



Invasion note

Jumping ship: a stepping stone event mediating transfer of a non-indigenous species via a potentially unsuitable environment

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Abstract

The smooth shelled blue mussel, *Mytilus galloprovincialis* Lmk (Bivalvia: Mollusca) arrived in Pearl Harbor, Oahu, Hawai'i on 22 June 1998 as a member of the fouling community of the USS Missouri, and mussel spawning activity was observed within 2 h of the vessel's arrival. Small mussels (<10 mm shell length, approximately 6 weeks post-metamorphosis) were collected on 30 September 1998 from a submarine ballast tank in Pearl Harbor, indicating that a successful recruitment event had taken place very soon after the first arrival of the species at this location. We suggest that even if *M. galloprovincialis* is not able to establish permanently within Pearl Harbor, the fact that it has been able to successfully spawn and recruit to another shipping vector within the Harbor indicates that a 'stepping stone' model of range expansion from temperate to temperate region via an intermediary subtropical environment is quite feasible for this species. Data from worldwide distributions of mussels of the family Mytilidae indicate that preferred habitats are eutrophic continental shelf regions, which suggests that successful establishment within Pearl Harbor is possible. However, oceanic coral-reef environments are not preferred habitat types, suggesting that *M. galloprovincialis* is not likely to become widely distributed in the Hawaiian Islands.

Introduction

The present paper describes an example of a novel stepping stone mechanism of invasion by which a species can move from one environment, via a non-colonizable environment, to a new and potentially hospitable environment. Adult smooth shelled blue mussels (*Mytilus galloprovincialis*) from northwest North America (a cool temperate environment) were identified as invaders into the subtropical environment of Pearl Harbor, Oahu, Hawai'i. Subsequent to this, we identified a successful recruitment event of *M. galloprovincialis* to another vessel in Pearl Harbor. The spawning event and the subsequent successful

recruitment event to another potential vector indicate that even if *M. galloprovincialis* does not become established permanently in Pearl Harbor it is possible for this species to use the locality as a stepping stone for further invasions. The stepping stone mechanism of invasion reported here illustrates how important prevention of even temporary invasions can be to the overall processes involved in anthropogenic introductions worldwide.

M. galloprovincialis Lamarck is one of three species of smooth shelled blue mussels, the other two being *M. edulis* Linnaeus and *M. trossulus* Gould. *M. galloprovincialis* is the most widely distributed of the three species and typically occurs in warm

temperate regions of full salinity. It is found naturally on Atlantic coasts of southwest Europe (Gardner 1992, 1994; Seed 1992), in the Mediterranean Sea (Quesada et al. 1995), and in Australia and New Zealand (McDonald et al. 1991; Hilbish et al. 2000). This species has a history of successful invasions into new regions. Known or putative records of invasions include Japan (Wilkins et al. 1983), Hong Kong (Lee and Morton 1985), South Africa (Grant and Cherry 1985), and California (Geller 1999). Evidence (which is sometimes circumstantial) indicates that all of these invasions have occurred within the last 150 years, and most of them during the 20th century. The vector of introduction of *M. galloprovincialis* into these regions is suspected to be hull fouling and/or ballast water. This species, like so many other marine invertebrates, is a member of the fouling community, has a larval life duration of approximately 4 weeks, and is tolerant of environment stress. These attributes mean that it has the potential to be widely distributed as an invader by shipping (Carlton 1989; Carlton and Geller 1993).

Evidence from these invaded locations indicates that *M. galloprovincialis* is a competitively dominant species and is likely to have either established itself alongside pre-existing competitors or to have displaced an established species. For example, there is no record of *M. galloprovincialis* from Japan prior to the 1930s but it now occurs extensively throughout much of the country (Wilkins et al. 1983), and similarly, there is no record of this species from South Africa prior to the 1960s but it is now widely distributed throughout the region. An analysis of length–frequency distributions of smooth shelled blue mussels in Victoria Harbour, Hong Kong, indicated that *M. galloprovincialis* was probably introduced into this subtropical region in 1981 (Lee and Morton 1985). More recent evidence based upon molecular analysis of museum material indicates that the extensive populations of *M. galloprovincialis* throughout California have become established since the late 1800s and have competitively excluded the native *M. trossulus* from much of its southern range on the Pacific coast of North America (Geller 1999). Although exact details are unknown, it appears that once established this species can spread extensively from the initial point source(s) of introduction. Thus, based on information from a variety of different geographic locations, *M. galloprovincialis* should be viewed as an important marine invasive species with the ability to readily become established and/or to

out-compete native species in many warm temperate regions and also some subtropical regions of the world.

Materials and methods

Mussel collection

Three large mussels (each \approx 90 mm shell length) were collected from the hull fouling community of the *USS Missouri*, when the vessel arrived in Pearl Harbor, Hawai'i on 22 June 1998 (Bishop Museum collection number BPBM MO254424). Small mussels (in the range 2–8 mm shell length) were collected from the ballast tank of a US Navy submarine in Pearl Harbor, Hawai'i on 30 September 1998 (Bishop Museum collection number BPBM 257065). Ethanol preserved samples were sent to School of Biological Sciences, Victoria University of Wellington, New Zealand, for analysis. For comparative purposes, specimens of *M. edulis* were received from Wales, UK, *M. edulis* and *M. trossulus* were received from Newfoundland, Canada, and *M. galloprovincialis* were collected from Wellington Harbour, New Zealand.

DNA extraction and PCR amplification

Total DNA was extracted according to Rawson and Hilbish (1995) from entire small mussels (including their shell) or from small tissue samples of larger mussels. DNA was amplified by PCR using the primers JH-5: 5'-GTA GGA ACA AAG CAT GAA CCA-3' and JH-54: 5'-GGG GGG ATA AGT TTT CTT AGG-3', designed from part of the 'giant exon' of the polyphenolic adhesive protein locus (Rawson et al. 1996). PCR products obtained using this primer set can be used to distinguish among *M. edulis*, *M. galloprovincialis*, and *M. trossulus* by their size differences (Rawson et al. 1996). PCR amplification was performed in a final volume of 25 μ l containing 50 ng of total cellular DNA, 0.5 μ M of each primer, 250 μ M of each dNTP, 1.5 mM MgCl₂, 1 \times PCR reaction buffer (20 mM Tris-HCl, pH 8.4; 50 mM KCl), and 0.25 units Taq DNA Polymerase (Gibco BRL). Samples were denatured at 94 °C for 3 min followed by 30 cycles at 94 °C for 40 s, 55 °C for 40 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR products were checked for yield on 1% agarose gels and sized with a 100 bp PCR Molecular Ruler (Biorad) standard.

Results

Nuclear DNA from smooth shelled blue mussels was amplified using primers JH-5 and JH-54 (Rawson and Hilbish 1995). These primers amplify species-specific target fragments which differentiate the three taxa *M. edulis*, *M. trossulus* and *M. galloprovincialis* (Rawson et al. 1996). PCR products were larger than stated in the literature when sized with the 100 bp PCR Molecular Ruler or when compared to the *Msp* I digested pBR322 DNA that was employed by Rawson and Hilbish (1995). The correct size of the amplified DNA regions is obtained after subtracting the length of the two primers (total of $21 + 21 = 42$ bp) from the PCR products. Samples from Wales (UK) exhibited 350 or 380 bp fragments characteristic of *M. edulis*, DNA amplified from mussels from Wellington (New Zealand) resulted in a 300 bp product characteristic of *M. galloprovincialis*, and Newfoundland (Canada) samples of *M. edulis* and *M. trossulus* yielded the 350 bp band characteristic of *M. edulis* or exhibited the 240 bp band characteristic of *M. trossulus* (Figure 1). PCR products of all nine Pearl Harbor mussels were 300 bp in length (in some individuals a very faint 500 bp fragment can also be seen). We therefore conclude that these Pearl Harbor blue mussels are all *M. galloprovincialis*.

It should be noted that all amplifications of the Welsh, Canadian and New Zealand mussels, and of some of the Pearl Harbor mussels, resulted in additional non-specific fragments. These bands gave a weak signal, were between 600 bp and 2 kb in size, and thus did not interfere with the diagnostic bands. The non-specific bands are probably the result of the 3'-end of the JH54 primer not corresponding exactly to the 'giant exon' of the polyphenolic adhesive protein.

Discussion

Before arriving in Pearl Harbor the *USS Missouri* was moored at Bremerton, Puget Sound, Washington, on the Pacific coast of the USA. Bremerton is a military reserve area with a naval decommissioning yard which is in close proximity to a *M. galloprovincialis* aquaculture operation that was probably the source of the hull fouling mussels. In preparation for its voyage to Hawai'i the *USS Missouri* was transferred to Astoria, Oregon, where it was moored for 9 days at a distance of

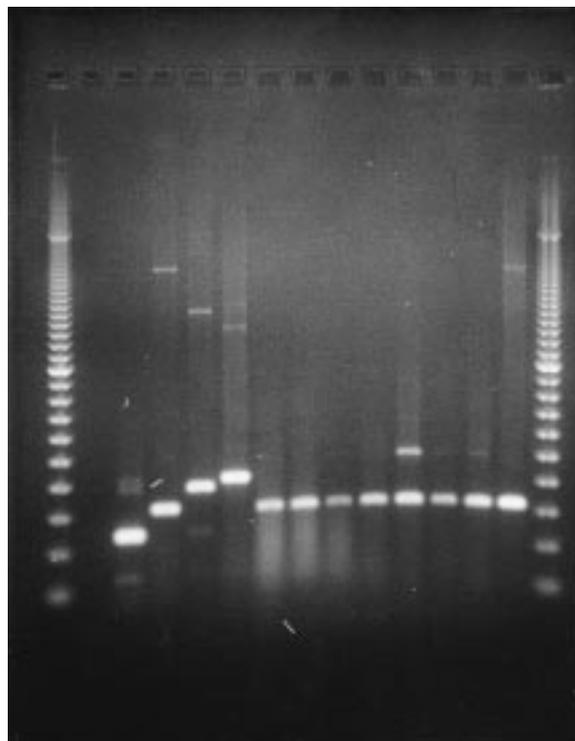


Figure 1. One percent agarose gel showing PCR products characteristic of the three *Mytilus* species. Lanes 1 and 15, 100 bp PCR Molecular Ruler (Biorad); lane 2, negative control; lane 3, *M. trossulus*; lanes 4 and 14, *M. galloprovincialis*; lane 5, *M. edulis* (350 bp fragment); lane 6, *M. edulis* (380 bp fragment); lanes 7–9, large Pearl Harbor *M. galloprovincialis*; lanes 10–13, small Pearl Harbor *M. galloprovincialis*.

19.5 km up the Columbia River in an attempt to prevent biological introductions to Pearl Harbor (Brock et al. 1999). Salinity data recorded from around the *USS Missouri* while moored in the Astoria River indicate that the deepest part of the vessel's hull (at ~ 10 m depth and where mussel fouling was greatest) was subject to salinity in the range 2–10 ppt, i.e., brackish but not fresh water (Belt Collins Hawaii 1998). After this attempt to kill the fouling community the vessel was towed for approximately 2 weeks across the Pacific Ocean before arriving at Pearl Harbor on 22 June 1998. Approximately 2 h after its arrival in Pearl Harbor staff of the Bishop Museum observed spawning activity of mussels associated with the fouling community of the *USS Missouri* (DeFelice and Godwin 1999). Temperature change is a mechanism well known to induce spawning activity in mussels (Buchanan and Babcock 1997; Utting and Spencer 1997) and it seems likely that

mussels which survived the brackish water immersion in Oregon and the trip to Hawai'i were responding in this fashion to the elevated temperature of Pearl Harbor compared with the cooler temperatures of Washington and Oregon. Thus, spawning of *M. galloprovincialis* of the hull fouling community of the *USS Missouri* would have begun almost immediately upon arrival in Pearl Harbor and observations indicate that it is likely to have continued for several weeks, and possibly for a period of months. Water temperature in Pearl Harbor at this time of year is approximately 26 °C (B. Nedved, University of Hawai'i, pers. comm.), a temperature which would reduce the usual larval span of 3–4 weeks to perhaps 2–3 weeks (Chipperfield 1953; Gardner, pers. obs.). Thus, settlement of the first *M. galloprovincialis* spat derived from the fouling community of the *USS Missouri* in Pearl Harbor is unlikely to have occurred before early July 1998.

The newly settled mussels were collected on 30 September 1998 from the ballast tanks of a submarine in dry dock within Pearl Harbor, its home base. The size of these mussels (2–8 mm shell length) indicates that they are very young, probably only 4–6 weeks old given the environmental conditions conducive to rapid bivalve growth in Pearl Harbor. This gives a total age for these animals of about 9 weeks (a likely maximum of 3 weeks larval duration and 6 weeks of juvenile duration). Thus, they must be derived from a successful spawning event which occurred about the last week of July, 1998. This timing is consistent with the continued spawning activity of the hull fouling mussels of the *USS Missouri* approximately 5 weeks after the vessel's arrival in Pearl Harbor. According to naval personnel, the submarine had been in Pearl Harbor for the previous year and its ballast tanks had received a thorough cleaning 4 months prior to the discovery of the juvenile mussels analysed in the present study. The proximity of the submarine docks to the *USS Missouri* (about 1 km apart) and the biological evidence strongly suggest that the newly settled *M. galloprovincialis* collected from the submarine recruited from a successful spawning event of the mussels still attached to the *USS Missouri*.

M. galloprovincialis has had its range extended by shipping traffic into the Pacific subtropical environment of Pearl Harbor, and having reproduced and recruited to another suitable vector within the Harbor (in this case a submarine, but other vectors are just as possible), it could be transported to other localities as an invasive species. The importance of such a 'stepping stone' mechanism of invasion for this species

is unknown, but given the species's history of invasions might well prove to be significant. Invasions of *M. galloprovincialis* are not restricted to warm temperate regions, but include Hong Kong (Lee and Morton, 1985), indicating that the distribution of this species can extend into subtropical environments. Taylor (1997) points out that the number of species of the family Mytilidae is strongly associated with eutrophic environments. For example, there are four to seven times more species on continental shores than on oceanic atolls. Taylor (1997, p.184) notes that 'There are, for instance, twice as many species (of the family Mytilidae) recorded from Hong Kong as for the entire Hawaiian Islands. Mytilid species are frequently major space-occupying organisms on hard substrates on shores in eutrophic areas, but are generally subordinate in oceanic coral-reef environments.' With regard to predicting the possible outcome of the introduction of *M. galloprovincialis* into Pearl Harbor it appears that the eutrophic environment of the harbor itself is possibly a suitable environment for the establishment of permanent populations. However, as the environment changes outside the immediate harbor it is possible that *M. galloprovincialis* will be much less successful in spreading into the oceanic coral-reef habitat, in accordance with Taylor's (1997) general observations.

An effort was made to kill the temperate fouling community of the *USS Missouri* before the vessel was transferred to Pearl Harbor. In their study of the efficacy of the treatment Brock et al. (1999) conclude that the immersion in brackish water and the subsequent transoceanic towing of the vessel proved to be 'extremely effective' in killing the fouling community. However, 11 marine taxa in the fouling community were alive when the vessel arrived in Pearl Harbor (Brock et al. 1999), and as the present paper demonstrates, at least one species in this new environment was able to spawn and successfully recruit to another vector of potential transfer. Eighty-three days after the arrival of the *USS Missouri* all hull fouling organisms were reported to be dead (Brock et al. 1999). We believe that this reflects not only the efficacy of the attempt to kill the fouling community (which was, in fact, only partially successful: Brock et al. 1999) but also the pronounced differences in the ecological and hydrographic conditions in Pearl Harbor compared to the prior habitat of these organisms in the Northwestern USA (a point not mentioned by Brock et al. 1999). The important point of the present study is the identification of a novel mode of translocation of this species, namely

a 'stepping stone' process whereby the species is introduced into an area for sufficient time to allow spawning and the subsequent recruitment to a suitable vector for translocation to another, perhaps more favourable region. Thus, even if *M. galloprovincialis* does not ultimately survive in Pearl Harbor (it has not been reported in several recent surveys of the area), the 'stepping stone' process recorded here illustrates how important prevention of even temporary invasions can be to the overall processes involved in anthropogenic marine introductions worldwide. Furthermore, this study demonstrates the important role of a range of tools (molecular, cytogenetic, etc.) in investigating and reconstructing the histories of biological invasions (Geller 1996; Holland et al. 1999; Holland 2000).

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References

- Belt Collins Hawaii (1998) Findings and conclusions on the use of Port of Astoria Pier 1 for low salinity treatment of marine fouling organisms attached to the ex-*USS Missouri*. Report submitted to the US Coast Guard, 433 Ala Moana Boulevard, Honolulu, HI 96813 by Belt Collins Hawaii, 680 Ala Moana Boulevard, Honolulu, HI 96813, 4 pp
- Brock R, Bailey-Brock JH and Goody J (1999) A case study of efficacy of freshwater immersion in controlling introduction of alien marine fouling communities: the *USS Missouri*. *Pacific Science* 53: 223–231
- Buchanan S and Babcock R (1997) Primary and secondary settlement by the greenshell mussel *Perna canaliculus*. *Journal of Shellfish Research* 16: 71–76
- Carlton JT (1989) Man's role in changing the face of the ocean: biological invasions and implications for conservation of nearshore environments. *Conservation Biology* 3: 265–273
- Carlton JT and Geller JB (1993) Ecological roulette: the global transport and invasion of nonindigenous marine organisms. *Science* 261: 78–82
- Chipperfield PNJ (1953) Observations on the breeding and settlement of *Mytilus edulis* (L.) in British waters. *Journal of the Marine Biological Association of the United Kingdom* 32: 449–476
- DeFelice RC and Godwin LS (1999) Records of marine invertebrates in Hawaii from the hull of the *USS Missouri* in Pearl Harbor, Oahu. *Bishop Museum Occasional Papers*, 59: 42–46
- Gardner JPA (1992) *Mytilus galloprovincialis* (Lamarck) (Bivalvia: Mollusca): the taxonomic status of the Mediterranean mussel. *Ophelia* 35: 219–243
- Gardner JPA (1994) The structure and dynamics of naturally occurring hybrid *Mytilus edulis* Linnaeus, 1758 and *Mytilus galloprovincialis* Lamarck, 1819 (Bivalvia, Mollusca) populations: review and interpretation. *Archiv für Hydrobiologie, Monographische Beiträge* 99: 37–71
- Geller JB (1996) Molecular approaches to the study of marine biological invasions. In: Ferraris JD and Palumbi SR (eds) *Molecular Zoology*, pp 119–132. Wiley-Liss, New York
- Geller JB (1999) Decline of a native mussel masked by sibling species invasion. *Conservation Biology* 13: 661–664
- Grant WS and Cherry MI (1985) *Mytilus galloprovincialis* in southern Africa. *Journal of Experimental Marine Biology and Ecology* 90: 179–191
- Hilbish TJ, Mullinax A, Dolven SI, Meyer A, Koehn RK and Rawson PD (2000) Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): routes and timing of transequatorial migration. *Marine Biology* 136: 69–77
- Holland BS (2000) Genetics of marine bioinvasions. *Hydrobiologia* 420: 63–71
- Holland BS, Gallagher DS, Hicks DW and Davis SK (1999). Cytotaxonomic verification of a non-indigenous marine mussel in the Gulf of Mexico. *Veliger* 42: 280–282
- Lee SY and Morton BS (1985) The introduction of the Mediterranean mussel *Mytilus galloprovincialis* into Hong Kong. *Malacological Review* 18: 107–109
- McDonald JH, Seed R and Koehn RK (1991) Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. *Marine Biology* 111: 323–333
- Quesada H, Zapata C and Alvarez G (1995) A multilocus allozyme discontinuity in the mussel *Mytilus galloprovincialis*: the interaction of ecological and life-history factors. *Marine Ecology Progress Series* 116: 99–115
- Rawson PD and Hilbish TJ (1995) Evolutionary relationships among the male and female mitochondrial DNA lineages in the *Mytilus edulis* species complex. *Molecular Biology and Evolution* 12: 893–901
- Rawson PD, Joyner KL, Meetze K and Hilbish TJ (1996) Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. *Heredity* 77: 599–607
- Seed R (1992) Systematics, evolution and distribution of mussels belonging to the genus *Mytilus*: an overview. *American Malacological Bulletin* 9: 123–137
- Taylor JD (1997) Diversity and structure of tropical Indo-Pacific benthic communities: relation to regimes of nutrient input. In: Ormond RFG, Gage JD and Angel MV (eds) *Marine Biodiversity: Patterns and Processes*, pp 178–200. Cambridge University Press, UK
- Utting SD and Spencer BE (1997) The hatchery culture of bivalve mollusc larvae and juveniles. Laboratory leaflet no. 68. Ministry of Agriculture, Fisheries and Food, Lowestoft, UK, 32 pp
- Wilkins NP, Fujino K and Gosling EM (1983) The Mediterranean mussel *Mytilus galloprovincialis* Lmk. in Japan. *Biological Journal of the Linnean Society* 20: 365–374