

Bivalve aquaculture transfers in Atlantic Europe. Part B: Environmental impacts of transfer activities

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Abstract:

For centuries human populations have moved live shellfish around the world for consumption or aquaculture purposes; being relayed from their area of origin for growout or sale. This is in contrast to the inadvertent anthropogenic spreading of species via e.g. ballast waters. There are inherent risks associated with transfer of shellfish including introducing of alien species, diseases, pests, bacteria and viruses associated with the translocated species in addition to the potential impact on genetic integrity and biodiversity of local stocks. Many examples of severe ecological impacts have been documented worldwide owing to the intentional or unintentional translocation of animals. It is therefore important to develop risk reduction methods which have not yet been documented to be incorporated into current fish health or environmental legislation. This part of the study describes the impacts of transfer activities of cultured bivalve shellfish along the European Atlantic coast; identifies hitch hiker species, fouling organisms or infectious agents which can be translocated with a target species. Further, the study highlights the need for thorough, standard risk reduction measures designed to minimise the impact on ecosystems worldwide. In a companion paper details of actual transfer activities in Atlantic Europe are presented and all levels of legislation dealing with transfer activities on a global, regional and national scale are carefully reviewed.

Highlights

► We provide a full list of threats related to bivalve transfer activities. ► We draw the attention to the large spectrum of non-target species transferred with bivalves. ► We show the impact of transfers as potential vectors for the introduction of alien species. ► We provide detailed recommendations for a suitable risk assessment for impacts reduction.

65 1. Introduction

66

67 The intentional movement of shellfish around the world is an activity that has a long history (Wolff &
68 Reise 2002). The objective is mainly economic; to develop a sustainable food supply, to replenish a
69 depleted stock or to start a new culture. The translocation of different shellfish species includes early
70 life stages from hatcheries, nursery sites or from the wild to new culture or wild fishery sites, often
71 crossing national and ecosystem boundaries, creates the potential for negative impact on the
72 environment. These impacts can occur through the introduction of shellfish and associated organisms
73 which can include non-indigenous species, fouling organisms, potentially toxic algae, viruses, bacteria,
74 disease agents, parasites, or the same species with a different genetic makeup. This can lead to an
75 intermixing of wild and cultured or indigenous and introduced stocks with resultant impact: e.g. lower
76 genetic integrity, subsequent poor recruitment and productivity, influenced by factors including
77 sterilization, reduced fitness, meat yield and fecundity. Further, intermixing may have effects
78 on competition, risk of predation, diversity and polymorphism, and on physiological and morphological
79 traits (Dethlefsen 1975; Tiews 1988; Ambariyanto & Seed 1991; Camacho et al. 1997; Calvo-
80 Ugarteburu & McQuaid 1998; Taskinen 1998; Wegeberg & Jensen 1999; Wegeberg & Jensen 2003;
81 Desclaux et al. 2004). As described in detail in the companion Part A of this paper the movement of
82 bivalves for aquaculture purpose can be differentiated into *transfers* and *introductions* (Beaumont
83 2000). According to this the movement of individuals outside the distributional range of the species is
84 defined as an introduction, whereas a movement within species range would be referred as a transfer.
85 In this study the focus is on the ecological effects of transfer activities.

86 Although most movements of shellfish are driven by economic reasons (Mortensen et al. 2006), stock
87 transfers are also made because of shortfalls in local supply to replenish or enhance indigenous wild
88 stocks. This reflects both the variable nature of recruitment to wild stocks; poor natural spat settlement
89 onto artificial collectors or the inability to produce a consistent hatchery supply of seed, results in the
90 lack of availability of high value commercially cultivated juvenile shellfish. Typical transfers of shellfish
91 in Europe include native and Pacific oyster seed from hatcheries to nursery or adults and ongrowing
92 sites, clam seed from hatcheries to ongrowing sites, scallops from natural spat collection sites to
93 ongrowing sites, mussels from natural seed beds to ongrowing sites, and shellfish relayed for
94 depuration or held at a dispatch centre prior to sale for human consumption.

95 Introductions as well as transfers, in the course of normal trade, have been responsible for the
96 establishment of several harmful and nuisance non-native species including fouling organisms. Once
97 established at a new locality these may continue to be moved by various means or by natural
98 expansions of their range. McKindsey et al. (2007) provided detailed information on the implications of
99 bivalve aquaculture and the introduction and spread of exotic species hitchhiking as fouling organisms
100 (e.g. barnacles, bryozoans, macroalgae, snails and even oysters). This study describes in contrast,
101 the transfer and introductions of bivalve mollusc species and refers to their potential impacts;
102 considering genetic effects, associated carrier parasites, pests, diseases, biotoxins, cysts, larvae and
103 eggs. It also considers the introduction and spread of species' travelling with consignments, on and
104 within the layers of bivalves' shells, in intervalval water, and within the tissues of the softbody. In many
105 cases of introductions and transfers of bivalve molluscs for cultivation, no serious attempt has been
106 made to avoid unwanted organisms. The export of half-grown Pacific oysters, *Crassostrea gigas*, spat
107 from France to Ireland in 1993 is an outstanding example. Examination after deposit of the oysters,
108 which had been certified "free from other species", revealed numerous other species: several fouling
109 organisms, other bivalve species (which may potentially carry pathogens or parasites) and 67 species
110 of phytoplankton, including dinoflagellate cysts (O'Mahony 1993; Minchin et al. 1993). Most of these
111 accompanying organisms would perish with time or have no or minor effects on cultivated species in
112 their new environment. However, sometimes new species may cause permanent or long-lasting
113 fouling problems, competition for space or food, or in extreme cases – disease. While fouling macro-
114 organisms may be relatively easy to find and identify if in appropriate numbers, microorganisms will be
115 more troublesome.

116 In a companion paper (Mühlbauer et al. subm.) details of actual transfer activities in Atlantic Europe
117 are presented and all levels of legislation dealing with transfer activities on a global, regional and
118 national scale were carefully reviewed.

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2. Effects of transfer activities

2.1. Transfer-effects of macro parasites and pests

Bivalve shells are a target of shell boring polychaets, such as *Polydora ciliata* inhabiting the shell of blue mussels, oysters, scallops and clams. This polychaete weakens shell strength (Kent 1981), increases energy requirements, impairs the overall health of the mussel (Kent 1979; Ambariyanto & Seed 1991), and harms in e.g. mussels the mantle tissues mainly responsible for reproduction (Wachter, 1979). Thus is classified as harmful to the host at least at high infestation rates (Michaelis 1978). A weakening in shell strength, the increased energy demand, the decline of reproductivity, and on occasions increased mortality, can severely impact both wild and cultivated mollusc populations. Other macro parasites inhabit organs and tissues of bivalves' softbody. From the German Bight for example, from two (affecting oysters) to ten (affecting mussels) different macro parasite species are reported to be common (Thieltges 2006). They belong to different phyla, inhabit various tissues and organs and cause a variety of symptoms. The intensity of the infestations can vary according to the conditions of the habitat. Blue mussels show the highest infestation rates at intertidal areas, followed by subtidal and offshore areas (Buck et al. 2005, Brenner et al. 2009). Other areas within the distributional range of blue mussels (*Mytilus edulis*) and close relatives (*Mytilus galloprovincialis*, *Mytilus trossulus*) show comparable numbers of parasite species, however, with a different species spectrum. Some parasite species are extensively found within the distributional range whereas others are restricted to relatively small areas. Thus, a movement of infested mussels amongst different areas and habitats to uninfested areas may support the transfer of parasites and pests between tidal levels e.g. from intertidal to subtidal areas, or from areas with high parasite diversity to areas showing a limited spectrum of species.

The role and effects of macro parasites on the health status of their hosts are still debated intensively. For *Mytilicola spp*, including *Mytilicola intestinalis* and *Mytilicola orientalis*, the characteristics range from being a pest with severe negative impacts (Odlaug 1946; Meyer & Mann 1950; Dethlefsen 1975), to only being a commensal organism feeding on unutilized fractions of the mussel's gut (Calvo-Ugarteburu & McQuaid 1998). Descriptions of other common parasitic species are more consistent. Metacercarias of trematods found in the digestive gland of blue mussels are described as reducing growth (Taskinen 1998; Calvo-Ugarteburu & McQuaid 1998), general health (Calvo-Ugarteburu & McQuaid 1998), reproductive ability (Coustau et al. 1993), and hamper feeding of the mussel (Thieltges 2006). Independently of the final evaluation of the resulting health effects of different parasite species, a spreading of these species should be generally avoided, whether by statute or industry voluntary codes of practice. Some parasites can impact commercial marketability and value by reducing shell strength or affecting the meat appearance and integrity of mussels (e.g. *P. ciliata*). Others like *M. intestinalis* cause aesthetic problems due to their markable colour and impressive size. And some just provoke distaste like the shell-inhabiting crabs of the genus *Pinnotheris*.

2.2. Transfer-effects of micro parasites (Protozoa) and diseases

In addition to macro parasites, bivalves are both host and vector of micro parasites, e.g. *Marteilia*, *Bonamia*, *Microcytos* and *Perkinsus* species. As these parasites severely affect the health of host shellfish, in contrast to macro parasites, they are listed under the mandate of the World Organisation of Animal Health (OIE 2010) and current shellfish health legislation (EC/2006/88). Prior to transfer activities, a shellfish area must be declared free of these listed diseases when destined for an area of equal or greater health status. For decades, outbreaks of e.g. Bonamiasis and Marteiliosis have led to dramatic losses in the French oyster industry and still a simple inspection for listed pathogens prior to transfer is not guaranteed to prevent the introduction, spread or containment of disease.

In the 1960s, *C. gigas*, was deliberately introduced from Japan to France and since then to much of the coastal regions of Europe. It was seen as a disease free, good growing alternative to *Ostrea edulis* and *Crassostrea angulata* whose stocks suffered severely under *Bonamia* and *Marteila* infections. The Pacific oyster is scientifically proven non-susceptible to *Bonamia* and so movements were routinely made around Europe with little control. In the 1990s, a transfer of *C. gigas* was made from France to

173 Ireland and deposited in the sea, prior to inspection for susceptible or hitch hiker species. After the
174 event, non indigenous species and indigenous species capable of transmitting serious disease were
175 found; including the pest *M. orientalis*, and *O. edulis* which is capable of transmitting *Bonamia*
176 (Minchin 1996).

177 Further, there is a lack of knowledge on the life cycles of even the best known bivalve pathogenic
178 agents. *Marteilia refringens* seem to go through several stages in a complex life cycle (Grizel et al.
179 1974; Perkins 1976). In Mortensen (2000) *O. edulis*, were kept free from *M. refringens* in tanks using
180 water from the wild oyster beds. However, these oysters became immediately infected once moved
181 out on oyster beds by some unknown intermediate host or stage of *M. refringens* present in the
182 environment. The most serious oyster pest in Europe, the protozoan *Bonamia ostreae* also illustrates
183 the problem. At first sight it seems not to have a complicated life cycle like *Marteilia*. *Bonamia*
184 propagates by binary fission until the host cell, the oyster haemocyte, bursts. But despite a number of
185 studies, there remain unanswered questions. It is not known why small oysters are unaffected, but the
186 oyster died due to the parasite when they approach sexual maturity. A life cycle with a phase in the
187 ovarian cycle has been suggested (van Banning 1990), but it is still not fully understood. Also, the host
188 range of many agents is largely unknown, and extensive studies are necessary to identify potential
189 host species. Thus, it remains unknown which bivalve species may be susceptible, which species
190 might be vectors, in which stage the parasite may be dispersed, or which species might be
191 intermediate hosts.

192 There is a tendency to link the pathogenic agents to the species in which they are first described, but
193 this may often be wrong. When the protozoan *Microcytos mackini* was identified as the causative
194 agent of Denman Island Disease of Pacific oysters, *C. gigas* in British Columbia, Canada, the agent
195 was first linked to this oyster species, but later similar organisms were observed also in flat oysters, *O.*
196 *edulis*, and *Olympia* oysters, *O. lurida*, in the US, and Sydney rock oysters, *Saccostrea commercialis*,
197 in Australia. The causative agents were identified as two different *Microcytos* species (Farley et al.
198 1988). Later experiments showed that *M. mackini* was pathogenic also for the oysters *Crassostrea*
199 *virginica*, *O. edulis* and *O. lurida* (Bower et al. 1997). The example illustrates that what may seem as
200 one disease in one species may appear in different areas, and be caused by different, but related
201 parasites, which themselves may be pathogenic for different host species. This complicates the one
202 disease-one host-one area management approach, which is commonly applied. Even when we have
203 documented that a specific agent is actually pathogenic, there are often great uncertainties concerning
204 the infectious dose of agents, influence of environmental factors on disease, etc.

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206 2.3. Transfer-effects of pathogenic agents, bacteria and viruses

207 The survival of bacteria in seawater and their presence in bivalves varies with exposure to
208 environmental factors such as temperature, salinity, organic loading and is influenced on seasonal and
209 spatial scales (Hernroth 2003). The bivalves' response towards ingested microbes is to eliminate
210 them. However, it has been shown that e.g. *Salmonella typhimurium* can survive more than two weeks
211 after being injected into the circulating system of mussels (Hernroth 2003). *Salmonella* species can
212 cause enterocolitis, enteric fevers such as typhoid fever, and septicemia with metastatic infections in
213 humans. Seawater is the natural habitat of the *Vibrio* bacteria, feared as pathogens in fish and
214 shellfish (Shao 2001). *Vibrio* can cause severe infections in humans after consumption of raw or
215 undercooked shellfish and contaminated food. A special hazard is caused by *Vibrio vulnificus*, where
216 severe infections can occur through skin lesions (Blake et al. 1979). *Vibrio* species are associated with
217 both human health problems and with the pathogenicity and mortality of mollusc species. In France,
218 the rearing of *C. gigas* is the main aquaculture activity and has recurrently suffered large scale
219 summer mortality phenomenon for the last 15 years. Several *Vibrio* strains belonging to *V.*
220 *aestuarianus* and *V. splendidus* have been found to be associated with diseased juvenile oysters
221 suffering from the summer mortality syndrome. Most of these strains exhibit also harmful effect when
222 living bacteria or their extracellular products are experimentally injected to healthy oysters confirming
223 their pathogenicity.

224 Like bacteria, viruses are predominantly concentrated in the digestive glands, but can also be
225 absorbed through the gills of bivalves (Abad et al. 1997). Certain viruses such as the Norovirus are
226 even more persistent and can remain infectious for weeks to months in seawater or in sediment

227 (Gantzer et al. 1998). Although they are inherently unable to multiply in bivalves, shellfish are efficient
228 vehicles for transmission of pathogenic viruses to humans. Epidemiological studies have revealed that
229 human enteric viruses are the most common pathogens transmitted by consumption of bivalve
230 shellfish (Lees 2000; Lipp & Rose 1997). Among these, hepatitis A virus (HAV) is the most serious
231 viral infection linked to the consumption of bivalves. In Italy, estimates suggest that approximately 70
232 % of HAV cases are caused by shellfish consumption (Salamina & D' Argenio 1998). The relatively
233 long incubation period following initial infection (average 4 weeks), complicates the traceability of the
234 viral source. Thus, HAV infections caused through shellfish consumption are probably underreported
235 or even remain undiscovered.

236 Norovirus and serotypes of the adenovirus group are associated with gastroenteritis. These viruses
237 have been recorded in seawater and shellfish in many different countries, such as Greece, Spain,
238 Sweden and the UK (Formica-Cruz et al. 2002). In particular overall viral infections caused by the
239 Norovirus (gene group II) have shown a remarkable increase, as registered by the Robert-Koch
240 Institute (RKI 2000). This increase, may be because Norovirus infections must be reported by law.
241 However, the rapid course of the illness within a few hours complicates appropriate counter measures.
242 In general, viruses can be surprisingly inert. After the finding of the fish pathogenic infectious
243 pancreatic necrosis virus (IPNV) in scallops, *Pecten maximus* (Mortensen et al. 1990), the subsequent
244 study of the fate of IPNV in scallops (Mortensen et al. 1992, Mortensen 1993) showed that the virus
245 was taken up during filtration, persisted for long periods of time, and was shed into the water by
246 contaminated scallops. No viral propagation was found, and in nature, the virus excreted from
247 contaminated bivalves would rapidly be diluted in seawater. Scallops and other bivalves should
248 probably still be considered potential vectors also of fish pathogenic viruses. One example is the virus
249 causing gill disease, which eradicated the susceptible populations of Portuguese oyster, *C. angulata*,
250 from the French coast, while the resistant Pacific oyster, *C. gigas*, remained only slightly affected by
251 the disease (Comps et al. 1976; Comps 1988). It has been hypothesised that *C. gigas*, which was
252 actually introduced to France just before the first outbreaks, was actually the vector, being adapted to
253 the virus through generations of coexistence in Japan. The risk of disease transmission becomes
254 greater when there are true biological vectors, where a pathogenic agent maintains its normal function
255 and even propagates. Considering the above-mentioned coexistence between any animal and its
256 microorganisms, the microecological balance may be disturbed during an introduction or transfer.
257 From the introduced scallop's point of view, there may be unknown reservoirs, intermediate or
258 alternative hosts of pathogenic agents in its "new" environment (Mortensen 2000). From the point of
259 view of the inhabitant of the recipient environment, the "newcomer" may pose a threat, bringing new
260 microorganisms, which are potentially pathogenic agents for them.

261 In a recent example, oyster spat from hatcheries contaminated with the Oyster Herpes Virus (OsHV-
262 1), was moved routinely for years around France and further afield uncontrolled, with little attention to
263 inspection, consignment carrier status, or for the presence of hitch hikers. Lately, an extremely
264 pathogenic variant, OsHV-1 μ var, was identified as the causative agent of high mortality especially in
265 France (Segarra et al. 2011), which prompted the EC Commission to consider the variant as an
266 emerging disease (EC) 350/2011.

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268 2.4. Transfer-effects of biotoxins, cysts, larvae and eggs

269 The main food source for bivalves is phytoplankton and thus the potential for accumulating algal toxins
270 is high. Several human diseases have been reported to be associated with many toxin-producing
271 species of dinoflagellates, diatoms, nanoflagellates and cyanobacteria that occur in the marine
272 environment (CDC 1997). Marine algal toxins become a problem primarily because they may
273 concentrate in shellfish and fish that are subsequently eaten by humans (CDR 1991; Lehane 2000).
274 These toxins cause severe syndromes of poisoning (e.g. Amnesic Shellfish Poisoning (ASP),
275 Diarrheal Shellfish Poisoning (DSP), Paralytic Shellfish Poisoning (PSP), Neurotoxic Shellfish
276 Poisoning (NSP)) and, on occasion, death. In addition to accumulating poisons, filtering bivalves can
277 function as a vector for the distribution of reproductive cysts of toxin-producing algal species. These
278 cysts may survive in unfavourable conditions for years buried in the sediments (Tillmann & Rick 2003)
279 and can, after being re-suspended and translocated in e.g. the intervalval water of molluscs, build up
280 new populations in formerly unaffected areas (Mons et al. 1998). Thus, this type of transfer may result

281 in human health risks, fishery and culture closures and commercial losses. The transportation of toxin-
282 producing algal species and their resting cysts, either in a ship's ballast water or through the
283 movement of shellfish stocks from one area to another, provides a possible explanation for the
284 increasing trend of harmful algal blooms (Hallegraeff 1995; McMinn et al. 1997). As part of the controls
285 to protect public health, European regulation requires a monitoring programme of shellfish relaying
286 and production areas to be established to check for the possible presence of toxin producing plankton
287 in the water and biotoxins in the shellfish flesh (EC Regulation 854/2004).

288 Comparable to the diversity of species living as commensals on the shells of bivalves, numerous
289 species are present in their intervalval water. Many species from different phyla such as bacteria,
290 viruses, fungi, or ciliophora use bivalves as a host, whereas others (or other species from the same
291 mentioned phyla) are filtered actively as food (e.g. micro algae) or enter the molluscs accidentally
292 through the incurrent water flow. Depending only on the size of organism many species and especially
293 their larval stages, cysts or eggs can be present in bivalves. Since live bivalves are usually
294 translocated dry, trapped species can travel together with their temporary host over large distances.
295 For example, egg capsules of the American oyster drill, *Urosalpinx cinerea*, have been found in the
296 Oosterschelde, an area of shellfish culture in The Netherlands. Most probably *U. cinerea* was
297 introduced within transferred shellfish from south-east England (ICES 2008).

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299 2.5. Genetic effects of transfers

300 It is becoming increasingly important to identify species being transferred or introduced, not simply
301 morphologically, but using appropriate statistically significant screening, including specific and
302 sensitive molecular tests. It is also recognised that the gene pool of the broodstock used to provide
303 progeny for cultivation or augmentation of wild, should not act as vectors of disease, compromise or
304 reduce genetic integrity of indigenous populations, result in interbreeding, reduced reproduction or
305 introduce traits not conducive to growth and survival. To predict the genetic consequences of
306 transfers, information on genetic composition of species to allow their identification and differences
307 between source and recipient populations is vital (Beaumont 2000). This may be expressed by
308 morphological, allozyme and DNA based data on genetic differentiation of populations and sub-
309 species.

310 Other considerations are the numbers of individuals transferred and whether they are wild stock or a
311 hatchery product. Loss of genetic diversity is difficult to avoid in hatchery conditions although there are
312 also ecological advantages using disease-free or sterile hatchery seed. Examples are given in
313 Beaumont (2000) on how mitochondrial DNA data indicate significant genetic consequences of the
314 introduction of *Argopecten irradians* from the USA to China, and *Patinopecten yessoensis* introduced
315 from Japan to Canada. Beaumont (2000) recommends that potential risks and consequences of
316 hybridisation should be experimentally assessed before introductions of scallops are carried out.
317 However, hybridisation is unpredictable and can lead to loss of genetic diversity or the breakdown of
318 co-adapted gene complexes, resulting in a poor commercial product. The use of sterile triploid
319 scallops for introductions to avoid hybridisation and reduce ecological impact has merit but reversion
320 to diploidy may occur. There is also the risk that introductions breeding with indigenous stock could
321 result in reduced future fecundity.

322 *Crassostrea gigas* was introduced in Europe as an alternative to the Portuguese oyster (*C. angulata*)
323 following the viral disease that caused the collapse of the Portuguese oyster populations. Currently
324 there is contact between the species worldwide; between France and the south of Portugal and
325 between Japan and Taiwan. In these regions hybrids have been found. This hybridisation has an
326 impact on the *C. angulata* population in Southern Europe. Pacific oyster spat is mainly obtained from
327 settlement from the wild, however about 20 % of pacific oyster spat is derived from hatcheries.
328 Hatcheries mainly produce triploid spat, which is not yet considered as a safe genetic confinement
329 tool, as triploids do occasionally breed. The effect of the partial sterility of triploids is poorly known.
330 Another threat to wild populations is the use of tetraploid broodstock if they escape from quarantine,
331 as their fitness relative to diploids and the impact of their breeding with diploids is still unknown
332 (GENIMPACT 2007). Another impact has recently been recognised resulting from the reproduction
333 and spread of Pacific oysters in the wild, invading ecosystems to replace indigenous species and
334 causing a problem to shellfish farmers because of extensive wild and uncontrolled spatfall. This non-

335 indigenous species which was originally introduced to enhance and expand aquaculture production
336 has become established in many European countries to the extent of now being considered a pest,
337 not only to farmers and wild fisheries, but also to leisure industries by impacting beaches and pier
338 areas.

339 The European flat oyster (*O. edulis*) occurs naturally from Norway to Morocco in the North-Eastern
340 Atlantic and in the whole Mediterranean basin. The species was also introduced in the United States,
341 from Maine to Rhode Island (1930's and 40's) and in Canada (about 30 years ago). Mediterranean flat
342 oysters have more genetic variability than the Atlantic population. The North American populations
343 were derived from the Atlantic population. Most flat oysters are grown from wild captured seed but e.g.
344 in the UK and Ireland hatcheries can produce flat oyster spat. Hatchery cultured spat can result in a
345 reduced genetic variability, if care is not taken in selecting broodstock. This can result in reduced
346 variability of the natural populations. The technology exists to produce polyploid flat oysters, however
347 this resource is not currently utilised. No large scale selective breeding programmes have been
348 initiated for *O. edulis*. However, some experiments to improve resistance to *B. ostreae* have been
349 carried out. Results show a higher survival rate and a lower prevalence of this parasite in selected
350 stocks, but also a reduced genetic variability in mass selected populations (Lapègue et al. 2006).

351 The mussel species *M. edulis* and *M. galloprovincialis* are widely distributed within Europe. *Mytilus*
352 *edulis* is found to be homogeneous throughout its range while *M. galloprovincialis* is genetically
353 subdivided into a Mediterranean and an Atlantic group. *Mytilus trossulus* also exists in discrete areas.
354 In places where two or more of these species occur together, hybrids are found. Information on the
355 distributions of mussel species and their hybrids is gradually improving (Dias et al. 2008a; 2008b). The
356 potential influence of environmental conditions on growth and shell morphology makes it difficult to
357 distinguish the species and their hybrids based on shell shape alone. Recent research on the
358 distribution of the *Mytilus* species in Europe has been greatly facilitated by molecular tools which,
359 based on the animal's DNA, are able to reliably distinguish between species and hybrids in both wild
360 and cultivated populations (Dias et al. 2008b).

361 *Mytilus galloprovincialis* was identified in cultivation areas in Scotland and *M. trossulus* alleles occur at
362 the Irish east coast (Kijewski et al. 2009) in waters which were formerly described as inhabited by pure
363 *M. edulis* populations (Gosling et al. 2008). This has raised questions relating to the risks associated
364 with transfers of seed and the consequential sustainability of blue mussel cultivation in certain
365 countries. Forensic investigation of the occurrence of *M. trossulus* in a few sea lochs in Scotland
366 indicates that the distribution of *M. trossulus* appears to be consistent with the species having been
367 moved from place to place during transfers of mussel stock for cultivation purposes. Where *M.*
368 *trossulus* has been moved out of the original Scottish site to areas of full strength salinity seawater, *M.*
369 *trossulus* have reportedly died and not spread through natural settlement. It has not yet been found in
370 wild populations, even where adjacent cultivation ropes contain large proportions of *M. trossulus*. The
371 majority of mussel production sites in Scotland produce *M. edulis* and work is ongoing to
372 systematically manage out *M. trossulus* from the Scottish index site to minimise any risk of its spread
373 within Scottish waters.

374 Further, the three main cultivation methods for mussels (bottom culture, suspended culture and pole
375 culture (or bouchot method) have their own specific growth requirement. Therefore, there may be a
376 genetic impact due to genotype-specific mortality in areas where aquaculture is the major source of
377 mussel biomass.

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379 2.6. Transfer-effects on biodiversity

380 Many non-native species introductions have not been registered and may have had no impact on
381 receiving environments (Gollash 2004). However, many introductions into the marine environment
382 have been classified as invasive (Kettunen 2009). The impacts identified have been wide ranging and
383 include impacts on native habitats and species. More specifically, it has been documented that
384 species can have direct impacts by excluding native species and thereby reducing biodiversity. The
385 introduction and transfer of marine molluscs from fisheries and aquaculture includes the risk of
386 transporting competitors, predators, parasites, pests and diseases which have compromised intended
387 molluscan culture and wild fisheries.

388 The expansion of *C. gigas*, throughout northern latitudes of Europe has been well documented (Reise
389 1998; Drinkwaard 1999a; 1999b; Smaal et al. 2005). The spread has been rapid and has resulted in
390 very high recruitment of the oysters in marine habitats. In some areas the diversity of species
391 associated with *C. gigas* has been demonstrated to be higher than that of ambient habitats (Kochman
392 et al. 2008). While species diversity may be comparable or higher on short spatial scales, the invasive
393 nature of *C. gigas* is such that habitat heterogeneity is greatly reduced over large spatial scales. There
394 is the additional risk of transfer of the highly pathogenic oyster herpes virus with the potential of
395 causing high mortality in naïve wild and cultivated populations of *C. gigas*.

396 The manila clam, *Ruditapes philippinarum*, originated from Asia, was introduced into France in the
397 1980's for aquaculture purposes, including Arcachon Bay. For economic reasons, this aquaculture
398 was unsuccessful and was rapidly abandoned; however, the species subsequently found good
399 environmental conditions to reproduce naturally and expanded in the wild. Ten years later, this exotic
400 clam species was more abundant than the native one, *Ruditapes decussatus*. This situation can be
401 explained by superior recruitment and rapid growth to outperform the indigenous species. Since 1992,
402 the biomass of the introduced clam *R. philippinarum* has been exploited by fishermen (Dang et al.
403 2010).

404 Still uncertain is, how the Manila clam out-competes the indigenous species and contributes to the
405 modification of biodiversity in Arcachon Bay. Both species colonize the same habitat and with time, the
406 ratio between the 2 species was modified to the benefit of the Manila clam. The competition is
407 probably not direct for space and food but associated with the fishing activity. The stock exploitation
408 impacts more drastically the European species because of its low capacity to recolonize the habitat
409 compare to those of the indigenous species (Auby 1993).

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412 3. Recommendations

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414 There is a need for comprehensive health surveillance strategies involving procedures to
415 systematically look for early signs and assess the adverse effects on the health-status of a country.
416 The priority should be prevention and to establish the absence of a problem, but have the facility to
417 detect one if it exists. Therefore, it is necessary to develop a plan to evaluate and establish the status
418 of a country or area and be able to control a problem if it occurs, e.g. via surveillance and eradication if
419 a disease or invasive organism is found. This may be undertaken by voluntary industry codes of
420 practice or by statute, depending on the status of each country and what it aims to control. It is
421 essential to identify the risks associated with aquaculture production and to introduce methods to
422 minimise and control them. These may be associated with the introduction of disease, pests,
423 parasites, fouling organisms or adverse effects associated with movements or transfers of bivalve
424 shellfish, equipment and sea water associated with the transfers.

425 The requirements, legal or otherwise, depend on their value; the impact on sustainability and whether
426 controls are considered to be possible. Measures should be in place to measure their success and to
427 point to further steps, if deemed necessary. It is vital to involve industry, policy makers and scientists
428 in the development of all strategies and procedures to ensure that each embrace them and contribute
429 effectively to their success. In development of a new Animal Health Law (SANCO/7221/2010 working
430 document) regarding movements of animals for trade and measures for disease control, the
431 conclusions of the chief veterinary officers emphasise the importance of surveillance as a key element
432 of animal health policy. They give priority to preventive approaches, early detection and quick
433 response; notification which in turn enables timely control and eradication when feasible. Also, clear
434 objectives of such a system should be established to generate and manage reliable, transparent and
435 accessible epidemiological & surveillance data connected into an appropriate informatics system.

436 Risk-based animal health surveillance under Council Directive 2006/88/EC is designed to prevent and
437 control certain diseases in aquaculture animals and products; including measures on suspicion of, or
438 during an outbreak of disease. Member States must ensure that a risk-based animal health
439 surveillance scheme is applied in all farms and mollusc farming areas. The aim of the schemes is to
440 identify and mitigate risks, instigate good site biosecurity measures, to detect any increased mortality
441 and the presence of listed or emerging diseases- where susceptible species are present.

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4. Conclusion

Moving shellfish within and between countries and ecosystems, poses a high risk of ecological impact, to genetic integrity and to the introduction and spread of invasive species and pathogenic agents. There should be a presumption against routine introductions and transfers of molluscan shellfish. Transfers should only occur through necessity, e.g. in the promotion of free trade and only be made following a full risk assessment to demonstrate negligible risk. As global communication continues to develop it becomes increasingly important to develop a more dynamic and transparent global approach, to controls with standardised guidelines, including aspects such as risk assessment, management advice, and the identification and application of research goals.

In general, all possible alternatives on the local level e.g. employing hatchery or spat collection methods should be investigated before consideration of transfers as a last resort. If there are good commercial reasons for the transfer of a species, a robust standard of risk assessment should be applied, prior to release, to ensure that ecosystems are protected. Risk based surveillance is now an animal health requirement under Council Directive (EC) 2006/88 for the prevention and control of certain diseases, and models produced by each country should be designed to identify and quantify risks of disease introduction and spread. Transfers of shellfish are made routinely at all levels, by countries of differing environmental and disease status, highlighting the real risks of introducing listed and non-listed pests, parasites and diseases. There is a need for continued coordination in the development and application of legislation and codes of practice within and between countries; to minimise introduction and spread of invasive species and pathogenic organisms. Several tools, e.g. ICES Codes of Practice (ICES 2005), EC regulations, The Animal Health Code from Office International des Epizooties, and common veterinary practice are designed in order to assess risk, and avoid introductions of pathogenic agents and exotic species with the consignments. However, even if all guidelines and recommendations are followed, it is impossible to predict all possible effects of transfers and introductions, and to predict which disease problems may follow. The spread of pathogens frequently occurs ahead of the diagnostics. Learning from introductions and transfers of other bivalve species is therefore essential, to enable a proper risk assessment.

The strategy and principles to be followed by EC Directives involve; such that the burden to the public and private sectors is proportionate, finding the balance between control of non-wanted organisms and over-regulation and ensuring that regulation and surveillance is based on a transparent assessment of risk. An essential part in the development of any risk based assessment model is to ensure that it accurately identifies and quantifies those risks associated with all farms within a zone and provides early detection of possible impacts. Risk assessment requires regular review as industry practices evolve, increasing or decreasing risk on farm sites. Each farm is to receive a ranking (high, medium or low) based on criteria developed at a surveillance work stream workshop, frequency of inspection to be determined by the ranking of each site (Annex III of (EC) 2006/88).

A full risk assessment should include possible effects of diseases (parasites, viruses and bacteria), genetic contamination and hitch hiking species. The risk assessment should be undertaken to ensure safety to ecosystems, as the long term environmental and financial costs from introductions is unquantifiable in the long term. Consultation on applications should be vigorous, be universally applied and be objective; and there should be a presumption against them, unless good scientific evidence proves otherwise. Further, consideration should be given to the risk to native stocks from interbreeding and to the resultant progeny invading ecosystems being possibly infertile, creating an imbalance within an ecosystem. At the final destination a proper quarantine facility should be established to monitor transferred bivalves. The facility must be authorised as an Aquaculture Facility and all movements of live animals into the facility are to be recorded. If high risk were assessed, consideration should be given to growing the animals through the F1 generation to assess reproductive behaviour and the danger of disease transmission, prior to release.

However, there is little health protection against non listed species where there is little scientific knowledge of their impact or susceptibility to diseases. A full risk assessment would require a complete list of all non-target species to identify non-native species that might be imported into an area with a consignment of shellfish. For this goal it will be necessary to have a good knowledge of the

496 marine biodiversity in shellfish areas and to be able to distinguish exotic species from indigenous
497 fauna and flora. So in this context it is important to have monitoring networks. Monitoring programs
498 developed for other purposes (i.e. for microbiological contamination, toxins and for EU Directives as
499 water framework directive and marine strategy directive) can provide useful information and with some
500 limited adjustments could be improved to include exotic species recording.

501 However, the presence of “usually harmless – potentially harmful” organisms leads us to the problem
502 on the existence of “stowaways”, and the action of mechanical vectors. One organism will always carry
503 another, and it seems impossible to obtain “clean” animals, in spite of long quarantines. An example of
504 stowaways is hidden organisms in a consignment of bivalve spat. Frequently, batches contain more
505 species than those they are supposed to contain, even if the batches have been (roughly) inspected,
506 cleaned and graded. Mechanical vectors are passive carriers, which are not needed for the
507 propagation of the species being carried. So even if all potential precautions were conducted properly
508 the effects of transfers and introductions of bivalve molluscs are to some extent unpredictable. Moving
509 molluscs, there is a risk of introducing pathogenic agents or of disturbing the balance between
510 potentially pathogenic agents and host species in the recipient ecosystem.

511 Overall, impact rather than financial considerations should be the prime concern. If a company wishes
512 to profit from a transfer or introduction it should be prepared to undertake the proper scientific
513 assessment of risk prior to release, as long term impacts can be serious and wide-ranging. Here, the
514 guidelines on best environmental practice (BEP) for the regulation and monitoring of marine
515 aquaculture as defined in the Monitoring and Regulation of Marine Aquaculture Programme
516 (MARAQUA) (Read et al. 2001) for the European Union as well as for all countries defined by the FAO
517 (FAO 1999), should be taken into account. These guidelines also include best available techniques
518 (BAT) and best management practices (BMP).

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525

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