar in time 1, time 2, and unpolluted control, we could roughly take the average value (1.9$^0/_{00}$) as the base level.

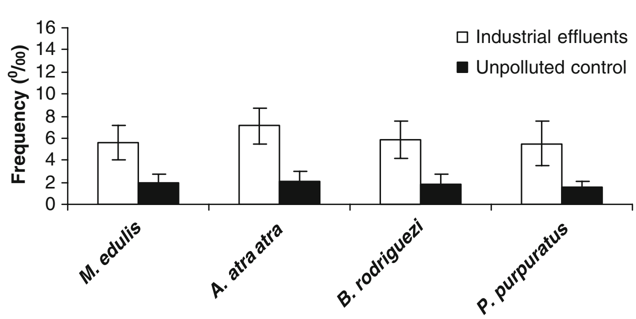

Fig. 2 Mean micronuclei frequency (per mill) and standard deviation for the four *Mytilidae* species sampled near the industrial effluents (site 2, *white bars*) and in the control unpolluted beach Punta Este (site 3, *black bars*)

Decreased MN frequencies after effluent cessation were consistent with improved values of physical–chemical parameters in seawater after the interruption of the urban discharge (Table 1), as revealed by increased dissolved oxygen and decreased biological oxygen demand and dissolved reactive phosphorus (signals of eutrophication).

Discussion

The most remarkable result in this study was a rapid recovery of normal cytogenetic records in a mussel species (*M. edulis*) after interruption of urban discharges, without any additional management strategies such as cleaning the affected area or others. In controlled conditions, Majone et al. (1987) found persistent increased micronuclei frequencies in mussels for more than 1 month after interrupting treatment with mitomycin C. Our results showed that less than 6 months was enough to allow mussels to recover baseline micronucleus frequencies after cessation of urban effluents. This result is encouraging because it demonstrates that genotoxic damage, detected by micronuclei tests, can revert spontaneously in the wild by simple interruption of the pollutant source.

We are aware that the main shortcoming of the paper is the lack of details on the pollution status of the sampling sites due to the fact that there are no pollution surveys in this coast. Only some physiochemical data, such as pH and biochemical oxygen demand, have been measured one time after the recovery intervention. Previous evaluation of water quality, where only bacterial records were made, reported total and fecal coliforms in the area (Izquierdo et al. 2003), those values exceeding the allowed sanitary levels in 91% water samples taken along the year before effluent cessation.

Genotoxicity biomarkers must be an integral part of the suite of biomarkers considered as exposure to genotoxic agents may exert a damage beyond that of the individual and may be active through several generations (Magni et al. 2006). This study provides some additional information useful for pollution-biomonitoring purposes. First, as the effect of pollution on cytogenetic abnormalities can revert in less than 6 months, periodic surveys should be considered in shorter periods (may be monthly) if MN tests are intended to be employed for routine monitoring of coastal ecosystems (Smolders et al. 2002; Izquierdo et al. 2003); otherwise, sporadic pollution events would remain undetected. Moreover, high levels of inter-individual variability of the responses of the aquatic organisms should be considered before applying these biomarkers for routine monitoring (Burgeot et al. 1996; Bolognesi et al. 2004). Seasonality is one of the factors accounting for biomarker variation (Solé et al. 1995; Bolognesi et al. 2004) and should also be considered, for example, in implementing year-round biomonitorization protocols. Finally, in the present study, micronuclei frequencies were very similar for the four Mytilidae species considered in each site (with higher and lower abnormality records in polluted and unpolluted sites, respectively). Therefore, although *M. edulis* is more popular for cytogenetic studies, any of them could be a potential bioindicator. Although species-specific characteristics have to be generally considered when monitoring the health status and possible toxic effects of the contaminant load in marine animals (Nyman et al. 2003), the cosmopolite group of mussel species could be considered collectively as a suitable universal indicator for in situ biomonitoring of coastal pollution. Local species could be employed to monitor coastal areas, avoiding risks of genetic introgression associated to specimens transfer to the monitoring sites (García-Vázquez et al. 2007), as would be required if only one target species is considered (Mersch and Beauvais 1997).

Bivalve aquaculture initiatives are promoted by the Argentine government, with interest of the private sector for the culture of the flat oyster *Ostrea puelchana* and the mussel *M. edulis*. Aquaculture of mussels has been proposed as an alternative or complement of artisanal shellfish harvest (Narvarte et al. 2007). The location of new hatcheries is one of the first points under consideration. The Patagonian coastline is almost totally unpolluted except near urban settlements (Gil et al. 1999; Izquierdo et al. 2003), and aquaculture may endanger virgin locations, some of them declared natural reserves and protected areas like the nearby Peninsula Valdes. Alternative usage of sites already anthropized could be envisaged if environmental conditions in those sites are safe and pollution does not compromise hatchery productivity and consumer health. The results found in this study may open the possibility of envisaging the use of anthropized areas for mussel culture after interrupting pollutants. However, many other analyses are necessary before starting aquaculture in formerly polluted areas, even if they are apparently clean. Persistent chemicals such as heavy metals, PCB, and specific pesticides produce an unpredictable long-term hazard in the marine environment. Evidence of long-term adverse effects of pollution in marine animals, for example heavy metals, has been demonstrated (Domingo 1994). Although from our results it seems evident that cytogenetic damage revealed with micronucleus test was a reversible process, additional complementary tests to evaluate long-term chronic ecotoxicological effects, largely unknown for aquatic biota until now (Fent 2004), should also be envisaged.

Another issue can be the effect of mussel aquaculture on the recuperated environment. Aquaculture has enormous economic potential but its main drawback is the phosphorus pollution it generates. In Table 1, we can observe that dissolved reactive phosphorus has been reduced by almost one half, from a level even higher than that existing in closed systems like marine aquariums down to a level which ranges within normal values (Trépanier et al. 2002). If aquaculture facilities were installed in the area, phosphorus load would increase again with subsequent eutrophication risks (Read and Fernandes 2003). Other impacts of similar nature can also be expected derived from the metabolic activity of cultured mussels. On the other hand, aquaculture encompasses a risk of disturbance of the existing wild community. The perceived risks are often associated with interactions between cultured and native stocks, and the adverse effects to ecosystems; public health issues are also a matter of concern (Svåsand et al. 2007). The siting of aquaculture facilities plays a major role in determining the impact of farmed stocks on wild populations (Triantafyllidis et al. 2007). Escapes or introgression of domestic individuals in wild populations is not the only risk associated with shellfish cultivation: human-induced disturbance from farming operations may also contribute to the biological patterns around mollusk cultures (Forrest and Creese 2006). A balance between socioeconomic benefits and environmental issues should be reached for sustainable shellfish culture.

In conclusion, this monitoring study demonstrated that the cessation of the marine disposal of sewage sludge was enough to allow recovery of normal cytogenetic records (micronucleus test) in mussels in 1 year, without additional treatment of the affected area. Although a combination of different indicators should be employed before considering the area totally recovered, this result is encouraging for opening the possibility of usage of recovered coastal systems as mollusk aquaculture sites.

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References

Ayllon, F. and E. Garcia-Vazquez. 2000. Induction of micronuclei and other nuclear abnormalities in European minnow *Phoxinus phoxinus* and mollie *Poecilia latipinna*: an assessment of the fish micronucleus test. *Mutation Research* 467: 177–186.

Bolognesi, C., G. Frenzilli, C. Lasagna, E. Perrone, and P. Roggieri. 2004. Genotoxicity biomarkers in *Mytilus galloprovincialis*: wild versus caged mussels. *Mutation Research* 552: 153–162. doi:10.1016/j.mrfmmm.2004.06.012.

Burgeot, T., S. Woll, and F. Galgani. 1996. Evaluation of the micronucleus test on *Mytilus galloprovincialis* for monitoring applications along the French coasts. *Marine Pollution Bulletin* 32: 39–46. doi:10.1016/0025-326X(96)89526-9.

Costello, M.I. and P. Read. 1994. Toxicity of sewage sludge to marine organisms: a review. *Marine Environmental Research* 37: 23–46. doi:10.1016/0141-1136(94)90061-2.

Depledge, M.H. 1999. Recovery of ecosystems and their components following exposure to pollution. *Journal of Aquatic Ecosystem Stress and Recovery* 6: 199–206. doi:10.1023/A:1009927201663.

Domingo, J.L. 1994. Metal-induced developmental toxicity in mammals: a review. *Journal of Toxicology and Environmental Health* 42: 123–141.

Fent, K. 2004. Ecotoxicological effects at contaminated sites. *Toxicology* 205: 223–240. doi:10.1016/j.tox.2004.06.060.

Forrest, B.M. and R.G. Creese. 2006. Benthic impacts of intertidal oyster culture, with consideration of taxonomic sufficiency. *Environmental Monitoring and Assessment* 112: 159–176. doi:10.1007/s10661-006-0359-3.

García-Vázquez, E., Danancher, D., Marteinsdottir, G., and Svåsand T.s.e. 2007. Monitoring tools for evaluation of genetic impact of aquaculture activities on wild populations. In: *Genetic impact of aquaculture activities on native populations. Genimpact final scientific report*, eds. T Svåsand, D Crosetti, E García-Vázquez, E Verspoor, 27–140. (EU contract n. RICA-CT-2005-022802). 176 p. http://genimpact.imr.no/.

Gil, M., M. Harvey, and J.L. Esteves. 1999. Heavy Metals in intertidal surface sediments from Patagonian coast, Argentina. *Bulletin of Environmental Contamination & Toxicology* 63: 52–58. doi:10.1007/s001289900947.

Hawkins, S.J., P.E. Gibbs, N.D. Pope, G.R. Burt, B.S. Chesman, S. Bray, S.V. Proud, S.K. Spence, A.J. Southward, and W.J. Langston. 2002. Recovery of polluted ecosystems: the case for long-term studies. *Marine Environmental Research* 54: 215–222. doi:10.1016/S0141-1136(02)00117-4.

Izquierdo, J.I., G. Machado-Schiaffino, F. Ayllon, V.L. D'Amico, L.O. Bala, E. Vallarino, R. Elias, and E. Garcia-Vazquez. 2003. Assessing polution in coastal ecosystems: a preliminary survey using the micronuclei test in the mussel *Mytilus edulis*. *Ecotoxicology and Environmental Safety* 55: 24–29. doi:10.1016/S0147-6513(02)00041-6.

Magni, P., G. De Falco, C. Falugi, M. Franzoni, M. Monteverde, E. Perrone, M. Sgro, and C. Bolognesi. 2006. Genotoxicity biomarkers and acetylcholinesterase activity in natural populations of *Mytilus galloprovincialis* along a pollution gradient in the Gulf of Oristano (Sardinia, western Mediterranean). *Environmental Pollution* 142: 65–72. doi:10.1016/j.envpol.2005.09.018.

Majone, F., R. Brunetti, I. Gola, and A. Levis. 1987. Persistence of micronuclei in the marine mussel, *Mytilus galloprovincialis*, after treatment with mytomicin C. *Mutation Research* 191: 157–161. doi:10.1016/0165-7992(87)90147-3.

Mersch, J. and M.-N. Beauvais. 1997. The micronucleus assay in the zebra mussel, *Dreissena polymorpha*, to in situ monitor genotoxicity in freshwater environments. *Mutation Research* 393: 141–149.

Moss, A., J. Brodie, and M. Furnas. 2005. Water quality guidelines for the Great Barrier Reef World Heritage area: a basis for development and preliminary values. *Marine Pollution Bulletin* 51: 76–88. doi:10.1016/j.marpolbul.2004.10.052.

Narvarte, M., R. Gonzalez, and P. Filippo. 2007. Artisanal mollusk fisheries in San Matias Gulf (Patagonia, Argentina): an appraisal of the factors contributing to unsustainability. *Fisheries Research* 87: 68–76. doi:10.1016/j.fishres.2007.06.012.

Nyman, M., M. Bergknut, M.L. Fant, H. Raunio, M. Jestoi, C. Bengs, A. Murk, J. Koistinen, C. Backman, O. Pelkonen, M. Tysklind, T. Hirvi, and E. Helle. 2003. Contaminant exposure and effects in Baltic ringed and grey seals as assessed by biomarkers. *Marine Environmental Research* 55: 73–99. doi:10.1016/S0141-1136(02)00218-0.

Read, P. and T. Fernandes. 2003. Management of environmental impacts of marine aquaculture in Europe. *Aquaculture* 226: 139–163. doi:10.1016/S0044-8486(03)00474-5.

Sanchez-Galan, S., A.R. Linde, J.I. Izquierdo, and E. Garcia-Vazquez. 1998. Micronuclei and fluctuating asymmetry in brown trout (*Salmo trutta*): complementary methods to biomonitor freshwater ecosystems. *Mutation Research* 412: 219–225.

Scarpato, R., L. Migliore, G. Alfinito-Cognetti, and R. Barale. 1990. Induction of micronuclei in gill tissue of *Mytilus galloprovincialis* exposed to polluted marine waters. *Marine Pollution Bulletin* 21: 74–80. doi:10.1016/0025-326X(90)90191-A.

Smolders, R., L. Bervoets, and R. Blust. 2002. Transplanted zebra mussels (*Dreissena polymorpha*) as active biomonitors in an effluent-dominated river. *Environmental Toxicology and Chemistry* 21: 1889–1896. doi:10.1897/1551-5028(2002)021<1889:TZMDPA>2.0.CO;2.

Solé, M., C. Porte, and J. Albaigés. 1995. Seasonal variation in the mixed function oxygenase system and antioxidant enzymes of the mussel *Mytilus galloprovincialis*. *Environmental Toxicology* *and Chemistry* 14: 157–164. doi:10.1897/1552-8618(1995)14[157:SVITMO]2.0.CO;2.

Studholme, A.L., O'Reilly, J.E., and Ingham, M.C. 1995. Effects of cessation of sewage sludge dumping at the 12-mile site. p. 257. NOAA Technical Report NMFS 124.

Svåsand, T., Crosetti, D., García-Vázquez, E., Triantafyllidis, A., and Verspoor, E.E. 2007. Symposium report. The international symposium on genetic impacts from aquaculture: meeting the challenge in Europe, 1–4 July 2007. Genimpact (EU contract n. RICA-CT-2005-022802) (http://genimpact.imr.no).

Trépanier, C., S. Parent, Y. Comeau, and J. Bouvrette. 2002. Phosphorus budget as a water quality management tool for closed aquatic mesocosms. *Water Research* 36: 1007–1017. doi:10.1016/S0043-1354(01)00286-X.

Triantafyllidis, A., Karaiskou, N., Bonhomme, F., Colombo, L., Crosetti, D., Danancher, D., García-Vázquez, E., Gilbey, J., Svåsand, T., Verspoor, E., and Triantaphyllidis, C. 2007. Management options to reduce genetic impacts of aquaculture activities. In *Genetic impact of aquaculture activities on native populations. Genimpact final scientific report*, eds. T Svåsand, D Crosetti, E García-Vázquez, E Verspoor, 161–174. (EU contract n. RICA-CT-2005-022802). 176 p. http://genimpact.imr.no/.

Woodfield, N.K., J.W. Longhurst, C.I. Beattie, and D.P. Laxen. 2003. Critical evaluation of the role of scientific analysis in UK local authority AQMA decision-making: method development and preliminary results. *Science of the Total Environment* 311: 1–18. doi:10.1016/S0048-9697(02)00675-7.

AUTHOR QUERIES

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