GENIMPACT

Evaluation of genetic impact of aquaculture activities on native populations.
A European network

WP1. Genetics of domestication, breeding and enhancement of performance of fish and shellfish

Blue mussel - *M. edulis*

Mediterranean mussel - *M. galloprovincialis*



A. Beaumont[1], T. Gjedrem[2] and P. Moran[3]

[1] University of Wales, Bangor, UK
[2] AKVAFORSK, Ås, Norway
[3] University of Vigo, Vigo, Spain

Beaumont A., Gjedrem T. and Moran P. (2006). Blue Mussel – *M. edulis* and Mediterranean mussel – *M. galloprovincialis*. In: "Genetic effects of domestication, culture and breeding of fish and shellfish, and their impacts on wild populations." D. Crosetti, S. Lapègue, I. Olesen, T. Svaasand (eds). GENIMPACT project: Evaluation of genetic impact of aquaculture activities on native populations. A European network. WP1 workshop "Genetics of domestication, breeding and enhancement of performance of fish and shellfish", Viterbo, Italy, 12-17th June, 2006, 6 pp. http://genimpact.imr.no/

A. Biology, ecology and genetics

Distribution

In Europe there are three species of mussel, all in the genus *Mytilus*: *Mytilus edulis* (blue mussel), *M. galloprovincialis* (Mediterranean mussel) and *M. trossulus* (Baltic mussel) (1, 2). *M. trossulus* is generally thought to be confined to the Baltic (3) and there is no significant fishery or aquaculture involving this species. Because of this, the genetic impact of human activity is negligible and the Baltic mussel will not be considered further in this leaflet. On the other hand there is very extensive mariculture of *M. edulis* and *M. galloprovincialis* almost throughout their distribution. There remains debate about the true taxonomic status of these two "species" because wherever their distributions overlap they can hybridise and their hybrids are fertile. Identification of *M. edulis* and *M. galloprovincialis* (and any hybrids) based on shell shape is usually uncertain because of the extreme plasticity of shape exhibited by mussels under environmental variation. Since 1995 a DNA-based genetic method that seems truly diagnostic for European populations of these two species (4, 5) has become available, but no large scale studies have yet been carried out to characterise the mosaic of populations of *M. edulis*, *M. galloprovincialis* and their hybrids that extend from the French Atlantic coast up to northern Scotland. The knowledge of the distribution of mussel species around Europe, as it was assessed in 1992, is given in Fig. 1 (1). Hybrids occur in regions where the species meet or overlap. Recent anecdotal evidence suggests that *M. galloprovincialis* could be present in the Netherlands, and that *M. edulis* has been introduced into culture on the Mediterranean coast of France.

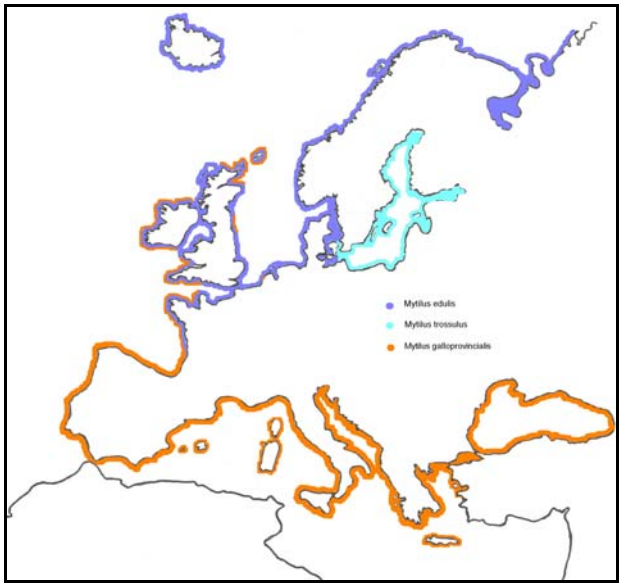


Fig. 1. Approximate distributions of *M. edulis*, *M. galloprovincialis*, and *M. trossulus* in Europe.

Biology

Mussels are bivalve molluscs and much is known of their biology (1) mainly because they have been an easy marine organism to collect and study. The two valves of the shell are equal in shape and size and are held tightly closed by a large posterior adductor muscle when the mussel is exposed to air. Feeding and respiration are carried out via currents of water directed across the gills. Food particles are trapped by cilia on the gills and carried in mucous strings to the mouth but this is a selective process and some particles are rejected as pseudo-faeces before entering the gut. The foot of a mussel is an important organ because it enables the mussel to attach by byssus threads to a solid substrate. Threads can be broken and replaced at will allowing mussels to re-orientate themselves within clumps or in rock cracks or crevices. Byssal attachment is an important, sometimes critical, factor in mussel aquaculture.

Maturation of eggs and sperm takes place in the gonad tissue that develops within the folds of the mantle and, depending on environmental temperature and food availability, mussels may spawn just once or several times each year. The sexes are separate in mussels, but there are no morphological differences between males and females. Even in ripe mussels where the mantle is packed with gametes (eggs or sperm) mantle colour is not always a reliable guide to the sex of an individual because eggs can range in colour from white to orange/pink. Two days after fertilisation, embryos develop into planktonic veliger larvae that are dispersed by ocean currents. Larvae settle after 4 to 8 weeks at a size of 250-300 microns shell length (7) and may have a secondary post-larval dispersal phase by "byssus drifting" up to a size of 2mm shell length (8). Due to their high fecundity, their extensive larval and post-larval dispersal capability, their ability to attach by byssus threads to non-specific substrates and also to one another, and their fast growth, mussels are often a very significant and abundant element of the ecology of many inter-tidal and sub-tidal habitats (1).

As with other bivalves, mussels are affected by "red tides", blooms of certain algae that are poisonous when consumed by humans and induce PSP (Paralytic Shellfish Poison), DSP (Diarrhetic Shellfish Poison) or ASP (Amnesiac Shellfish Poison). Mussels from affected areas cannot be sold and this causes major problems for mussel farming.

Population genetics

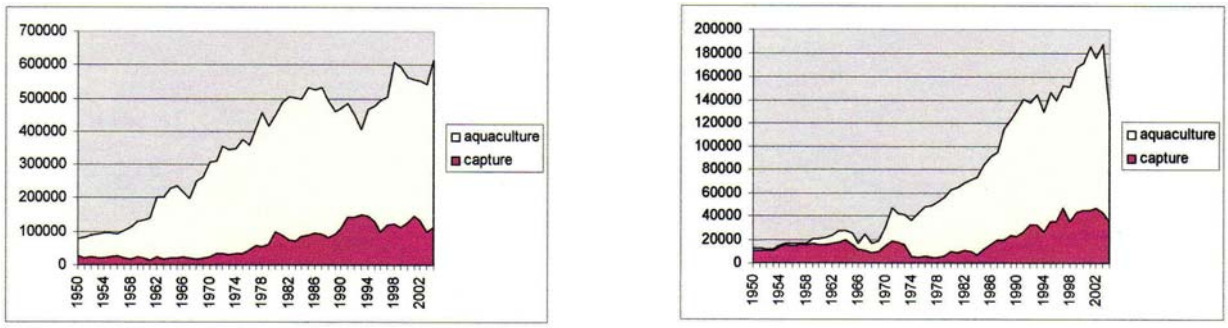
The genetics of mussels have been extensively studied. Allozymes were the main genetic markers used from the 1970s to the 1990s and their use culminated in the recognition of, and the world-wide distribution of, the three *Mytilus* species (2). From the early 1990s genetic studies have tended to focus either the hybrid zones between species (9, 10, 11), or the unusual mode of mitochondrial DNA inheritance (Doubly Uniparental Inheritance) discovered in 1994 (12, 13). Although some nuclear DNA markers have been developed for species identification (5, 14, 15) it is only very recently that any microsatellite loci have been isolated for mussels and these have yet to be employed in any extensive population study (16). The main conclusion from population genetic studies using allozymes was that *M. edulis* was genetically homogeneous throughout its range. On the other hand, *M. galloprovincialis* is genetically subdivided into a Mediterranean group and an Atlantic group with a break point at the well defined Almeria-Oran oceanographic front in the Mediterranean Sea (17).

The true extent of the mosaic of population structure across the huge hybrid zone of these two species is not known in detail except at one or two restricted locations (e.g. 10, 11). However, now that a simple and cheap diagnostic DNA-based marker (4, 5) is available, and some microsatellites have been published, a programme of extensive sampling of mussel populations around Europe is warranted.

B. Breeding and culture practices

Production

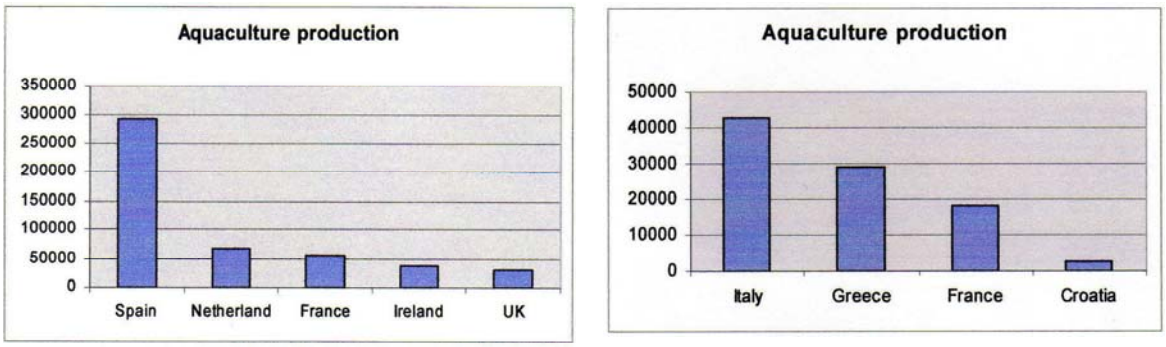
M. edulis and *M. galloprovincialis* are the most important mussel species in Europe. Total European tonnage of aquaculture and capture from 1950 – 2004 are given in Fig. 2. It should be noted that the statistics provided by FAO (6) treat mussels from the Atlantic coast of Spain as *M. edulis*, but, as the distribution map (Fig. 1) shows, the species in this region is *M. galloprovincialis*.



(a) *M. edulis* (b) *M. galloprovincialis*

Fig. 2. Total mussel tonnage in Europe: aquaculture and capture, 1950-2004 (6).

European aquaculture production for 2004 is given for main producing countries in Figure 3. Note that Spanish production given as *M. edulis* in Fig 3(a) should be as *M. galloprovincialis* in Fig. 3(b). Spain is by far the greatest producer of mussels by aquaculture (300,000 tonnes annually), greater than the combined total of other important mussel producing countries such as Netherlands, France, Italy, Ireland and UK (Fig. 3). European countries produced 38% of world production in 2003.



(a) *M. edulis*. (b) *M. galloprovincialis*

Fig. 3. European aquaculture production of mussels in 2004 from main countries (6).

Hatchery practices

Because natural spatfall has generally been sufficient to supply most or all of the mussel farmers' requirements in Europe, there has been little interest in developing hatchery culture of mussels. There is at least one commercial hatchery in New Zealand that regularly provides rope culture farmers with hatchery produced mussel spat, but there is no hatchery production of mussels in Europe. However, because of the recent expansion of the industry in northern

Europe and fierce competition for variable, possibly dwindling, seed mussel stocks, there is now interest in adapting well-tried oyster hatchery culture methods on mussels (19).

Grow-out

European aquaculture of mussels relies on natural spatfall and there are three main methods of culturing these spat up to market size: bottom cultivation, *bouchot* culture and the suspended rope method (18).

Bottom cultivation involves dredging mussel seed from areas where there is extensive natural annual spatfall and relaying the spat in low inter-tidal or sub-tidal lays in shallow sheltered productive areas. One-year-old seed dredged for relaying ranges in size from 10-30mm shell length. Larger, but slow growing, mussels can also be moved from high inter-tidal areas down to sub-tidal beds where growth rate is increased. This method is most widely used in the Netherlands but has been significantly exploited elsewhere (e.g. the Menai Strait, Irish Sea).

The traditional French bouchot method of mussel culture is carried out on wooden poles that are placed upright into the sea bed in the low inter-tidal region. Natural settlements of mussels are collected on sub-tidal poles or horizontal lines of coir rope during the spring and are transferred onto growing bouchots later in the year. Mussel spat can be mechanically loaded into a long "stocking" of natural fibre that can then be wound around the bouchot. As the stocking fibre rots, the mussels attach by byssus to the bouchot. Synthetic fibre stocking material can also be used because the mussels can force their way out through the mesh as they grow.

Suspended ropes for the culture of mussels can be deployed from large floating rafts or from sub-surface longlines. Raft culture is the commonest in sheltered Spanish rias (estuaries) while sub-surface longlines are used in both sheltered and more exposed sites. Natural settlement of spat is often sufficient to produce commercial quantities of mussels, but spat from elsewhere can also be applied directly to the ropes by using a version of the stocking method where the rope runs down the centre of the stocking. Mussels on bouchots or long lines can be harvested after about 18 – 24 months of growth.

Because these three methods place mussels into rather different environmental situations and predator exposure, it is possible that there may be genotype-specific differential mortality during culture.

Because of the long period of the year when the marketing condition of mussels is poor, due to the fact that they are spawning or spent, there is interest in the potential of sterile triploid mussels that could enable year-round marketing. This has been achieved with Pacific oysters and now most sold in the USA, and many in France, are triploid. Triploid mussels have been produced experimentally in the laboratory (20) and also at pilot commercial scale in Canada (21).

Selective breeding

Although environmental effects are very strong on mussel growth, genetic variation in growth rate has been documented (22). Reasonably high heritabilities have been estimated for larval and juvenile growth in mussels (23, 24) and a good selection response for growth rate achieved (25). These facts suggest that there is considerable capacity for breeding programmes to provide improved strains within both species for particular culture methods.

At present the CAWTHRON Research Centre in New Zealand is running a selective breeding programme and they report positive selection response. In Europe there is a need to develop

the necessary hatchery and nursery infrastructure to spawn and rear mussels before any selective breeding programme is possible. However, there seems to be much to be gained in domestication of the mussel.

C. Interaction studies

The precise distributions of the two commercially important European mussels (*M. edulis* and *M. galloprovincialis*), and the extent of their hybridisation, remain to be characterised for most areas of the European Atlantic coast. Without such basic information the genetic impact of mussel aquaculture, particularly in relation to species interactions, will be extremely difficult to assess.

It is thought that *M. galloprovincialis* is slowly spreading northwards invading territory once exclusive to *M. edulis* and that this may be partly a result of global warming. Whether or not it has been involved so far, it is likely that climate change will increase the rate of this process. It is debatable whether short-term (5-10 year) crisis management of species interactions is worth the effort in the face of longer term (50-100 year) climate change factors.

In areas where aquaculture activity is the major source of mussel biomass it is possible that there may be genetic impact due to genotype-specific mortality related to the particular culture method used.

Hatchery culture of mussels is in its infancy and, in the short to medium term, is unlikely to represent more than a small percentage of the natural spat production of mussels in Europe. For this reason there is negligible risk of genetic impact. However, in the longer term, if domesticated strains of either species are developed and extensively cultured there is certainly the potential for genetic impact.

Triploid mussels, if these prove to be a commercial commodity, could be produced in Europe. However, based on experience with the Pacific oyster and other bivalves, their genetic impact is likely to be low. Triploid bivalves are generally almost sterile and any gametes produced are aneuploid and infertile.

D. Conclusions / Implications

- It is essential to precisely characterize the true distributions of *M. edulis*, *M. galloprovincialis* and their hybrids in all regions, but especially where mussel aquaculture takes place.
- Based on such a survey, a series of sites should be identified that are to be genetically monitored on a regular basis to identify any changes in species composition over time.
- More microsatellite genetic markers should be developed for mussels to better identify (a) species differences, (b) the processes of hybridization and introgression (c) fine-scale within-species genetic variation and (d) potential genetic variation between natural, rope cultured and bottom cultured mussels
- Phenotypic and genetic parameters for quantitative traits are scarce and should be studied and response to selection for growth and survival should be investigated.
- Triploid mussels should be produced and tested for their potential genetic impact on native diploid stocks.

References

(1) Goslin E. (1992). Genetics of *Mytilus*. In*:* "The mussel *Mytilus*: ecology, physiology genetics and culture". E. Gosling (ed), Elsevier Amsterdam, 309-382.

(2) Koehn R.K. (1991). The genetics and taxonomy of species in the genus *Mytilus*. Aquaculture, 94: 125-14.

(3) McDonald J. H., Seed R. and Koehn R. K. (1991). Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Mar. Biol., 111: 323-333.

(4) Inoue K., Waite J.H., Matsuoka M., Odo S. and Harayama S. (1995). Interspecific variations in adhesive protein sequences of *Mytilus edulis, M. galloprovincialis*, and *M. trossulus*. Biol. Bull., 189: 370-375.

(5) Wood A.R., Beaumont A. R., Skibinski D.O.F and Turner, G. (2003). Analysis of a nuclear marker for species identification of adults and larvae in the *Mytilus edulis* complex. J. Moll. Stud., 69: 61-66.

(6) FAO (2006). Fish Stat plus. 2004. Aquaculture statistics. http://www.fao.org/fi/statist/FISOFT/FISHPLUS.asp

(7) Bayne B. L. (1965). Growth and the delay of metamorphosis of the larvae of *Mytilus edulis* (L.). Ophelia, 2: 1-47.

(8) Lane D. J., Beaumont A. R. and Hunter J. R. (1985). Byssus drifting and the drifting threads of the young post larval mussel *Mytilus edulis* (L.). Mar. Biol., 84: 301-308.

(9) Gardner J. P. A. (1994). The structure and dynamics of naturally occurring hybrid *Mytilus edulis* Linnaeus, 1758 and *Mytilus galloprovincialis* Lamark, 1819 (Bivalvia; Mollusca) populations: review and interpretation. Arch. Hydrobiol. Suppl., 99: 37-71.

(10) Daguin C., Bonhomme F. and Borsa P. (2001). The zone of sympatry and hybridisation of *Mytilus edulis* and *M. galloprovincialis*, as described by intron length polymorphism at locus *mac-1*. Heredity, 86: 342-354.

(11) Gilg M.R. and Hillbish T. J. (2003). Patterns of larval dispersal and their effect on the maintenance of a blue mussel hybrid zone in Southwestern England. Evolution, 57: 1061-1077.

(12) Skibinski D.O.F., Gallagher C. and Beynon C.M. (1994). Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. Genetics, 138: 801-809.

(13) Zouros E., Ball A. O., Saavedra C. and Freeman K. R.. (1994). An unusual type of mitochondrial DNA inheritance in the blue mussel *Mytilus*. Proc. Natl. Acad. Sci. USA, 91: 7463-7467

(14) Rawson P. D., Joyner K. L., Meetze K, and Hilbish T.J. (1996). Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. Heredity, 77: 599-607.

(15) Heath D.D., Rawson P.D. and Hilbish,TJ. (1995). PCR-based nuclear markers identify alien blue mussel (*Mytilus* spp.) genotypes on the west coast of Canada. Can. J. Fish. Aquat. Sci., 52: 2621-2627.

(16) Presa P., Perez M. and Diz A. P. (2002). Polymorphic microsatellite markers for blue mussels (*Mytilus* spp.). Conservation Genetics, 3: 441-443.

(17) 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. Mar. Ecol. Prog. Ser., 116: 99-115.

(18) Spencer B.E. (2002). Molluscan Shellfish Farming. Blackwell Science, 274pp Oxford.

(19) www.blueseedproject.com.

(20) Beaumont A.R. and Fairbrother J.E. (1991). Ploidy manipulation in molluscan shellfish: a review. J. Shellfish Res., 10: 1-18.

(21) Brake J., Davidson J. and Davis J. (2004). Field observations on growth, gametogenesis, and sex ratio of triploid and diploid *Mytilus edulis.* Aquaculture, 236: 179-191.

(22) Mallet A.L., Zouros E., Gartner-Kepay K.E. and Freeman K. R. (1986). Genetics of growth in blue mussels: family and enzyme-heterozygosity effects. Mar. Biol., 92: 475-482.

(23) Mallet A.L., Freeman K.R. and Dickie L.M. (1986). The genetics of production characters in the blue mussel, *Mytilus edulis*. I. Preliminary analysis. Aquaculture, 57: 133-140.

(24) Stromgren T. and Neilsen M.V. (1989). Heritability of growth in larvae and juveniles of *Mytilus edulis*. Aquaculture, 80: 1-6.

(25) Toro J.E., Alcapan A.C., Ojeda J.A. and Vergara A.M. (2004). Selection response for growth rate (shell height and live weight) in the Chilean blue mussel (*Mytilus chilensis* Hupe 1854). J. Shellfish Res., 23: 753-757.

(26) Brichette I., Reyero M.I. and Garcia C. (2001). A genetic analysis of intraspecific competition for growth in mussel culture. Aquaculture, 192: 155-169.