Reproductive and molecular ecology of the European lobster: Implications for conservation management

Submitted by

Charlie Dove Ellis

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

Abstract

The European lobster (*Homarus gammarus*) is an ecologically important benthic decapod which supports fisheries that are critical to the economic prosperity of coastal communities. However, populations across its range are pressured by rising exploitation, from which management has failed to prevent stock collapses in the recent past. Fisheries management of the species is significantly hindered by deficiencies in our knowledge of fundamental characteristics of population biology, including the connectivity and genetic diversity of stocks. As a result, the effectiveness of strategies designed to conserve recruitment and ensure harvests are sustainable is poorly understood. This thesis focuses on elucidating aspects of reproductive and molecular ecology in *H. gammarus* which can be used to inform and appraise conservation management initiatives, currently applied via both the regulation of capture and the wild release of hatchery-reared juveniles. The size-specific fecundity of reproducing females was defined around southwestern UK, and spatial variation in clutch size between populations was linked to a longitudinal gradient in oceanic temperature range across Northern Europe. The reconstruction of paternal genotypes show that single males fertilise individual clutches, which hints at demographic stability within a productive Atlantic fishery. Population genetic structure, investigated at a fine spatial scale in the same region, evidenced high connectivity and suggests that the localised interventions of an active hatchery do not lead to juveniles being released beyond areas they might naturally recruit via planktonic dispersal. However, genetic differentiation and isolation-by-distance at a broad geographic scale indicate that direct gene flow between remote populations is limited, so that (i) a failure to maintain spawning stock biomass may negatively affect local recruitment, (ii) the utilisation of non-resident broodstock for hatchery stocking may cause a loss of adaptive potential, and (iii) the recovery of depleted stocks is likely to be problematic. Finally, simulations indicated that genetic parentage assignment will prove accurate in distinguishing cultured individuals from natural stock among admixed populations in the wild, an important development that should facilitate the optimisation of hatchery stocking and lead to rigorous assessments of the conservation value of releasing lobsters reared in captivity.

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I reserve my final thankyou for you, the reader. I thank you in advance for taking the time to explore this product of my hard work. I hope that in reading it you obtain even a small amount of the satisfaction, learning and fulfilment that I have gained in writing it.

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Author's Declaration

All chapters presented in this thesis were written by C.D. Ellis under the guidance and supervision of D.J. Hodgson and A.G.F. Griffiths, with the exception of the chapter included as an Appendix, which was co-written by C.D. Ellis and S.T.V. Neenan under the supervision of D.J. Hodgson and T. Tregenza, and of which a previous draft was submitted to the University of Exeter as an MSc thesis by S.T.V. Neenan. All molecular analyses were conducted at the Penryn Campus of the University of Exeter, under the supervision of A.G.F. Griffiths. Fieldwork was conducted by C.D. Ellis, with additional sampling provided by collaborators from other academic institutes and the fishing industry, and with additional laboratory and animal-holding facilities courtesy of the National Lobster Hatchery, Padstow. C.D. Ellis received studentship funding from the European Social Fund, and from The Worshipful Company of Fishmongers for research on lobster population genetics and paternity. Specific contributions to chapters are as follows:

Chapter 2

C.D. Ellis researched the subject matter and wrote the chapter under the supervision of D.J. Hodgson and A.G.F. Griffiths. R.C.A. Bannister, C.L. Daniels and D.P. Boothroyd all provided comments on the chapter manuscript.

Chapter 3

C.D. Ellis collected and processed all samples, analysed the data and wrote the chapter. H. Knott provided assistance with sample collection and processing, M.J. Witt provided temperature data, and A.-L. Agnalt and O. Tully provided previously-analysed egg count data. D.J. Hodgson provided useful guidance with data analysis and manuscript preparation, while C.L. Daniels provided comments on the chapter manuscript.

Chapter 4

C.D. Ellis conducted fieldwork, collected samples, carried out all genetic analyses, analysed the data, and wrote the chapter. H. Knott assisted with the

collection of samples. A.G.F. Griffiths helped design the experiments and provided guidance on the processing of samples and analysis of data. C. André and H. Knutsen contributed unpublished genomic DNA sequences for microsatellite primer development. D.J. Hodgson provided guidance on the analysis of data. A.G.F. Griffiths, D.J. Hodgson, T.K. Sørdalen, C. André and H. Knutsen all provided useful comments on the chapter manuscript.

Chapter 5

C.D. Ellis conducted all fieldwork and sample collection around Cornwall, processed all samples, analysed the data and wrote the chapter. Various other researchers and industry stakeholders provided samples from elsewhere in the UK and Europe. C. André provided previously-used raw genotype data and DNA sequences for microsatellite development. M. Collins provided support with data analysis. A.G.F. Griffiths provided guidance with sample processing, data analysis, and manuscript preparation. D.J. Hodgson and C.L. Daniels provided comments on the chapter manuscript.

Chapter 6

C.D. Ellis conducted all fieldwork, sample collection and processing to obtain microsatellite genotypes, and conducted data analysis and wrote the chapter. A.G.F. Griffiths and D.J. Hodgson provided guidance with data analysis and manuscript preparation.

Appendix

C.D. Ellis and S.T.V. Neenan designed and carried out the experiments, analysed the data and co-wrote the appendix chapter. T. Tregenza helped conceive the experiments, while various staff and volunteers of the National Lobster Hatchery assisted with maintenance of the animals and aquarium systems and the collection of some data. D.J. Hodgson provided useful advice on data analysis and preparation of the manuscript. D.P. Boothroyd provided comments on an earlier version of the chapter manuscript.

Lobsters bred in such abundance around Britain's coastline that they were fed to prisoners and orphans and ground up for fertiliser; servants sought written agreements from their employers that they would not be served lobster more than twice a week.

Bill Bryson, 'At Home: A Short History of Private Life'

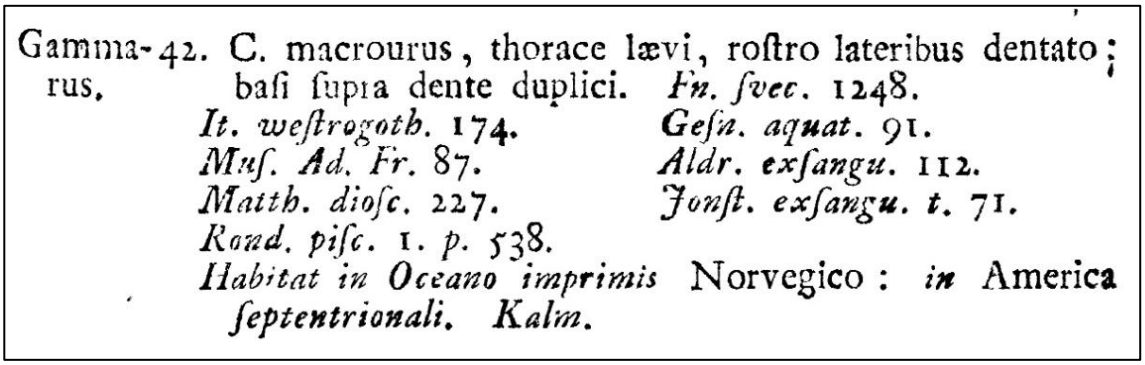
Photo: Katie Sindle / National Lobster Hatchery

Chapter 1: Introduction

This thesis investigates facets of reproductive and molecular ecology in wild populations of European lobsters. A major component of this research examines genetic data from nuclear microsatellite loci to assess fundamental aspects of clutch fertilisation, genetic diversity, and the spatial bounds of populations. Throughout, the patterns observed in empirical data are discussed in regard to relevant ecological processes, and are framed against current efforts to manage and conserve lobster fisheries, particularly those reliant on the release of captive-reared lobsters. All chapters contain an introduction, methodology and discussion specific to each study, and chapter two is an extensive literature review of current understanding of the performance and potential of lobster hatcheries, so this introduction presents the motivation and outline of the thesis in addition to some basic information on lobsters and human utilisation through fisheries and aquaculture.

Lobsters and conservation

The European lobster (*Homarus gammarus*, Linnaeus, 1758; Figure 1) is a large decapod crustacean from the coastal seas of the Northeast Atlantic, historically ranging from northern Norway and the Skagerrak, around continental Europe, to northern Morocco, including Britain and Ireland and the Azores, and throughout most of the Mediterranean and western parts of the Black Sea (Butler et al., 2013; Spanier et al., 2015; Figure 2). The species has been targeted as a seafood commodity for at least 500 years (Spanier et al., 2015), principally via the deployment of baited pots. Although the earliest management regulations designed to sustain *H. gammarus* fisheries date back almost 170 years, overexploitation has caused historic and contemporary stock collapses throughout extensive portions of the known spatial range (Dow, 1980; Spanier et al., 2015), from which population recovery has been slow or absent (Kleiven et al., 2012).



Gamma-rus. 42. C. macrourus, thorace lævi, roſtro lateribus dentato; baſi ſupra dente duplici. *Fn. ſvec.* 1248. *It. weſtrogoth.* 174. *Geſn. aquat.* 91. *Muſ. Ad. Fr.* 87. *Aldr. exſangu.* 112. *Matth. dioſc.* 227. *Jonſt. exſangu. t.* 71. *Rond. piſc.* 1. *p.* 538. *Habitat in Oceano imprimis* Norvegico: *in* America *ſeptentrionali. Kalm.*

Figure 1. The original binomial classification of the European lobster, from the 10th edition of Carl Linnaeus's *Systema Naturae*, published in 1758. The description roughly translates as, *"Smooth thorax; toothed sides of the rostrum; abdomen above double teeth. Habitat: chiefly in the ocean; Norway to Northern America."* The species was named as *Cancer gammarus* by Linnaeus but was reassigned to the new genus *Homarus*, described by Friedrich Weber in 1795, to which Henri Milne-Edwards added the only extant congener, *H. americanus*, in 1837.

Widespread and historic fishing effort and prolonged attempts at captive culture have ensured that the general biology of *H. gammarus* is relatively well known among marine invertebrates. However, some significant information gaps remain on the species' ecology and life-history which hinder the establishment of appropriate and enforceable fisheries legislation and the protection of key habitats (Addison & Bannister, 1994; Bannister et al., 1998; Linnane et al., 2001; Mercer et al., 2001; André & Knutsen, 2010; Ellis et al., 2015a). Effective stock management and ecosystem-level protection are both required to mitigate rising fishery pressure and global seafood demand (Fisheries and Aquaculture Department, 2016a), and are most likely to safeguard *H. gammarus* populations where they are soundly evidence-based. Where scientific data on wild *H. gammarus* are lacking, some studies are available based on individuals reared in captivity or the closely related American lobster, *Homarus americanus* (Milne-Edwards, 1837), which inhabits the eastern coasts of Canada and the USA and supports the world's most productive lobster fisheries (Wahle et al., 2013). However, there are likely to be some important differences between the two *Homarus* species, and between cultured and natural ontogenies.

Figure 2. Map of the range of European lobsters. A topographic map of Western Europe and Northern Africa, with the known recent range of the species (Butler et al., 2013) superimposed in yellow. Reproduced with permission from the International Union for Conservation of Nature.

Lobster lifecycle

In maturity

Homarid lobsters have an easily identifiable and charismatic morphology, with an exoskeleton of a fused cephalothorax from which the 10 legs emerge ventrally, and an articulated abdomen leading to a broad tail fan used for

rearward propulsion swimming when threatened (Wahle et al., 2013). Most characteristically, the two foremost legs are modified into large and powerful claws, with strong dimorphism in size and serrations (Elner & Campbell, 1981). Adult lobsters generally favour subtidal habitats at depths of less than 50m which provide crevices and voids in which they can shelter, such as rocky reef, boulder and cobble ecotypes, although also persist on soft sediments which allow burrowing (Wahle et al., 2013; Skerrit et al., 2015). Lifetime migratory behaviour is poorly understood in *H. gammarus*, though seasonal home ranges of adults may cover >1km^2 (Moland et al., 2011; Skerrit et al., 2015). *Homarus* spp. are cryptic throughout their lives, vacating home shelters to forage using chemosensory hairs and antennae during periods of poor visibility (Cooper & Uzmann, 1980; Moore et al., 1991). Foraging and omnivorous feeding behaviours are linked with prey availability and metabolic rate (Cooper & Uzmann, 1980; Skerrit et al., 2015). Dietary composition varies with ontogeny and stage in the moult cycle (Sainte-Marie & Chabot, 2002), though typically includes crabs, echinoderms, gastropods, bivalves, polychaetes, macroalgae and fish carcasses used as trap bait, which may contribute substantially to subsistence in heavily fished areas (Cooper & Uzmann, 1980; Saila et al., 2002; Grabowski et al., 2010; Wahle et al., 2013). *Homarus* spp. have no known size limit and show negligible senescence (Klapper et al., 1998; Elmore et al., 2008), though direct aging of individuals, which has been historically limited and prone to uncertainty (Sheehy et al., 1999; Kilada et al., 2012), may soon be realised via annual growth bands preserved in calcified gastric structures which are retained across moults (Kilada et al., 2015).

Reproduction

Estimates as to lobsters' size at the onset of sexual maturity (SOM) are prone to variation from differences in morphological, physiological and functional indices of maturation (Tully et al., 2001; Wahle et al., 2013), but probably also due to spatial variation between populations (Wahle et al., 2013). Female fecundity is size-specific (Tully et al., 2001; Agnalt et al., 2007; Agnalt, 2008; Ellis et al.,

2015b), with vast differences in egg production between first-time spawners and the largest females (e.g. <5,000 and >40,000 eggs, respectively – Agnalt, 2008), who show no reduction in reproductive potential despite presumably attaining significant ages. Lobsters are functionally iteroparous (having multiple reproductive cycles), although may effectively be semelparous (having a single reproductive event before death) in areas where fishing mortality is high (Fogarty & Gendron, 2004; Wahle et al., 2013), since minimum landing size (MLS) limits are typically aligned loosely to SOM (Tully et al., 2001). Adult *Homarus* spp. are normally solitary and territorial, but cohabit briefly during mating in the late summer and autumn (Wahle et al., 2013). The male's spermatophore is stored by the female until being used to externally fertilise the eggs during extrusion onto the ventral abdomen and pleopod appendages, where they are fixed throughout a long gestation of approximately 10 months (Latrouite et al., 1981; Atema & Voight, 1995; Aiken et al., 2004; Agnalt et al., 2007). Most egg development occurs in spring in response to rising temperatures, with the clutch hatching over one to two weeks during summer (Agnalt et al., 2007). Most females spawn and moult in alternate years, though mating during without moulting, moulting and extruding eggs in the same year, and respawning without re-mating have all been reported (Latrouite et al., 1981; Atema & Voight, 1995; Agnalt et al., 2007).

Pelagic larval life-stages

Pre-larval *Homarus* spp. hatch from developed eggs and cling to the remaining clutch for several hours until the mother frees them by shaking her abdomen and tail at night, by which time most have completed the moult to Stage I, the first of three fully planktonic larval instars (Phillips & Sastry, 1980, Wahle et al., 2013). Hatch is synchronised with spring tides, which may aide dispersal or simply provide the planktonic larvae with the relative cover of new moon darkness (Ferrero et al., 2002). Stage I larvae are positively phototactic and rheotactic (Schmalenbach & Buchholz, 2010), potentially to ensure they are retained in areas conducive to benthic settlement (Øresland & Ulmestrand,

2013), and are omnivorous and opportunistic feeders, able to filter-feed phytoplankton as well as actively hunting zooplankton (Phillips & Sastry, 1980). The significant logistical difficulties of capturing wild larvae means that planktonic development has only been properly assessed in captive environments, where larval duration (Stages I-III; Figure 3a) is principally governed by temperature and food availability (Gruffydd et al., 1975; Mackenzie, 1988). Most *H. gammarus* larvae become free-swimming and readily identifiable as clawed lobsters after two to three weeks, upon metamorphosis to Stage IV (Figure 3b), the first post-larval life-stage (Arnold et al., 2009; Daniels et al., 2010). Survival to this stage in aquaria is typically 5-25%, but is highly variable and reflective of genetic influences (Jørstad et al., 2005a, 2009; Moland et al., 2010).

Figure 3. Larval and post-larval stages of the European lobster. Hatchery-reared *H. gammarus,* at the first larval instar (Stage I – at left, top and side profiles), and the first post-larval instar (Stage IV – at right, side profile). Images scaled approximately 5:1, courtesy of the National Lobster Hatchery.

Benthic settlement

Within a few days of Stage IV metamorphosis, post-larvae begin diving to and from the seabed in search of substrates suitable for settlement (Wahle et al., 2013), with success likely to be limited by habitat availability and both inter- and intra-specific competition (Wahle & Steneck, 1991; Linnane et al., 2000a; Ball et

al., 2001; Incze et al., 2003; Jørstad et al., 2009). Juvenile ecology is mostly cryptic over the first one-to-two years (the 'early benthic phase', EBP; 5-25mm CL), but is much better understood in *H. americanus* than *H. gammarus* (Wahle et al., 2013), which have only been found sporadically in the wild (e.g. Linnane et al., 2000b), despite some co-ordinated attempts (e.g. Howard & Bennett, 1979; Mercer et al., 2001; Linnane et al., 2001). Juvenile *H. americanus* favour complex cobble and fringing habitats, from which thousands of settled post-larvae have frequently been collected, using methods that have failed to locate any *H. gammarus* juveniles in similar habitats at European locations (Wahle & Steneck, 1991; Mercer et al., 2001; Linnane et al., 2001; Inzce et al., 2003; Selgrath et al., 2007).

Experiments with hatchery-reared *H. gammarus* juveniles in mesocosm and wild environments have shown that cobbles, gravels and shells provide readily inhabitable interstitial spaces and that cohesive sediments support the construction of extensive tunnel systems (Howard & Bennett, 1979; Wickins et al., 1996; Linnane et al., 2000a; Jørstad et al., 2001, 2009). Predation, particularly by demersal fish, is a significant risk until shelter has been found (van der Meeren, 2000). Juvenile *Homarus* spp. are able to survive and grow on a diet of plankton only (Barshaw, 1989), but will also forage for food in the vicinity of the burrow (Wickins et al., 1996; Mehrtens et al., 2005), but are not encountered regularly until retained by conventional fishing gear at a carapace length of around 50mm.

European lobster fisheries

The period 2009-2013 (the most recently reported) saw record reported landings of European lobster, averaging 4917 tons per annum, of which over 77% was from the coastal fisheries of the UK (including the Channel Islands and Isle of Mann) and Ireland (Fisheries and Aquaculture Department, 2016a; Figure 4). While the majority of landings are attributable to commercial fishers, mainly working from vessels under 10m in length on trips which last a single day (CEFAS, 2014), capture by recreational fishers can contribute a major

proportion of fishing mortality in some areas, and reported figures for both sectors may seriously underestimate actual landings of *H. gammarus* (Kleiven et al., 2012). Unsustainable fishery extraction has caused widespread declines in *H. gammarus* abundance, with Scandinavian populations in particular suffering a severe collapse between the 1930s and 1970s, when landings declined by 90-99% in Sweden, Norway and Denmark (Dow, 1980; Agnalt et al., 2008; Kleiven et al., 2012).

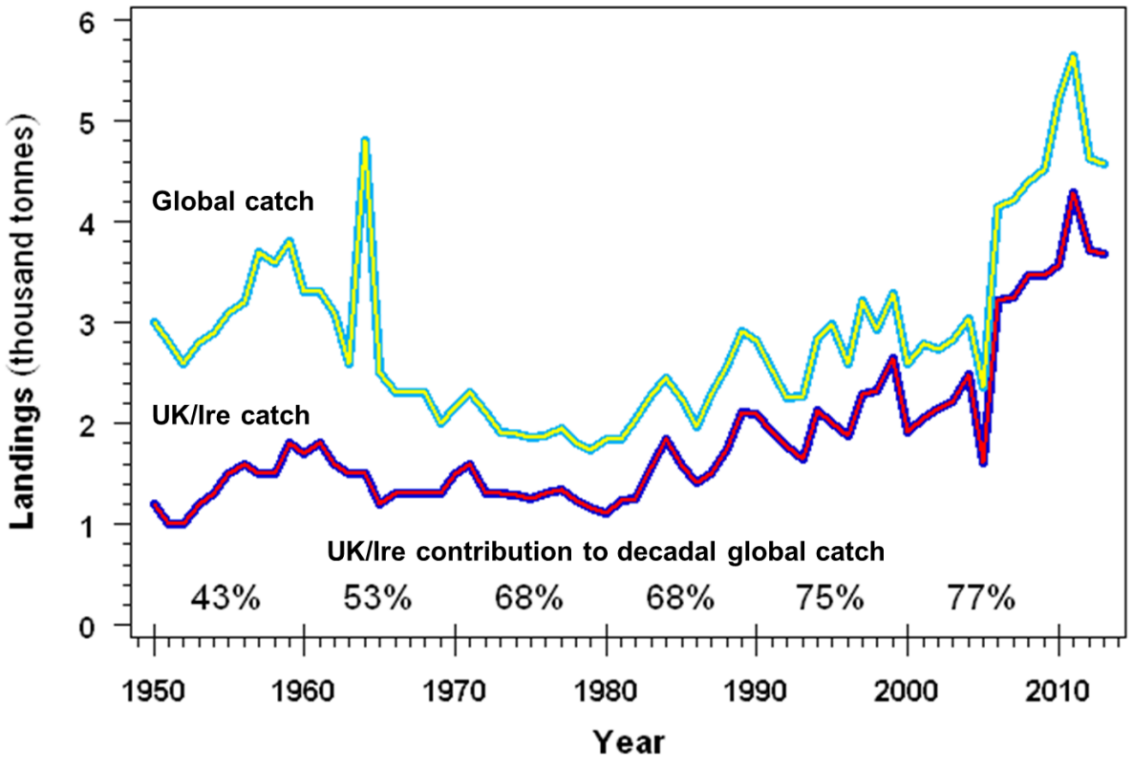


Figure 4. Recorded landings of *Homarus gammarus* from 1950-2013. Total catch of European lobsters, globally (blue/yellow plot) and solely from the UK* and Ireland (navy/red plot). Percentage figures show the proportion of the decadal global catch attributed to the UK* and Ireland. The spike in global landings in 1964 is caused by a recorded 2,200 t catch registered to Turkey, which is presumably erroneous as it is more than an order of magnitude greater than the national average from adjacent years in that decade. Increased landings since 1980 partly reflect improvements in data collection (e.g. Greece has average landings averaging 236 t since 1982, with a minimum of 89 t, yet zero landings are recorded before 1982). All data is courtesy of the Fisheries and Aquaculture Department (2016a) of the FAO. * = including the Channel Islands and Isle of Mann.

Lobster catchability and recruitment both appear to be improved by periods of increased water temperature (Dow, 1980; Sheehy & Bannister, 2002), although

in applications of conservation management or the identification of endogenous causes of fluctuations in abundance, the usefulness of landings data is seriously hindered by incomplete reporting (see Figure 4 caption), changes in fishing effort or locations, and bias inherent in the capture process (Addison & Bell, 1997; Browne et al., 2001; Bowlby et al., 2008; Kleiven et al., 2012; Sundelof et al., 2013). Even short-term stock assessments are largely absent for *H. gammarus* in most regions, although such studies yield limited information given temporal variations in population size or activity (Bowlby et al., 2007), and dynamic behaviours, such as the resident and migratory demographics observed in *H. americanus* (Dunnington et al., 2005; Bowlby et al., 2008). The lack of a method with which to reliably detect juvenile *H. gammarus* has inhibited the assessment of recruitment dynamics (e.g. Wahle & Steneck, 1991; Incze et al., 2003) that have been used to inform fisheries management for *H. americanus* (Wahle et al., 2013).

Lobster fishers and industry stakeholders have always played important roles in the proposal, implementation and assessment of conservation management, though as with many marine fisheries, the preservation of the industry has often taken precedence over that of the resource in the policies and practices of government and fishery managers and stakeholders (Dow, 1980). European lobsters are not currently subject to EU Total Allowable Catch (quota) regulation, though an 87mm CL MLS is applied throughout European waters. Tagged hatchery releases have shown this size may be attained within four to five years, but that at least seven year classes recruit to fishery MLS annually (Bannister et al., 1994; Sheehy et al., 1999). Spatial variabilities and uncertainties of SOM and the survivability of clutches from first-time breeders mean that MLS may not allow sufficient reproductive opportunity to sustain lobster populations where fishing pressure is high (Tully et al., 2001), and several alternative strategies have been pursued at local or regional levels which also aim to maintain a critical mass of spawning stock. These include closed seasons, closed areas, restrictions on the amount and type of gear, increased minimum size limits and landing bans on breeding individuals, as

identified by the bearing of eggs or a minor mutilation of the tail fan (v-notch) exacted by fishers (Hoskin et al., 2011; Butler et al., 2013; Moland et al., 2013; Øresland & Ulmestrand, 2013). Surprisingly, given the size-specific nature of egg production, maximum landing sizes have not been employed until the recent introduction of a moratorium on landing females of 155mm CL or more in Scotland (Marine Scotland, 2015). The enforceability of these legislations varies and is generally hampered by the spatial fragmentation of administrative boundaries, since it often relies on the establishment of catch provenance, a serious challenge where fishers work across multiple jurisdictions.

European lobster aquaculture

Context and concept

With most capture fisheries stagnating under intense pressure due to growing human populations, aquaculture sectors have become an increasingly important means of meeting demand, and are expected to become the predominant mode of seafood production in coming years (Fisheries and Aquaculture Department, 2016c). There are three major aquaculture sectors: product enhancement, resource enhancement and full grow-out (Butler et al., 2013; Radhakrishnan, 2015). Product enhancement, the captive on-growth of wild-captured stock to improve marketability (Radhakrishnan, 2015), is limited in *H. gammarus* as it is protected by fishery MLS and unsuited to communal rearing techniques (Wahle et al., 2013), although some industry stakeholders store lobsters over periods of weeks or months to take advantage of seasonal variation in market price. Full grow-out, the captive culture of wild or hatchery stock in aquaria and/or sea-based containers to marketable sizes, has been attempted in recent years. Rearing is complicated by the willingness of lobsters to cannibalise in the confines of captivity (Cooper & Uzmann, 1980), although technological progress has been made (e.g. Drengstig & Bergheim, 2013; Daniels et al., 2015). Aquaria-based grow-out has yet to realise economic viability (Kristiansen et al., 2004; Drengstig & Bergheim, 2013), but potential may exist in the on-growth of

hatchery-reared juveniles in sea-based containers (Beal et al., 2002; Benavente et al., 2010; Daniels et al., 2015), which avoid most of the rearing costs associated with aquaria-based operations. The majority of aquaculture-based initiatives using *H. gammarus* have focussed on the potential of resource enhancement (Wahle et al., 2013); the improvement of wild capture fishery harvests via the release of hatchery-reared juveniles (stocking), either by restoring depleted or locally extinct stocks (restocking), or by augmenting natural recruitment to increase/sustain harvest yields (stock enhancement – Bell et al., 2005, 2006, 2008). The following chapter provides an extensive review of the progress of European lobster stocking, so only a brief overview is given here.

Hatchery stocking of European lobsters

Hatchery stocking aims to overcome recruitment limitations in wild populations by rearing offspring through vulnerable life stages in aquaria-based life support systems, in which survival is assumed to be greatly elevated from that in the wild due to the absence of interspecific predation, before wild release at a less vulnerable life-stage (Nicosia & Lavalli, 1999; Jørstad et al., 2005a). In principal, hatchery stocking is best suited to aquatic species which demonstrate high fecundity but low survivability of offspring, and which are incompatible with methods of full grow-out aquaculture (Addison & Bannister, 1994; Lorenzen, 2005, 2008). The technique should be well suited to *H. gammarus*, a high-value species with a history of stock collapse and early life-stages which are presumed to be considerable recruitment bottlenecks in nature (Wahle & Steneck, et al., 1991; Bannister & Addison, 1998).

Aquaculture-based rearing of *H. gammarus* has been attempted for over 150 years, and has driven the majority of scientific research on the species (Nicosia & Lavalli, 1999). Rearing success has improved considerably as technological and scientific advances have been made (Nicosia & Lavalli, 1999), although it remains highly variable (Jørstad et al., 2005a) and near-total mortality can afflict some cohorts or periods of the rearing season. For many years the progress of

lobster stocking initiatives has been hindered by a lack of life-history knowledge that would allow the optimisation of rearing environments and release protocols (van der Meeren, 2005), and while some rigorous monitoring of released lobsters has been achieved (Bannister & Addison, 1998), this is arguably not of the requisite capacity on which to base overall assessments of the economic and ecological value of hatchery stocking.

Study motivation

Given the collapses suffered by many stocks across the species' range, the need to improve our understanding of *H. gammarus* ecology is particularly urgent to ensure currently healthy populations can be conserved effectively. The same need applies to hatchery stocking, for which improved management and monitoring is required to provide rigorous evaluation of potential and realised impacts on lobster populations and fisheries. This thesis is a collection of studies which address specific knowledge or methodological deficits relating to European lobster ecology and conservation management.

Outline of thesis

The aim of this thesis is to make a meaningful contribution to existing information on the European lobster, so that fisheries management and conservation initiatives are better equipped to safeguard the species' future. Particular attention has been paid to ensure that experimental findings are related to their implications for, and potential applications by, organisations currently attempting to contribute to lobster fisheries sustainability, especially via means of hatchery stocking. One such organisation, the National Lobster Hatchery in Cornwall, UK, have provided collaboration and a focus for the objectives of this research.

Briefly, the ambitions of this thesis are as follows: to review the performance of hatchery stocking as a tool for the conservation of lobster fisheries, and to highlight what further information is required to ascertain the impact of lobster

stocking; to characterise the size-specific fecundity relationship of lobsters in Cornwall, and assess whether variation in this trait across the species range, postulated to be caused by methodological inconsistencies, may alternatively be explained by heterogeneity in environmental temperature; to elucidate the fertilisation ecology of individual clutches in European lobsters, in order to quantify the frequency of multiple paternity, and any proportional or spatial skew in sire representation; to define the population genetic diversity and population structuring of European lobsters, at a broad scale across the range, and at a fine scale throughout Cornwall and the Isles of Scilly, and; to evaluate the power and error rates of distinguishing hatchery-reared stock from natural conspecifics among admixed populations using parentage assignment as a mode of genetic tagging.

Overview by chapter

In Chapter 2, an extensive review of the scientific literature provides the basis for an overview of the achievements and limitations of monitored trials of hatchery stocking of the European lobster. Recent findings from other stocked species, especially issues which have not been considered or assessed in lobsters, are summarised in order to present a blueprint for future investigation required to properly manage and assess the impacts of lobster stocking.

Chapter 3 presents a regional measurement of female fecundity for European lobsters in Cornwall, a vital parameter for the estimation of egg production and reproductive potential in this important local fishery. This size-specific relationship is compared to others obtained from across the northern portion of the species range in order to test whether apparent spatial variation in clutch size observations may be indicative of the evolutionary divergence of populations under geographic and environmental heterogeneity, as opposed to methodological inconsistencies, an alternative hypothesis postulated elsewhere.

In Chapter 4, the paternal contributions to individual egg clutches are reconstructed via microsatellite genotyping, to elucidate whether European lobster broods are typically sired by single or multiple males. In recent years, the widespread availability of molecular markers have led to a number of studies of paternity in crustaceans, which have discovered that paternity dynamics vary between species and often show fluidity and spatial variation within species. Only a pair of unconfirmed reports of European lobster fertilisation structure exist, so this study aimed to reveal the paternity dynamics of lobsters from an important regional fishery subject to hatchery stocking. Aside from providing knowledge of a basic and important aspect of lobster reproduction, the results of this study have implications for the power and potential of parentage-based tagging.

Chapter 5 addresses the molecular ecology of the European lobster, information of which is scant and/or collected using outdated methods. A panel of microsatellite markers, three of which are newly developed, are utilised to assess the population genetic diversity, differentiation and structure of lobster samples encompassing both a micro-geographic scale and an extensive portion of the species' total distribution. Because it has not been possible to make physical observations of larval dispersal, genetic characteristics can be used to imply the boundaries of lobster populations and the connectivity between them. Such information is crucial to the creation of informed conservation management strategies, and especially to ensure hatchery interventions do not disrupt natural population structure.

In Chapter 6, the microsatellite genotypes obtained in the two preceding chapters are used to investigate whether parentage assignment may present a viable alternative to restrictive physical tags in the identification of hatchery-reared lobsters in the wild. I test whether the available markers provide the power required to allocate released individuals to hatchery parents while excluding natural stock, and how this is affected by the composition of admixed stocks. The validation of an affordable molecular

method to identify the offspring of particular lobsters would present a pathway to enable robust, comprehensive and comparative study of the impacts of hatchery stocking and other fisheries management strategies.

Chapter 7 draws the findings of this thesis together to help place them in the wider context of European lobster ecology, conservation and hatchery stocking, highlighting where limitations in the research warrant further study and proposing which further work should be prioritised in the near future.

A complete bibliography combining references from all chapters is found at the end of this thesis, after an appendix with supplementary material.

Chapter 2: European lobster stocking requires comprehensive impact assessment to determine fishery benefits

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Abstract

Historically, hatcheries in Europe and North America attempted to contribute to the conservation and enhancement of clawed lobster stocks, but lacked monitoring programmes capable of assessing success. In the 1990s, this perspective was changed by the results of restocking and stock enhancement experiments that inserted microwire tags into hatchery-reared juvenile European lobsters (*Homarus gammarus*) before release. This allowed recapture in sufficient numbers to prove that lobsters had survived and recruited to the mature fishable stock. However, evidence of recruitment still failed to answer key questions about the ultimate ecological and economic benefits. As a result, a growing number of lobster stocking ventures remain hindered by a lack of clear evidence of the effects of their stocking schemes. This review evaluates these experiments and related studies on other fished species, summarizes key findings, and identifies data and knowledge gaps. While studies of fitness in cultured lobsters provide some of the most encouraging results from the wider field of hatchery-based stocking, the limitations of physical tagging technology have significantly hindered appraisals of stocking impacts. We lack fundamental knowledge of lobster ecology and population dynamics, especially among pre-recruits, and of the impact of stocking on wild lobster population genetics. We advocate the use of genetic methods to further our understanding of population structure, rearing processes, and stocking success. We also recommend that more focused and comprehensive impact assessments are required to provide a robust endorsement or rejection of stocking as a viable tool for the sustainable management of lobster fisheries.

Introduction

Capture fisheries make crucial contributions to the world's wellbeing and prosperity. The global value of fisheries was estimated at over €65 billion per annum in 2010, ca. 10% of the world's population are dependent on fish-related jobs, and seafood products are a vital source of protein and micronutrients for 3 billion people (Fisheries and Aquaculture Department, 2016b). Commonly, however, conventional management fails to prevent the overexploitation of stocks. Interventions that use hatchery technology to improve or re-establish the productivity and sustainability of capture fisheries, which can be categorized as "stocking", are, therefore, worth considering. For many aquatic species, the survival of juveniles in aquaculture facilities is several orders of magnitude higher than in the wild, allowing increased recruitment above natural levels (Lorenzen, 2005). Stocking schemes aim to improve and sustain capture fisheries and are categorized as either "restocking" (the release of cultured juveniles to restore spawning biomass) or "stock enhancement" (the recurrent release of cultured juveniles to overcome recruitment limitations) (Bell et al., 2006). Lorenzen (2008) advocates that aquaculture-based enhancement of stocks ranks alongside regulation of fishing effort and restoration of key habitats as a principal means by which wild fisheries can be sustained and improved.

With many capture fisheries under intense pressure, aquaculture technologies have become an increasingly important means of seafood production, largely through the full grow-out of marketable fish, but also by restocking and stock enhancement of wild populations. Hatchery stocking is undertaken worldwide and has been most successful in large-scale schemes coordinated and funded by government or industry. For example, the government-financed programme in Japan alone involves the enhancement of >80 marine species (Kitada, 1999) and is estimated to account for 90% of the chum salmon (*Oncorhynchus keta*) fishery, 50% of the kuruma prawn (*Penaeus japonicus*) and red sea-bream (*Pagrus major*) catch, 30% of the flounder (*Paralichthys olivaceus*), and almost all the scallop harvest (Kitada *et al.,* 1992; Kitada and Kishino, 2006). However, the contribution of stock enhancement to global fisheries production has remained small (~2%), and few case studies have been declared outright successes (Lorenzen, 2008). Overall, the available literature appraising the

impact of stocking is heavily biased towards certain finfish; Araki and Schmid (2010) found that 62% of genetic-based stocking impact studies evidenced salmonids, flatfish, and bream, despite these groups accounting for only 5% of the catch tonnage of enhanced fisheries.

For many years, the progress of stocking enterprises was hindered by a lack of appropriate research into wild life histories and by a lack of effective methods for distinguishing released individuals from wild conspecifics. As a result, robust evaluation of the economic and ecological benefits of stocking has been impeded, restricting impetus within the industry. Extensive knowledge of the ecosystem, species biology, and population-specific data is required for the design of successful stocking programmes. For example, of eight species across a variety of taxa cultured in Japan reviewed by Kitada (1999), six showed significant variation in the effectiveness of stocking with differing release locations and/or release densities. The method, timing, and recipient habitat of releases and the density, size, and conditioning of released animals can all have significant effects on survivability (van der Meeren, 2000; Ball et al., 2001; Stunz & Minello, 2001; Svåsand et al., 2004; Leber et al., 2005; Hamasaki & Kitada, 2008a; Ochwada-Doyle et al., 2010).

The focus of this review is the European lobster (*Homarus gammarus* L.), an ecologically and economically important decapod crustacean ranging from northern Norway to Morocco and the eastern Mediterranean (Triantafyllidis et al., 2005). Global catches of European lobster have been increasing since the 1980s, with recent recorded pot-caught landings reaching 5,913 t in 2011 (Fisheries and Aquaculture Department, 2016a). Compared to many finfish or the recent very large landings of the American lobster (*Homarus americanus*) in North America (e.g. 50,000 t in Maine – Steneck & Wahle, 2013), European lobster landings are small and come from sparse stocks. The species is of very high value, however, fetching an average market price of €12.50 kg^{-1} at the time of writing (Fish Information and Services, 2014). Therefore, lobster populations are disproportionately important to local fishing communities and regional economies as well as fulfilling key roles in the maintenance of healthy and diverse marine ecosystems (Mann and Breen, 1972; Breen & Mann, 1976). Aquaculture-based augmentation of wild Homarid lobster populations has been

attempted on both sides of the North Atlantic for over 150 years using numerous release strategies and life history stages (Nicosia & Lavalli, 1999). Because enhancement of existing populations was difficult to identify, few of these experiments have been assessed in terms of benefits to fisheries (Addison & Bannister, 1994; Nicosia & Lavalli, 1999). Lobster hatcheries have provided most of the recorded information on clawed lobster life history (Nicosia & Lavalli, 1999), but significant voids still exist in our understanding of the species' basic ecology.

The basic technology to rear lobsters through the planktonic phases has long been available. This lifestage is presumed to be an important recruitment bottleneck due to predation in the wild (Richards & Wickins, 1979; Bannister & Addison, 1998). However, efforts to trial the stocking of lobsters were renewed in Europe throughout the 1980s-1990s in response to three key drivers. First was a severe collapse of the fishery throughout Scandinavia from 1930 to 1970 due to overexploitation and inadequate management, which saw landings decline 99% in Denmark, 92% in Norway, and 90% in Sweden, all but wiping out a once-thriving export commodity (Dow, 1980; Agnalt et al., 1999; Fisheries and Aquaculture Department, 2016a). This led to aspirations to restock depleted populations as well as to enhance stocks where uncapped potting effort rose in response to new continental export opportunities, such as the UK (Bannister, 1986). Second, it was demonstrated that hatchery-reared lobsters acquired benthic, shelter-seeking behaviours (Cobb, 1971; Cooper & Uzmann, 1980; Botero & Atema, 1982) that might decrease their vulnerability to wild predators and hence improve survival (Howard, 1980, 1988). Third, the development of coded microwire tagging (CWT) technology (Jefferts et al., 1983) allowed cultured juvenile lobsters to be distinguished from wild conspecifics after release (Wickins et al., 1986; Bannister & Addison, 1998).

Experimental lobster stock enhancement programmes were launched to release large numbers of juvenile lobsters onto known lobster grounds at a range of sites in France (Henocque, 1983; Latrouite & Lorec, 1991), the UK (Burton, 1992; Bannister et al., 1994; Cook, 1995), and Norway (Agnalt et al., 1999, 2004; Agnalt, 2008). Coded microwire tags were inserted into late-stage juveniles prior to release, and their recapture provided the first definitive

evidence that cultured lobsters were able to survive in the wild. In both UK stock enhancement and Norwegian restocking trials, cultured lobsters were shown to attain adult sizes (Bannister et al., 1994; Agnalt et al., 1999) and add to spawning-stock biomass (Bannister et al., 1994; Agnalt, 2008). Restocking also showed that released lobsters could augment rather than simply displace natural stocks (Agnalt et al., 1999, 2004). While most of these studies declared the renewed lobster stocking efforts as tentatively successful, it was also proposed that production costs and lobster market values did not make the observed recapture rates economically viable (Whitmarsh, 1994; Moksness et al., 1998).

In this review, we summarize current practices in lobster stocking and reappraise the measurement of stocking success and the practices of monitored stocking trials. We then highlight critical issues for lobster stocking, including hatchery production methods, understanding the ecology of lobsters in the wild in order to optimize success of released lobsters, and genetic considerations. Finally, we address the problem of comparing stocking to alternative management strategies and conclude by suggesting future research directions.

Hatchery rearing of European lobsters

The rationale for current European lobster cultivation is typical of hatchery enterprises. Fishery stakeholders are attracted to stocking where other management options are limited or unappealing. Intensive developments in husbandry, infrastructure, and stakeholder engagement are required to establish a lobster hatchery, and significant gaps remain in our understanding of aspects of the biology and ecology of *H. gammarus*. Nevertheless, severe stock depletions, high market value, and well-functioning rearing technology continue to encourage new lobster stocking efforts in Europe (Svåsand et al., 2004).

Female lobsters, bearing eggs fertilized naturally in the wild, are typically bought or loaned from fishers or merchants and are held until the larvae have hatched. Larvae are normally reared communally through the planktonic lifestages (Zoea larval stages I–III and post-larval stage IV) in tapered hoppers or

Hughes/Kreisel cones in which upwelling air and/or water reduces settling and cannibalization (Richards & Wickins, 1979; Beard et al., 1985; Grimsen et al., 1987; Beard & Wickins, 1992; Burton, 1992; Cook, 1995; Nicosia & Lavalli, 1999; Daniels et al., 2010). Survival of the planktonic phase is highly sensitive and variable even in the captive environment, and although individual batches may attain survival >50%, typically 10-15% of stage I larvae reach the onset of benthic behaviours a few days after moulting to Stage IV (Burton, 1992; Nicosia & Lavalli, 1999; Daniels et al., 2010). The absence of interspecific predation suggests that cultured larval survival is likely to far exceed that of wild larvae, although the scarcity with which wild conspecifics are found (Nichols & Lovewell, 1987) means that no reliable estimates of natural survival exist for comparison. Once they attain stage IV, post-larvae have a much greater swimming ability and are generally then separated into individual holding compartments for on-growing before being released into wild environments at an early benthic juvenile phase.

Over 1.4 million cultured juvenile European lobsters have been released by known stocking programmes between 1983 and 2013. Of these releases, 90% can be classified as stock enhancement of existing commercial fisheries around the UK, Ireland, and France, and 10% as restocking heavily depleted populations in Norway, Germany, and Italy (Table 1). Approximately 255,000 released lobsters (mostly in Norway and the UK) were grown on to late juvenile stages (12-21 mm carapace length (CL) – Latrouite & Lorec, 1991; Burton, 1992; Cook, 1995; Bannister & Addison, 1998; Agnalt et al., 1999; Schmalenbach et al., 2011) and tagged to allow wild survival to be monitored. More recently, stock enhancement programmes in Orkney, Scotland, and Cornwall, England and restocking trials in Roma, Italy have released some 900 000 untagged juveniles at earlier life-stages (stage V+, >5 mm CL – D. Shearer, pers. comm; G. Nascetti, pers. comm.).

Table 1. Summary of major and/or widely reported stock enhancement projects for European lobsters, 1972-2013.

Location (*Hatchery*, Area)	Release years	Monitoring years	Release age/stage	Number released	Number recaptured	Recapture ratio (% of released)	Source reference
France (Ile de Sein; Ile d'Yeu; Ile de Houat)	1972-1977	-	Stage 5 to 1 year	~265,000	-	-	Henocque (1983)
France (Ile de Sein; Ile d'Yeu; Ile de Houat)	1978-1983	1980-1983	~ 1 year	†1,300	0	-	Latrouite & Lorec (1991)
UK (*MAFF*, Bridlington; *NWSFC*, Aberystwyth; *SFIA*, Ardtoe & Orkney)	1983-1990	1985-1994	~ 1 year	*90,925	1,471	1 : 62 (1.6%)	Bannister et al. (1994); Cook (1995); Burton (1993); Bannister & Addison (1998)
France (Ile de Sein; Ile d'Yeu; Ile de Houat)	1984-1987	1987-1989	~ 1 year	*25,480	22	1 : 1,158 (0.1%)	Latrouite & Lorec (1991)
Norway (Kvitsøy)	1990-1994	1992-2001	~ 1 year	*127,945	7,950	1 : 16 (6.2%)	Agnalt et al. (2004)
Ireland (Galway; Wexford)	1993-1997	-	Stage 4-5	~292,000	-	-	Browne & Mercer (1998)
Germany (Helgoland)	2000-2005	2001-2009	~ 1 year	*~5,400	487	1 : 11 (9.0%)	Schmalenbach et al. (2011)
UK (*OSFH*, Orkney)	2000-2013	-	Stage 4-10	~747,000	-	-	D. Shearer, pers. comm.
UK (*NLH*, Cornwall)	2002-2013	-	Stage 5-10	~150,000	-	-	This paper
Italy (*CISMAR*, Viterbo)	2010-2013	-	Stage 4+	~10,000	-	-	G. Nascetti, pers. comm.
TOTAL	**1983-2013**	**1985-2009**	**Stage 4 to ~1 year**	**~1,714,947 (249,750 tagged)**	**9,930**	**1 : 25 (4.0%)**	**-**

***** = tagged. **†** = *Homarus gammarus* × *Homarus americanus* hybrids; "phenotypically marked", but omitted from tagged release total.

Assessments of lobster stocking success

Monitored stocking trials

Long after the development of the requisite technology to rear lobsters through the larval phases for release as juveniles, the success of early stocking programmes still could not be formally evaluated (Addison & Bannister, 1994). Ecdysis (exoskeletal moulting) precludes the use of externally-fixed markers in lobsters, particularly juveniles which moult frequently. As a result, there was no lasting method to discriminate between hatchery-reared and wild individuals. Whether released animals survived and actually enhanced natural stocks

(instead of displacing them) was unproven, proponents of stocking were unable to demonstrate whether the method provided any benefits to fisheries (Addison & Bannister, 1994).

Flawed attempts to recognize recaptured hatchery-reared individuals led to the trial release of 1300 *H. gammarus* × *H. americanus* hybrid juveniles in France during the 1970s (Latrouite & Lorec, 1991), despite no evidence for their ecological suitability and the scheme relying on local fishers identifying precise morphological variations in surviving hybrids. Extensive interannual fluctuations in landings inhibited the usefulness of fishery capture statistics in quantifying stocking success (Le Gall et al., 1983), but the advent of the first suitable internal tagging methods in the early 1980s encouraged three groups in France, Norway, and the UK to commit significant resources to new experimental stocking programmes (Bannister & Addison, 1998). These projects (Table 1, entries 3-5) reared and released a total of 244,350 late-stage juveniles. The insertion of magnetized, batch-coded CWTs offered the prospect of detecting survivors and evaluating the contribution of stocking to fisheries. Since these experiments concluded in the late 1980s or early 1990s, only one further scientific assessment of *H. gammarus* stocking has been reported: 5400 one-year-old lobsters were tagged with visible implant elastomers (VIE – Uglem et al., 1996) and released during 2000-2005 on the German island of Helgoland (Schmalenbach et al., 2011). Monitoring of these projects has enabled the identification of cultured lobsters through to adult sizes and currently provides all of the data available with which to assess the effectiveness of lobster stocking in Europe (Latrouite & Lorec, 1991; Burton, 1992; Bannister et al., 1994; Cook, 1995; Bannister & Addison, 1998; Agnalt et al., 1999; Agnalt et al., 2004; Schmalenbach et al., 2011).

There were many differences of detail in relation to release sites and methods, local fishing effort and legislations, and monitoring patterns both within and among the groups undertaking European stocking trials. However, hatchery rearing protocols were largely shared and, with little information about the habitat requirements of prerecruit lobsters, all groups released juveniles into areas populated by adults. Release numbers were maximized but dispersed in relatively small batches to reduce potential competitive interactions. Each group

released a succession of annual juvenile cohorts over four or more years and, in most cases, monitored stocks and landings for at least a comparable period to estimate survival and the proportion of tagged lobsters in the fishable stock (Latrouite & Lorec, 1991; Burton, 1992; Bannister et al., 1994; Cook, 1995; Bannister & Addison, 1998; Agnalt et al., 1999; Agnalt et al., 2004; Schmalenbach et al., 2011).

In France, Norway, and the UK, recaptured lobsters fitted with CWTs were detected using magnetic detectors on board potting vessels or at quayside landing stations (Bannister & Addison, 1998), while VIE-tagged lobsters in Germany were identified visually by fishers and divers (Schmalenbach et al., 2011). The recapture profiles of release cohorts typically illustrated common sequences of growth, accumulation, and decay over the monitoring period. Annual recaptures were largely on the scale of tens to hundreds, cumulating to a total of 9930 individuals across all monitored projects, mostly recaptured 3-10 years after release as sub-adults or adults in the size range 50-120 mm CL (Burton, 1992; Bannister et al., 1994; Cook, 1995; Bannister & Addison, 1998; Agnalt et al., 1999; Agnalt et al., 2004; Schmalenbach et al., 2011). Released lobsters generally showed high site fidelity (e.g. recaptured within 6 km of release sites – Bannister & Howard, 1991), and many of the adult females carried fertilized eggs, although whether these were sired by wild or cultivated males was not assessed.

Recapture rates

Monitoring of hatchery-reared European lobster recruitment has shown that releases in the order of 100 tagged juveniles have typically yielded single-figured numbers of recaptures (Table 1) (Bannister & Addison, 1998; Agnalt et al., 2004; Schmalenbach et al., 2011). These nominal recovery rates were regarded as indicators of the potential contribution to the local fishery, but also of the potential economic rates of return (Whitmarsh, 1994).

Stocking trials in France provided the least encouraging total recapture figures (Table 1), although these results can be somewhat discounted due to deficiencies in their monitoring programmes. Although CWTs were implanted

into 24,500 juveniles released around the French Atlantic coast during 1984-1987, monitoring began two years after the first releases, but lasted only three years (Latrouite & Lorec, 1991). Only 22 lobsters were recaptured, but the maximum recapture window (2-5 years for different release cohorts) appears insufficient in light of the recapture profiles of later trials elsewhere. At that same time in the UK, almost 91,000 year-old juveniles were tagged and released in four areas – Bridlington in England, Aberystwyth in Wales, and Ardtoe and Orkney in Scotland – where the natural stocks were depleted (though still more abundant than in Norway). Total recaptures were 1,471 over the 5-8 year monitoring period, with the regional recovery rates ranging from 1.3 to 2.4% (Bannister & Addison, 1998).

Higher recapture results came several years later from the heavily depleted lobster stock in the Norwegian archipelago of Kvitsøy. By 2001, 6.2% of the 128,000 coded-wire-tagged year-old juveniles released during 1990-1994 had been recaptured, and released lobsters outnumbered wild conspecifics amongst the legal-sized catch (Agnalt et al., 2004). Importantly, both the proportion of hatchery-reared lobsters in the fishable stock and catch per unit effort (CPUE) increased over the monitoring period, suggesting that cultured lobsters had enhanced existing stocks rather than replacing them (Agnalt et al., 1999; Svåsand et al., 2004). Most recently, off Helgoland, >9% of the 2000-2005 release cohorts had been recaptured by 2009, when 8% of the total landings comprised hatchery-reared lobsters (Schmalenbach et al., 2011). Of those lobsters released in 2001, 1 in 7 were recaptured, the highest rate recorded for any stocked *H. gammarus* cohort (Schmalenbach et al., 2011).

Projections and perceptions of success

The results of European projects have produced very different perceptions about the potential worth of lobster stocking. In France, the low number of recaptures caused an abrupt and premature termination of the monitoring programme (Latrouite & Lorec, 1991). In the UK, the results were welcomed as the first definitive proof of successful survival and recruitment of cultivated lobsters in the wild (Bannister, 1995; Bannister & Addison, 1998). However, modelling showed that recovery rates were too low to generate a positive net

value to the fishery, even when offsetting the costs of building a hatchery over a 25-year release period (Whitmarsh, 1994). In Norway, the high proportional contribution to the depleted stock was viewed positively (Agnalt et al., 1999; Svåsand et al., 2004), though production costs exceeded the value of recaptured lobsters here too (Moksness et al., 1998). In a global context, lobster stocking in Norway gave more efficient fishery yields than those of prawn or crab enhancement in the Far East (Hamasaki & Kitada, 2008b).

Although none of these monitored European stocking trials generated total recapture rates of even 10% of the number of lobsters released (Bannister & Addison, 1998; Agnalt et al., 1999; Nicosia & Lavalli, 1999; Agnalt et al., 2004; Schmalenbach et al., 2011), some studies have estimated more encouraging survival rates from speculative calculations of capture probability. For hatchery-reared lobsters in Helgoland, survival rate to the fishery minimum landing size (MLS) was estimated to be 30-40% using the Lincoln-Peterson method (Schmalenbach et al., 2011). When converted via an independent estimate of trap catchability, recapture numbers produced very high survival estimates of 50-84% for individual release sites in northeast England (Bannister et al., 1994). Norwegian recaptures provided more tangible evidence of success by showing that cultured lobsters contributed significantly to spawning biomass. Within 4-10 years of release, cultured females were estimated to account for 27% of egg production within the Kvitsøy population and showed no difference to wild females in measures of fecundity or egg development (Agnalt et al., 2007; Agnalt, 2008).

Fitness of hatchery-reared lobsters

Studies from stocked populations in Norway provide the only direct evidence of the fitness of cultured *H. gammarus* in the wild, with ecological and genetic indicators used to assess pre- and post-release fitness. Mature cultured females appear to perform as well as wild equivalents in terms of size-specific fecundity, weight of egg mass, egg size, and embryonic development (Agnalt, 2008), a crucial finding rarely achieved among other stocked species. Results have been less conclusive when rearing the offspring of wild and cultured broodstock together in competitive, “common garden” environments. The

progeny of cultured females recaptured around Kvitsøy, Norway experienced only 60% of the survival of the offspring of local wild females through both the larval and juvenile phases (Jørstad *et al.,* 2005a, 2009). While in isolation, this represents a damaging assessment of the fitness of cultured lobsters, results were confused by the performance of a second group of wild females that originated just 12 km away, but whose offspring were similarly outperformed by those of local natural females. Perhaps most tellingly though, the authors acknowledged that both wild and cultured males had access to mate with either cohort of females (Jørstad et al., 2005a, 2009), which may have significantly biased the categorization of offspring as wild- or hatchery-derived, particularly within a population where natural and cultured lobsters were fairly evenly represented (Agnalt et al., 2004).

Limitations of existing impact assessments

Existing assessments of lobster stocking success are susceptible to caveats and assumptions. The recapture numbers cited in Table 1 were not corrected for (i) tag loss, which would yield false negatives and underestimates of survival among tagged lobsters (Agnalt et al., 2004); (ii) emigration to adjacent areas, which would reduce the number of marked lobsters available for recapture (Cook, 1995); (iii) spatial mismatch between release and resampling sites; and (iv) imperfect recapture sampling by quayside monitoring teams.

These issues have not been factored into the lobster survival estimates of any impact assessment, suggesting that the recorded recovery rates cited in Table 1 were almost certainly underestimates. As such, pessimistic assessments of the economic viability of lobster stocking by Whitmarsh (1994) and Moksness *et al* (1998) were probably based on pessimistic estimates of the survival of cultured lobsters. More fundamentally, these economic assessments evaluated the viability of stocking programmes to be run purely as self-financing businesses and failed to account for the long-term potential of hatchery-reared lobsters to boost or restore local recruitment. Additionally, this appraisal technique fails to account for any potential benefits of raising the profile of lobsters and sustainable fishing among the public.

Summary of stocking performance and current hatcheries

Stocking has been proven to be a potentially effective method of fisheries remediation (Bannister & Addison, 1998; Svåsand et al., 2004). Despite uncertainties in the magnitude of the recovery rates, monitoring of *H. gammarus* releases have shown that hatchery-reared lobsters have survived, grown, and mated in the wild in considerable numbers and in multiple locations and ecotypes. However, there remains considerable scope to improve our knowledge of the ecological dynamics influencing stocked lobster survival and to standardize methods of lobster stocking and assessments of its impact.

Interest in undertaking European lobster stocking has soared in recent years as a tool to conserve and improve fisheries and even to mitigate proposed offshore developments (e.g. pipe-laying, wind farms, spoil dumping). Currently, there are two established hatcheries in the UK undertaking stock enhancement on a relatively significant scale. These programmes operate in the Orkney Islands and Cornwall (Table 1), where the continued pressure on lobster stocks and the economic importance of the fishery justify the concept of engaging in stock enhancement. They are responsible for over half of the reported releases of cultured lobsters into European waters in the past four decades, but neither programme has ever undertaken routine monitoring of their effects. This is mostly due to the prohibitive costs incurred in growing juveniles to sizes suitable for physical tagging and subsequent monitoring of the wild population for recaptures (D. Shearer, Orkney Lobster Hatchery, pers. comm.). For scientific support, they refer to the basic impact assessments already described; Orkney was one location of the 1980s mark-recapture trials, while Cornish enhancement endeavours are based entirely on the experimental results from outside Cornwall.

Both hatcheries have been active in undertaking research and developing technical innovations to more effectively and economically rear lobsters. They are aware that reducing expenditure per juvenile produced is a principal method of increasing their economic viability, alongside increasing the survival probability of hatchery-reared lobsters in the wild. These hatcheries also accept their obligation to validate the impact of their stocking programmes, but have

been unable to self-subsidize the comprehensive ecological research and monitoring required. What follows is a summary of several aspects of marine stocking that are critical to resolve in order to improve or perhaps even disprove the value of releasing cultured lobsters for stock management.

Critical issues for lobster stocking

Understanding lobster ecology

Knowledge gaps regarding the ecology and population dynamics of *H. gammarus* significantly obstruct the unbiased assessment of the performance of hatchery stocking. The most serious of these is the continued absence of methodologies for locating or capturing wild post-larvae and juveniles, despite coordinated efforts (e.g. Linnane et al., 2001; Mercer et al., 2001). As a result, it is unknown whether recruitment is density-dependent and, therefore, limited by habitat-specific carrying capacities (as it is in *H. americanus* – Wahle & Steneck, 1991, 1992; Wahle & Incze, 1997; Steneck & Wahle, 2013), and we have no understanding of how cultured lobsters compare to wild equivalents in basic behavioural, physiological, and morphological traits. Almost all published information on the biology of early benthic phase *H. gammarus* comes from studies based on cultured lobsters, the majority of which have occurred in aquaria environments (e.g. Wickins et al., 1996; Linnane et al., 2000a). Even when based in the wild (e.g. van der Meeren, 2000, 2005), observations of the behaviour and performance of hatchery-reared juveniles still may not accurately reflect the biology of natural juveniles in wild ecosystems.

Similarly, the planktonic larval phases are rarely collected in the wild, even in areas high in abundance of reproductively mature adults (S. Clark, Devon and Severn IFCA, pers. comm.). Light traps have proven useful for surveying wild larvae in Scandinavian fjords, which exhibit considerable water retention (Øresland and Ulmestrand, 2013), but have had limited success within the Bristol Channel in the UK due to strong tides and currents (S. Clark, Devon and Severn IFCA, pers. comm.). Elsewhere, continuous plankton recorder samples provide temporally and spatially extensive datasets of planktonic abundance, but decapod larvae are not routinely identified to species level (Richardson et

al., 2006). The absence of basic data on natural larvae and juveniles has inhibited the creation of demographic models that have been useful to predict the effect of stocking in other species (e.g. Lorenzen, 2005, 2006; Hervas et al., 2010).

There is a dearth of studies dedicated to operational variables and their influence on settlement success in hatchery-reared lobsters, and the lack of standardization in existing stocking trials makes their data unsuitable for analysis. Comparisons of different methodological aspects are likely to be biased by the presence of numerous uncontrolled covariates throughout the culture, release, and monitoring processes. Experimental features such as release methods have varied extensively within and among individual projects, with juveniles variously delivered onto benthic habitats by divers or water flume (Bannister et al., 1994; Burton, 2001), released offshore at the sea surface at night (Schmalenbach et al., 2011), and even released during the day into shallow waters off boats or along the intertidal shoreline (Agnalt et al., 1999). In isolation, the lower recapture rates recorded in the UK compared to Norway and Germany could, therefore, be interpreted as a sign that benthic releases yield lower settlement success than surface and shore releases. However, this is counter-intuitive to our expectation that delivering lobsters onto shelter-providing benthic substrates, avoiding pelagic predators, should increase settlement success. It is more likely that the lower UK recapture results arise from the higher abundance of the wild stock, as enhancing productive stocks has been less effective than restocking depleted populations in other decapod crustaceans (Hamasaki & Kitada, 2008b). However, this cannot be evaluated using existing data and should be investigated.

Improving tagging technology

Existing monitored stocking experiments have depended on the use of physical tags to detect recaptured lobsters, with first the coded microwire tag (CWT) in the 1980s and later the visible implant elastomer (VIE) from the late 1990s. These assessments provided the first empirical evidence of the performance of hatchery-reared lobsters in the wild, but there are important limitations to the use and effectiveness of these tags. Both tag types are normally injected into

ventral tissues of the upper abdomen, from where VIE tags have been shown not to alter behaviour or growth (Neenan et al., 2015). VIE tags are logged visually through translucent tissues (Uglem et al., 1996; Neenan et al., 2015), whereas CWTs must be retrieved by dissection after initial detection by magnetometer (Burton, 1992; Bannister et al., 1994). Large juveniles (7 months; 12-16 mm CL) show high tag retention (99%) and survival (97%) over three months when tagged with CWT and VIE and reared in aquaria (Uglem et al., 1996; Linnane & Mercer, 1998). Modern hatcheries typically release younger *H. gammarus* juveniles, however (post larval stage V-VI, 4-6 weeks old, 5-8 mm CL), which show reduced survival after tagging (83% for CWT; 68% for VIE) and significant tag migration (Uglem et al., 1996; Linnane & Mercer, 1998).

The lack of a suitable tag with which to mark juveniles from the first post-larval instar has prohibited any assessment of whether the considerable investment required to grow juveniles to sizes facilitating tagging is reflected in increased recruitment. Since the founding principle of stocking is to culture vulnerable lifestages in captivity, it is conceivable that lobster survival is suitably optimized at the onset of benthic settlement behaviours (i.e. post-larval stage IV-V). This principle, plus the opportunity to maximize numerical release outputs and avoid on-growing expenses, has meant that most active European hatcheries now release early juvenile stages as standard, even though the only evidence for the effectiveness of this strategy is inferred from localized increases in abundance of *H. americanus* in eastern Canada following releases of cultured post-larvae (e.g. Comeau, 2006; Côté & Cloutier, 2014). These results were obtained by the utility of before-after-control-impact (BACI) methods, where lobster abundance in release areas is compared to that in similar, unenhanced habitats over several years. BACI methods have proven useful in implying enhancement effects where hatchery-reared lobsters are not tagged (Comeau, 2006; Côté & Cloutier, 2014), although this style of monitoring produces data that lack the definitive evidence provided by the recapture of tagged individuals. Ideally, a new physical tag is required that is cheap and easy to apply, is capable of marking lobsters from the first post-larval phase to adulthood, and is visually detectable by fishers. This would enable a large number of juveniles to be tagged as standard release procedure and facilitate assessments of optimal

stocking protocols via low-cost and widespread monitoring by fishery stakeholders, who may be positively motivated by a visible tag. However, such a development is unlikely to be forthcoming, given the regular turnover of sclerotized body parts at ecdysis and the vast discrepancy in size between post-larvae and adults.

Attention is, therefore, turning to the potential of polymorphic genetic markers to assign parentage and replace or augment physical tags in future assessments of lobster stocking impact. Methods of genetic profiling can assign hatchery origin with a high degree of certainty (Jones & Arden, 2003) and have important advantages over established internal tags (Table 2). Tag loss can be effectively eliminated, individuals can be sampled sublethally on multiple occasions, and there are no restrictions on the release size of juveniles (Neenan et al., 2015). Genetic profiling can allow assessments of the recruitment performance of different groups, families, or even genotypes (Sekino et al., 2005; Tringali, 2006) and the extent to which wild and cultured animals integrate and interbreed in the environment. With genetic markers of sufficient quantity and variation, hatchery-derived lineages may even be tracked beyond the released generation by identifying the wild-born offspring of hatchery-reared parents, potentially enabling multigenerational assessments of stocking (Letcher & King, 2001; Blouin, 2003).

Employing genetic methods has already proven successful in the detection of hatchery-reared fish among enhanced wild populations of steelhead trout (*Oncorhynchus mykiss*) (Christie et al., 2012a, 2012b) and black sea-bream (*Acanthopagrus schlegelii*) (Jeong et al., 2007) and has been proposed as a method of establishing traceability for aquaculture-derived fish at the marketplace (Hayes et al., 2005). In one of the most positive impact assessments of fishery enhancement, microsatellite-based pedigree reconstructions showed that stocked *A. schlegelii* suffered no loss of heterozygosity, integrated with wild schools, and contributed 59% of individuals to an important fishery in Japan (Jeong et al., 2007). Similarly thorough evaluation is required to elucidate the long-term impact of stocking *H. gammarus*, although such investigations are not cheap or accomplishable

without archived tissues from which the genotypes of hatchery progeny can be deduced (i.e. maternal and egg samples).

Table 2. Summary of the expected performance of different tag types for use in impact assessments of European lobster stock enhancement. CWT and VIE performance is based on reported performance in previous uses, whereas genotype tag performance is based on theoretical performance and reports from other stocked species.

	Tag performance criteria								
Tag type	**Individual ID**	**No min. juvenile size**	**No tag loss**	**Sub-lethal sample**	**Fisher independent monitoring**	**Fisher social impact**	**Multiple generations traceable**	**Genetic fitness impact**	**Stock integration testable**
CWT	Yes ʃ	No	No	No	No*****	No*****	No	No	No
VIE	No	No	No	Yes	Yes	Yes	No	No	No
Genotype	No†	Yes	Yes‡	Yes	No*****	No*****	Yes	Yes	Yes

***** = Fishers may be utilized and socially impacted by monitoring, but cannot readily identify released individuals as part of routine fishing activities. ʃ = Standard tags are batch-numbered, but sequentially-numbered tags are available to identify individuals. † = Individual identification is possible but often requires a larger panel of genetic markers than is required to establish hatchery origin via parentage assignment, the most commonly used genotype-based method. ‡ = No tag "loss", but false negatives can be introduced by genotyping errors (e.g. flawed tissue collection or processing, the presence of null alleles, mistyping and mutation rates, etc.). Repeat sample processing and analysis of data can be used to estimate and/or correct this error rate.

The type and quantity of markers required for parentage assignments to accurately detect hatchery-reared lobsters from large-scale surveys of wild populations would be largely dependent on the population's genetic diversity, effective size, and gene flow, the broodstock turnovers and recapture survey methods employed, and whether multiple paternity frequently exists among individual broods (as has been found in *H. americanus* – Gosselin et al., 2005). Sampling only landed lobsters that are destined for the market may be a more practical survey method than in situ, on-board sampling of the catch (including undersized lobsters destined for return to the sea). The latter could be biased by the inclusion of single individuals sampled on multiple occasions, which would be indistinguishable from multiple individuals possessing genotypes that are identical by descent, although this approach does lend itself well to obtaining recapture data that could reveal the movements of stocked lobsters and the spatial impacts of stocking. Simulations and case studies have shown that parentage can be accurately assigned, even where systems boast hundreds or thousands of candidate parents, using as few as 60-100 SNPs

(Hayes et al., 2005; Anderson & Garza, 2006) or 7-15 microsatellites (Bernatchez & Duchesne, 2000; Letcher & King, 2001; Hayes et al., 2005; Jeong et al., 2007; Christie et al., 2012a), although this is also dependent on the overall power provided by the number and frequency of alleles (Bernatchez & Duchesne, 2000).

For *H. gammarus*, it may well be possible to base such parentage assignments on established and available genetic markers, such as the twelve microsatellites published by André and Knutsen (2010). However, where spatial population genetic structuring is minimal, hatchery broodstock turnovers are high, and multiple paternity occurs frequently within individual broods (all of which are possibly the case for *H. gammarus*), the number of markers required to resolve parentage may rise to become prohibitively costly. Next-generation genotyping resources, such as RAD tags and larger panels of SNPs, offer the resolution to overcome such obstacles (Baird et al., 2008; Hohenlohe et al., 2010), and for species such as Atlantic salmon (*Salmo salar*), microarray genotyping chips featuring many thousands of SNPs are now widely available (Affymetrix, 2014). The development and widespread utilization of such technology is likely to be beyond the financial means and expertise of independent lobster hatchery ventures, however. Still, there is a significant time-lag between captive rearing and potential recapture in the wild, and many universities and research facilities are now equipped with the capabilities to carry out a range of molecular genetic analyses. Therefore, even where no immediate plans exist to assess stocking, all lobster hatcheries should routinely archive tissue and several fertilized eggs from every brood female for potential future collaborative research opportunities.

Improving hatchery production

All hatcheries require the stable production of juveniles to enable release numbers to achieve stocking targets. Because facilities culturing lobster have experienced prolonged and sometimes unexplained periods of production failure, stabilizing juvenile output is required. Where cultured juveniles have no reduction in fitness, increasing both the quantity released and their chances of wild establishment can improve the effectiveness of stocking. Some significant

biotechnical advances have been made in recent years that improve lobster hatchery production and cost-effectiveness. While ovigerous females are plentiful in spring and summer, the separation of some at reduced water temperatures (~6°C) slows egg development and allows the rearing season to be extended. Anecdotally, this has been more effective and reliable than the upward manipulation of egg development and raises the possibility that stable year-round production may be possible. In trials of the so-called “green-water technique”, utilizing algal cultures and enriched live feed more than doubled survival to the first post-larval instar compared to standard rearing protocols (Browne et al., 2009). The larval and post-larval stages are particularly vulnerable to the effects of nutrient limitation; therefore, nutritional enrichments improve growth and survival, even in standard culture environments (Daniels et al., 2010; Schoo et al., 2014). Further improvements have arisen from the long-awaited innovation of multi-layered juvenile rearing systems, which increase hatchery capacity 40-fold compared to traditional single-layer vessels (Shellfish Hatchery Systems, 2013). As advancements continue, hatcheries are able to increase production and the overall economic viability of lobster stocking. By example, one *H. americanus* hatchery more than doubled its production costs from 2002 to 2013, although this enabled technical advances that increased annual production from 1,500 to 417,000 juveniles, slashing the investment per juvenile from over US$33 to just US$0.26 (Haché et al., 2014).

As well as ensuring they can produce the quantity of juveniles required, stocking projects must aim to ensure that the quality of cultured lobsters is sufficient to achieve long-term population enhancement. In Norway, the performance of recaptured lobsters has been promising in basic fitness traits, such as reproductive potential (Agnalt et al., 2008). Nevertheless, juveniles reared in captive conditions are frequently shown to have reduced suitability to the demands of life in natural ecosystems (e.g. Davis et al., 2004, 2005; Castro & Cobb, 2005). Ecological naivety is evident in the higher predation vulnerability of cultured *H. americanus* juveniles compared to wild conspecifics (Castro and Cobb, 2005). For *H. gammarus*, the continued failure to locate wild juveniles has prevented comparisons of fitness to that of cultured equivalents, an approach used widely for other stocked decapods (e.g. Davis et al., 2004, 2005;

Castro & Cobb, 2005; Ochwada-Doyle et al., 2010). Even so, studies have shown that juveniles reared in competitive communal environments grow faster than those raised in isolation (Jørstad et al., 2001), while previous exposure to predator odours gives cultured juveniles a superior ability to outcompete untreated cohorts for limited shelter spaces (Trengereid, 2012).

Although some cultured decapod juveniles have matched the predator avoidance of wild conspecifics regardless of acclimation regimes (Ochwada-Doyle et al., 2010), innate behaviours are likely to be complemented by targeted ecological conditioning before wild release. In hatchery-reared blue crabs (*Callinectes sapidus*), conditioning via controlled predator exposure significantly increases carapace spine length and subsequent post-release survival (Davis et al., 2004, 2005). The traditional hatchery culture of *H. gammarus* juveniles is isolated and largely devoid of environmental enrichment, but in recent years, attempts have been made to on-grow juveniles in sea-based submerged containers. This semi-wild environment appears to promote traits that are likely to have a positive impact on settlement success and adaption to the natural environment and offers significant potential as an acclimation step before the release of cultured lobsters. Survival often exceeds that of hatchery-reared cohorts (Beal et al., 2002; Benavente et al., 2010), and container-reared lobsters typically demonstrate altered behavioural responses and improved growth and pigmentation (Figure 1). Overall, the unnatural selection pressures of culture environments are a fitness concern that remains largely unaddressed in lobster hatcheries, and significant adjustments to existing rearing and conditioning protocols may well be required to increase the viability of current lobster stocking ventures (van der Meeren, 2005; Trengereid, 2012).

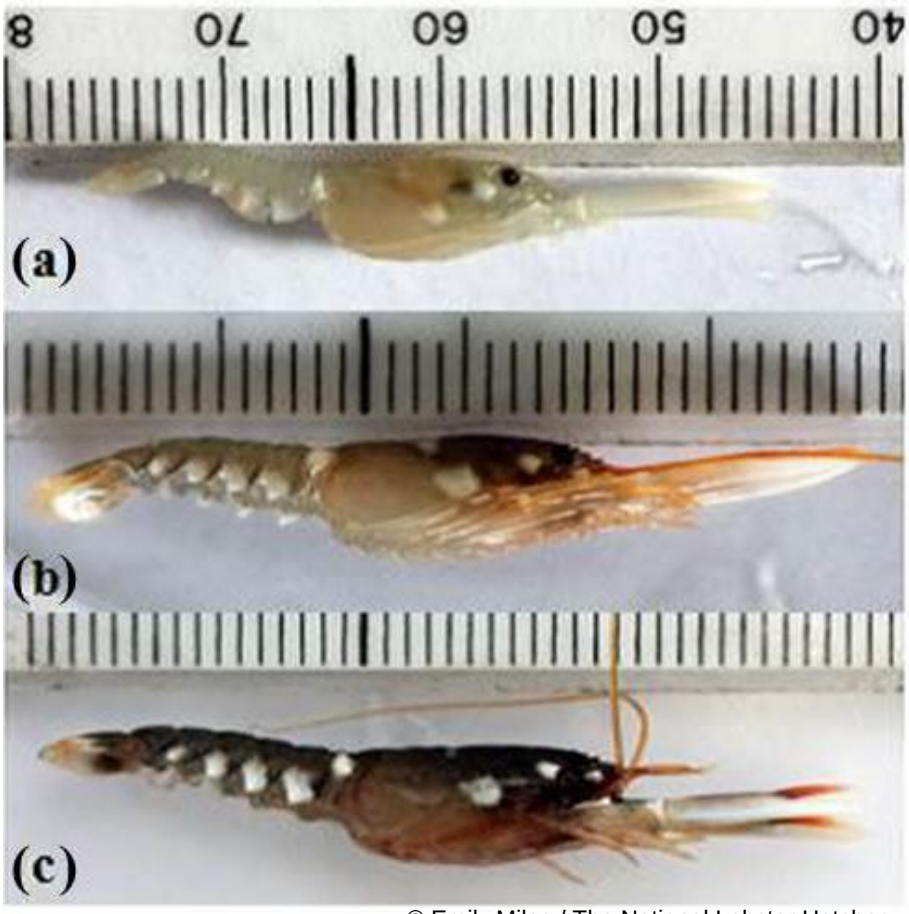

Figure 1. Cultured juvenile European lobsters on-grown in sea containers and in hatchery aquaria. Lobsters reared in an open bay (c) and estuary (b) show increased growth and pigmentation compared to equivalents reared only in the hatchery (a).

Ensuring effective genetic management

Poorly regulated fishing throughout most of the range of *H. gammarus* is likely to have seriously impacted the status of benthic ecosystems and significantly influenced the population genetics of European lobsters. Genetic management of the species has rarely been prioritized or even considered by fishery managers, and the pressures of intensive commercial fishing activities are likely to have impacted the genetics of lobster populations more profoundly than the limited activity of stocking schemes to date. However, mismanagement of lobster fisheries in general should not mean that ventures aiming to enhance and conserve these fisheries via hatchery stocking should not be expected to pursue rigorous standards of ecological accountability. While stocking is generally expected to increase short-term abundance of populations, troubling

recent data in other species suggests that negative genetic impacts may arise in target stocks, undermining fishery conservation objectives (Sekino et al., 2002, 2003; Bert et al., 2007; Kitada et al., 2009; Rourke et al., 2009; Hamasaki et al., 2010; Christie et al., 2012a, 2012b; Satake & Araki, 2012). It is increasingly apparent that the dual goals of short-term productivity and long-term conservation are not usually complementary and are difficult to achieve simultaneously (Satake & Araki, 2012).

Many authors have proposed ways in which stocking schemes can limit negative genetic impacts, and routinely comparing the genetic diversity and relative fitness of wild and cultured fish is commonly recommended (e.g. Blankenship & Leber, 1995; Shaklee & Bentzen, 1998; Bell et al., 2006; Gaffney, 2006; Bert et al., 2007; Tringali et al., 2008; Laikre et al., 2010; Lorenzen et al., 2010). For example, Bert *et al* (2007) suggest that stocking enterprises should study the species' regional population genetics, genotype broodstock at a resolution sufficient to distinguish their offspring, monitor the genetic variation of cultured juveniles and incoming broodstock, and use genetic assays to scan the wild population for both hatchery progeny and any flux in the larger gene pool. Many independent hatcheries are unable to fund such research or have prioritized investing in biotechnical innovations though, so genetic aspects of management have often been ignored (Bell *et al.,* 2006). This is largely the case among organizations stocking *H. gammarus* and requires rectifying to ensure that heavily exploited lobster fisheries are not subject to any deleterious effects via stocking.

Maintaining fitness and genetic diversity

Attaining long-term population growth and simultaneous conservation of the regional gene pool is unlikely where stocked animals have fitness disadvantages (Satake & Araki, 2012). Fitness disadvantages can arise in cultured individuals as a consequence of narrow genetic make-up or via inadvertent selection processes occurring in the hatchery environment that make cultured juveniles ill-suited to their natural ecosystem. Where released animals introduce heritable reductions in fitness, stocking has the potential to have negative impacts on wild stocks. This is reported most often where target

populations are small and/or show high levels of adaptation to local conditions (Lorenzen et al., 2012). Released animals often have reduced fitness for the natural environment compared to wild conspecifics; Araki and Schmid (2010) reviewed 39 studies that assessed fitness effects, of which 22 found that survival, growth, or reproductive success were reduced by hatchery rearing. Given the dissimilarities between hatchery and wild environments, traits that lead to high fitness in one may reduce fitness in the other. Trout (*Oncorhynchus mykiss*) raised in captivity have nearly double the reproductive success of wild-born fish when spawned in a hatchery, but their offspring suffer greatly reduced performance in the wild, where survival is less than a third that of the wild-origin cohort (Christie et al., 2012a).

A key principle of stocking is that offspring survival is relatively increased in the captive environment, which means that many released individuals may be closely related. Increasing the number of related individuals in a population generally decreases the overall genetic diversity and effective population size and increases the potential for inbreeding depression (Ryman & Laikre, 1991). Cultured individuals often show reduced genetic diversity (e.g. Sekino et al., 2002) and have low effective population sizes, especially where broodstock are captive-reared, are used to rear multiple generations of offspring, or where competitive processes lead to highly skewed reproductive success (Sekino et al., 2003; Shishidou et al., 2008). Parentage assignments in hatchery-reared flounder (*Paralichthys olivaceus*) revealed that almost all of the offspring were sired by one of six males, and that half of the twelve spawning females yielded no surviving juveniles at all (Sekino et al., 2003). Although the influence of stocking on population genetic diversity may be trivial compared to that caused by environmental or fishing pressures (Sugaya et al., 2008; Kitada et al., 2009), in some cases, it can be extremely damaging; stocking doubled the number of adult trout (*O. mykiss*) on spawning grounds in Oregon, USA, but actually cut the total effective population size by two-thirds (Christie et al., 2012b).

Wild-mated females have typically been utilized for *H. gammarus* stocking, with several hundred new broodstock sourced for each production season. Where broodstock are marketed for human consumption upon return to their donors, their repeated use is prevented. Whether achieved via the ease of accessing

readily-mated females or by enlightened genetic practices, these methods should have contributed to ensuring relatively high genetic diversity among progeny. However, family contributions have been found to be skewed in *H. gammarus* culture (Jørstad et al., 2005a), and how the genetic diversity of released lobsters compares to that within target populations requires evaluation using modern techniques.

Consideration of population structure and local adaptation

Genetic diversity is the principal origin of adaptive evolutionary potential (Frankham et al., 2011), so populations are increasingly vulnerable to environmental change where genetic diversity is eroded by the release of cultured individuals (Laikre et al., 2010). Where cultured animals lack hereditary adaptations to their release environment and interbreed with wild fish that are more suitably adapted, adaptive traits crucial to the species' fitness in that environment are likely to be eroded, reducing the overall fitness of the population. In recent studies on wild marine fish, molecular markers have helped reveal previously unforeseen levels of population structure and local adaptation to environmental heterogeneity (e.g. temperature and salinity), even at small geographical scales (e.g. Atlantic cod, *Gadus morhua* – Knutsen et al., 2003, 2011; Jorde et al., 2007; Atlantic herring, *Clupea harengus* – Lamichhaney et al., 2012; Limborg et al., 2012; Teacher et al., 2013a; sticklebacks, *Pungitius pungitius*, *Gasterosteus aculeatus* – Shikano et al., 2010; Shimada et al., 2011; Bruneaux et al., 2013; Atlantic salmon, *Salmo salar* – Griffiths et al., 2010). Although genotyping only 25 individuals per population can often provide accurate estimates of population-level differences in allele frequencies (Hale et al., 2012), even relatively basic genetic studies are generally complex and expensive. As a result, population genetic data are frequently absent or outdated for stocked marine species, and such studies on *H. gammarus* provide somewhat contradictory evidence or lack peer review.

Investigations of genetic structure and diversity in *H. gammarus* populations using polymorphic microsatellites, allozymes, and mitochondrial DNA (e.g. Jørstad & Farestveit, 1999; Jørstad et al., 2004a, 2005b; Triantafyllidis et al., 2005; Huserbråten et al., 2013) have attempted to delineate populations and

estimate gene flow within and between them. Observed restrictions in adult migration give the potential for considerable genetic isolation between *H. gammarus* subpopulations (Øresland & Ulmestrand, 2013), although the most recent research suggests that high genetic connectivity exists over relatively large spatial scales (≈ 400 km), even among semi-enclosed habitats (Huserbråten et al., 2013). These results, obtained via microsatellite DNA analysis of heavily depleted Scandinavian Skagerrak populations, suggest that larval dispersal must be high and must be the primary origin of gene flow (Huserbråten et al., 2013). Where larvae are distantly dispersed and cultured lobsters add significantly to the spawning biomass, the long-term impacts of stocking could extend far beyond the spatial boundaries over which releases occur.

Spatial heterogeneity in *H. gammarus* population genetic variation has been detected, however, particularly in regions isolated by oceanographic and topographic conditions, such as northern Norway and throughout the Mediterranean (Jørstad & Farestveit, 1999; Ulrich et al., 2001; Jørstad et al., 2004a, 2005b; Triantafyllidis et al., 2005) and even among populations from the comparatively unrestricted Atlantic coasts of Ireland, France, and Portugal (Ulrich et al., 2001). There appears to be an overall association between geographic distance and genetic variation (Ulrich et al., 2001), although considerable genetic differences can be found over modest spatial scales (e.g. 142 km between fjords, Jørstad et al., 2004a). Rapid recent developments in whole-genome genotyping methodologies and the field of bioinformatics now offer greater resolution and deeper insight into the extent of population structure and local adaptation. Studies utilizing these technologies throughout the range of *H. gammarus* will be critical for understanding the spatial scales that stocking may be expected to impact and for ensuring that lobster releases are non-detrimental.

Stocking vs. alternative management strategies

To date, lobster stocking in Europe has always been practiced in addition to legislative fishery management measures such as closed seasons, closed areas, gear restrictions, and landing bans on undersized, v-notched, or

ovigerous lobsters. However, assessments of the relative effectiveness of lobster stock enhancement and other alternative fishery management tools are either lacking or are too ambiguous to allow formative comparison between methods. Several conservation methods are often applied concurrently, which potentially gives a greater chance of safeguarding stocks, but it becomes difficult to appraise the relative strengths and limitations of individual components. The need for rigorous analysis of lobster stocking is particularly urgent, but so too is the analysis of other management measures in order to enable comparative assessments of fishery conservation tools.

Our understanding of the effects of most fishery management options is poor, but marine protected areas (MPAs) have recently demonstrated potential in sustaining exploited lobster populations. In the UK, the closure of waters off Lundy Island to all fishing activities led to a rapid increase in lobster abundance and mean body size (Hoskin et al., 2011), while in Norway, MPA designation increased lobster CPUE by 245% over four years, far beyond the 87% increase in control areas (Moland et al., 2013). Over 95% of lobsters caught, tagged, and rereleased into both Norwegian and Swedish MPAs remained within or very near to reserve boundaries in multiannual mark-recapture analyses (Moland et al., 2011; Øresland & Ulmestrand, 2013), while high genetic connectivity between these MPAs suggests that larval dispersal benefits may be extensive and far-reaching (Huserbråten et al., 2013). Arguably, thoughtfully designated MPAs have offered more conclusive stock conservation benefits than hatchery stocking to date, although MPAs do have an immediate negative economic impact on displaced fishers. However, employing the two methods simultaneously (i.e. releasing cultured lobsters into MPAs) may offer a powerful stock conservation method and provide quicker enhancement of adjacent fisheries.

Conclusions

The regulation of European lobster stocking has been largely *ad hoc* and lacks alignment with the robust frameworks established for the informed management of marine stocking ventures (e.g. Blankenship & Leber, 1995; Lorenzen et al.,

2010). Given that recent findings from the wider field of aquatic stocking show that the successful integration of cultured individuals into dynamic wild populations is a highly complex process, this is clearly unsatisfactory. While some deviation from best practice may have been the result of insufficient and fragmented planning by regulatory managers or hatchery operators, much more has been unavoidable. The inconclusive performance of previous lobster stocking projects in providing economically viable benefits to lobster fisheries has made it hard for active hatcheries to attract significant financial backing and industry support. However, the exhaustive monitoring and technical developments required to evidence economic viability are often economically unviable in their own right; as our understanding of the potential ecological considerations mounts, the costs associated with piloting a stocking programme increase (Blankenship & Leber, 1995; Lorenzen et al., 2010). In the absence of focussed guidelines or coordinated investment from industry or government, active hatcheries have been largely unable to address significant gaps in our scientific understanding of lobster biology that are integral to the informed management of stocking ventures and lobster fisheries themselves. As a result, hatcheries have been forced to focus on advancing production and revenue, conducting ecological research where possible along the way.

From existing studies designed to assess the potential of stocking *H. gammarus*, a proof-of-concept has been demonstrated. Based on recaptures of hatchery-reared lobsters achieving fishery minimum landing sizes and reproductive maturity in multiple locations, conclusions have been generally positive that stocking could represent a worthwhile fishery conservation method. However, these conclusions are undermined by a lack of consistent evidence that benefits are universal and cost-effective and by a series of inconclusive or damaging reports into the effects of stocking in other marine species. Nevertheless, in the wake of increased pressures on some fisheries and the regional collapse of others, interest in stocking programmes aimed at restoring or enhancing lobster populations has only increased in recent years. The societal decision whether to pursue stocking of European lobster populations requires evidence of both positive and negative impacts of hatchery releases, so a renewed evaluation of lobster stocking, utilizing the more thorough

assessment tools now available, is required to limit the ambiguity of that decision.

Impact assessments attempting to appraise the effect of European lobster stocking are significantly hindered by the elusive nature of wild juveniles and scarcity of other fundamental information on the ecology of natural populations and have, so far, been restricted to unfavourable juvenile tagging methods. Genetic methods should be employed to improve wider understanding of lobster biology and population ecology as well as to deliver assessments on the evolutionary fitness of cultured lobsters and the likelihood of their release to cause negative effects on natural populations. Genetic resources also require testing for their effectiveness in identifying cultured lobsters in the wild. Recent improvements in the quality and cost-efficiency of juvenile production could help make stocking a viable tool for improving the productivity and sustainability of lobster fisheries, although this requires thorough and strategic evaluation.

Overall, our understanding of the dynamics and potential of lobster stocking remains limited, and further research using contemporary methods is required to deliver informative impact assessments. Ideally, all lobster hatcheries should implement the following initiatives: (i) archive maternal and progeny tissues from all broodstock; (ii) establish a management strategy that will limit negative impacts of releases in the presence of population structure and local adaptation; (iii) conduct controlled temporal studies of lobster abundance in release areas, both before and after stocking; and (iv) link with a research institute or university to enable collaborative research. Implementation of these procedures would help raise the ethical and ecological standards of stocking ventures, would provide basic evidence of the effect of stocking on local abundance, would lay the foundations for more comprehensive assessments of the performance of stocked lobsters, and would facilitate partnerships with organizations capable of assessing population structure and stock boundaries throughout the species' range, as well as driving efforts to locate wild juveniles to resolve associated knowledge gaps.

Chapter 3: Geographic and environmental drivers of fecundity in the European lobster.

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Abstract

Fecundity in the European lobster (*Homarus gammarus*) has been shown to exhibit extensive spatial variation across northern Europe. Previously, this has been attributed to a lack of methodological standardization among samples. Instead, we show significant correlations between fecundity and both geographical and environmental drivers. We use linear mixed-effect models to assess the contribution of latitude, longitude, and measures of sea surface temperatures on the size-fecundity relationships of 1058 ovigerous females from 11 locations in the UK, Ireland, and Norway. We include new data for 52 lobsters from Falmouth, UK, the southwest limit of existing samples. Fecundity at mean female size correlated positively with eastings and greater annual ranges in sea surface temperature, but not with mean temperature or latitude. This contradicts the established latitudinal and mean temperature dependency reported for the closely related *H. americanus*. We postulate that proximity to stable Atlantic currents is the most likely driver of the relationship between fecundity and longitude. Mechanisms are discussed by which egg production or retention may be influenced by temperature range rather than by mean temperature. With further validation, we propose that temperature-correlated fecundity predictions will provide a valuable tool in ensuring that management thresholds are appropriate for the reproductive characteristics of lobster populations.

Introduction

Measures of egg production are vital parameters for estimating the reproductive capacity of marine populations, the maintenance of which is a key objective of fishery management. Knowledge of reproductive capacity is critical for informed management of exploited populations because it is required for models of stock and recruitment dynamics and can be used to define the maximum threshold for fishing mortality (Laurans et al., 2009). It is also important to determine the geographic scales over which the reproductive characteristics of managed species vary in order to apply commensurate stock conservation measures to each region (Tully et al., 2001; MacCormack & DeMont, 2003; Currie & Schneider, 2011).

A size-specific fecundity factor is well documented in populations of the European lobster (*Homarus gammarus,* L.), a prized decapod crustacean fished extensively throughout its range (e.g. Hepper & Gough, 1978; Bennett & Howard, 1987; Tully et al., 2001; Lizarraga-Cubedo et al., 2003; Agnalt et al., 2007; Agnalt, 2008). However, published estimates of mean fecundity have varied considerably among putative populations throughout northwest Europe (Agnalt, 2008), ranging from ~5200 eggs per oviposition in southeast Scotland (Lizarraga-Cubedo et al., 2003) to ~12 500 in southern England (Roberts, 1992) and southwest Norway (Agnalt, 2008), among females of 100 mm carapace length (CL).

Environmental determinants of fecundity variation have been identified in many marine species (Wright, 2013), including sea water parameters such as temperature, salinity (e.g. Gomez et al., 2013), and dissolved oxygen (e.g. Wu et al., 2003). Temperature (or latitude, as a proxy) has been found to correlate tightly with the exponent of size-specific fecundity variation in American lobster (*Homarus americanus*) (Currie & Schneider, 2011). It also aligns with reproductive traits in other lobsters, including Southern rock lobster, *Jasus edwardsii* (Annala et al., 1980; Gardner et al., 2006), and in fish inhabiting a similar range throughout the Northeast Atlantic, such as Atlantic cod, *Gadus morhua* (Thorsen et al., 2010; Wright et al., 2011a; Hansen et al., 2012) and Dover sole, *Solea solea* (Witthames et al., 1995; Mollet et al., 2013). We aimed

to test associations between *H. gammarus* fecundity and geographical and environmental factors, to assess whether they may contribute to the observed spatial variation in fecundity. Management has failed to prevent extensive and enduring stock collapses in the recent past (e.g. throughout Scandinavia in the mid-20th century – Dow, 1980; Agnalt et al., 1999), and where stock thresholds fail to reflect regional differences in fecundity, the management of pressured fisheries can be seriously undermined (Lambert, 2008; Morgan, 2008). Therefore, the identification of drivers that explain reproductive variation may be important in conserving lobster populations (Green et al., 2014).

Despite the established influence of ecological drivers in reproductive variation across a range of taxa, whether regional differences in *H. gammarus* fecundity may be driven by environmental factors has not been assessed. Observed variation in clutch size amongst clawed lobsters has been attributed to differences in the success of attaching the externally-incubated eggs (Currie & Schneider, 2011), the rate of egg loss over a lengthy incubation of 9-10 months (Wahle et al., 2013), and the retention of eggs during capture and subsequent handling and storage (Agnalt, 2008). Agnalt (2008) hypothesized that a lack of methodological standardization among studies may prevent the detection of population-level variations, but we aimed to assess whether the influence of thermal environment might be detectable within the observed variation of *H. gammarus* fecundity.

We hypothesized that a relationship would exist between temperature and fecundity among putative populations of *H. gammarus*. To test this hypothesis, egg counts of ovigerous females were collated from existing studies of fecundity in northern Europe. A new fecundity measurement was also made for females from the Atlantic peninsula of Cornwall, UK, an unassessed region at the southwest edge of the range of available data where the lobster fishery is vital in supporting 370 commercial potting vessels (S. Davies, pers. comm.; Cornwall IFCA, 2014). Parameters of the size-specific fecundity relationships of these samples were regressed against geographical and environmental covariates. We find longitudinal and environmental predictors of fecundity at mean size and discuss our findings in relation to lobster physiology, evolutionary ecology, and fishery management.

Material and methods

New samples

Animal acquisition and storage

Ovigerous female lobsters (*n* = 52) were caught in baited pots and collected directly from inshore fishers working in Falmouth Bay, southwest UK in January-March 2013. This was carried out with permission from the local authority, as the landing of ovigerous females within inshore waters is normally prohibited (Cornwall IFCA, 2014). A large and evenly distributed range in female sizes was requested because this improves the accuracy of estimates of size-fecundity relationships (Estrella & Cadrin, 1995). A broad size range was achieved, although legal landing restrictions meant that no females could be obtained less than the 90 mm CL jurisdictive minimum landing size. Most females were sampled immediately upon collection; where this was not possible, females were stored for a maximum of 3 d in a modern ~2000L recirculation system, where chilled temperatures (5-6°C), shelter provisions, and low stocking density (maximum 3 m^{-2}) ensured egg loss was negligible (daily net cleaning revealed that egg loss equated to <10 eggs $lobster^{-1}$ d^{-1}).

Physical fecundity estimation

Carapace length (CL) was measured using Vernier callipers, rounding down to the nearest whole millimetre, and the egg mass was collected by hand, as per Agnalt (2008). A subsample of the eggs was separated and counted manually, ranging from 517 to 708 individual eggs (mean = 606, ± 3.45). No repeat subsamples were taken because Agnalt (2008) showed that the correlation between two counts was >0.99 using even smaller subsamples [wet weights of 1-1.5 g, compared to 2.2-3.9 g (mean = 2.97 g, ± 0.05 g) in this study]. Egg development was similar among all females, with most clutches being partially “eyed”, although no formal measurements of development stage were taken.

Individual fecundity estimates were made by calculating the dry weight of the egg mass against that of the counted subsample; dry weight was preferred so that any variation in the amount of seawater incidentally gathered with the egg mass would not bias the measurement. All egg samples were dried in a drying

oven (UT6200, Thermo Electron LED, Germany) at 105°C for 24 h (±1h). Samples were moved into a sealed desiccating cabinet to cool before mass was measured to the nearest 1 mg by electronic balance (AE240 Balance, Mettler, UK). After an additional hour in the drying oven, sample mass was remeasured to check that it was stable and that drying had completed; all samples were deemed fully dried after this check because the difference in mass between the measurements was <1% of the total sample mass. The dry mass of the subsample of known egg count was used to determine the mean dry mass per egg as:

$$\text{Dry mass per egg (mg)} = \frac{\text{Subsample dry mass (mg)}}{\text{Subsample size } (n \text{ eggs})}$$

Fecundity estimates for each individual were then obtained from the total dry mass of eggs as:

$$\text{Fecundity } (n \text{ eggs}) = \frac{\text{Subsample dry mass (mg)} + \text{Remaining sample dry mass (mg)}}{\text{Dry mass per egg (mg)}}$$

Geographical survey

Data collection and statistical modelling

To test potential geographic and environmental drivers of fecundity variability in *H. gammarus*, data were collected from five studies assessing fecundity among 1009 individuals in 10 areas around the UK, Ireland, and Norway, plus the 52 individuals from Falmouth, southwest UK (Figure 1), measured by this study. Each regional sample location was assigned latitudinal and longitudinal coordinates from the approximate centre of the spatial range of sampling, as could be best deduced from study methodologies. Mean sea surface temperature (SST) data were obtained for each location the first day of each month during the year(s) of the study and one preceding year, since the majority of Homarid lobsters spawn in a biennial cycle (Tully et al., 2001; Comeau & Savoie, 2002; Agnalt et al., 2007). Using SST data, the mean temperature (mean SST of all months in all years) and temperature range (the mean difference between the mean SST of the three coldest months and the mean SST of the three warmest months of each year) were calculated for each

location. SST data were obtained via NASA's AVHRR Oceans Pathfinder from the Physical Oceanography Distributed Active Archive Center (PO.DAAC, 2014) for all locations except Falmouth, UK, for which SST data were only available via NASA's MODIS Aqua EOS-PM from the Goddard Space Flight Center (MODIS-Aqua, 2014) due to the recentness of the sampling.

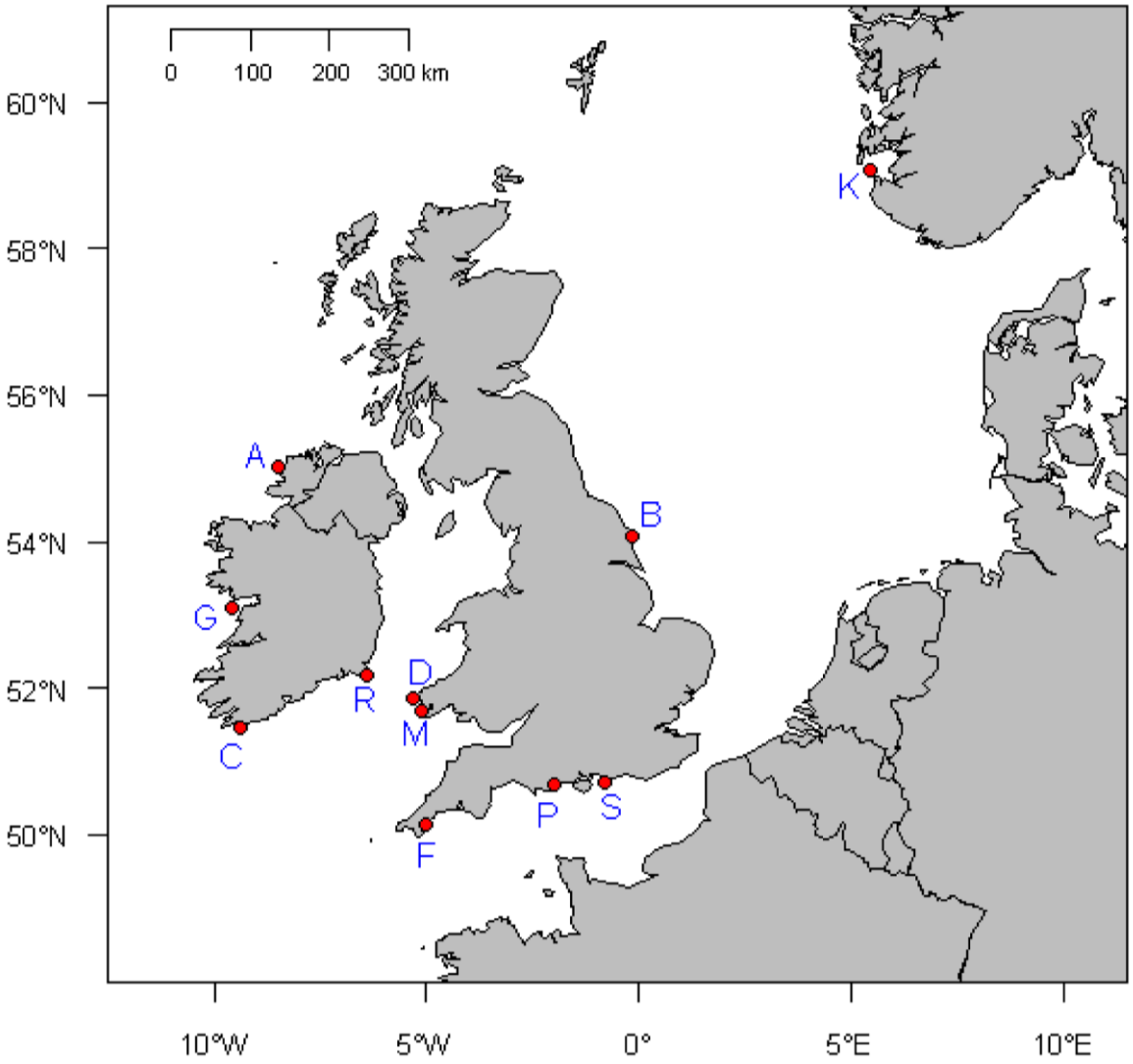

Figure 1. Map of regional fecundity samples. Map of the UK and Ireland, with continental Europe around the North Sea, showing the locations of regional fecundity samples. Fecundity in Falmouth (F) was assessed in this study, while other samples used to model correlations with temperature were: Arranmore (A), Galway (G), Cork (C), and Rosslare (R) from Tully *et al* (2001); St Davids (D) from Bennett & Howard (1987); Milford Haven (M), Selsey (S), and Bridlington (B) from Free (1994); Poole (P) from Roberts (1992); and Kvitsøy (K) from Agnalt (2008). See Table 1 for further information on regional samples.

SST was utilized instead of sea bottom temperature (SBT) because SBT was unavailable at the spatial and temporal resolutions required. While SBT may

present a more biologically relevant parameter for benthic lobsters, the use of SST was supported by a regression of 80 surface (mean = 1.8 m below surface) and bottom (mean = 3.3 m above seafloor) temperature measurements obtained by depth casts (ICES Data Centre, 2014) taken between 1998 and 2008 at fishable locations (within 15 km of the coast and <8+5 m depth) across the geographic range of the study. The relationship showed a highly significant correlation between surface and bottom temperatures (Pearson's product-moment correlation, $r^2 = 0.96$, $p < 0.01$).

General linear models (GLM) were constructed using R (R Core Team, 2012) to apply power (log-log), log-linear, and linear fits to the global relationship between fecundity (*F*) and female size (*CL*) across all 1061 individuals. Analysis of the distribution of residuals and comparisons of the log-likelihood ratio statistic and Akaike information criterion (AIC) of each model confirmed that the power fit, $\log(F) = \log(aCL)^b$, best described this relationship (see Supplementary material). Power law models have been favoured in other recent studies (e.g. Tully et al., 2001; Lizarraga-Cubedo et al., 2003; Agnalt, 2008) because they account for the volumetric nature by which the brooding capacity of the abdomen increases in length and width with increasing carapace length. The outlying data of three individuals for which fecundity estimates lay beyond 4 s.e. of the allometric relationship were removed from the analysis.

A linear mixed-effects model was constructed using the R package lme4 (Bates et al., 2014) to test the effect of the sizes of potential geographical and environmental drivers on lobster fecundity. Geographical factors assessed were latitude, longitude, and the interaction between the two, while environmental factors (analysed separately) were mean temperature, temperature range, and the interaction between the two. The relative strength of all geographical and environmental covariates was standardized via an adjustment to similar scales (mean = 0; s.d. = 1). From these models, coefficients of log(fecundity) at the mean size of all sampled females (F_{mean}) and the exponent of the size-specific fecundity power relationship (F_{slope}; *b* value) were extracted for each regional sample and then regressed in GLMs against the scaled geographical or environmental covariates. All combinations of models containing the effects of geographical or environmental factors on F_{mean} and F_{slope} in each regional

sample were compared using multimodel inference and model averaging in the R package MuMIn (Barton, 2013). Model-averaged effect sizes and AIC weights (the proportion of weight accumulated by all models containing the assessed variable) were extracted to evaluate the relative importance of each variable on F_{mean} and F_{slope}. Correlation between geographical and environmental factors was tested by linear regressions. The GLMs used for the regression of F_{mean} and F_{slope} against geographical and environmental parameters were weighted by the sample sizes studied in each lobster population to limit the influence of imprecise estimates on global relationships.

Some existing fecundity samples within the spatial range investigated were not analysed because raw data were unavailable (e.g. eastern and western Scotland – Lizarraga-Cubedo et al., 2003) or were collected before the backdated availability of SST measurements (e.g. northwest France – Latrouite et al., 1984). Data from another sample taken near Whitby in northeast England by Bennett and Howard (1987) were omitted because it was deemed likely that they were biased by considerable egg loss prior to fecundity estimation. The data included extremely low egg counts (e.g. <750 eggs) and yielded a very low correlation for the power-fitted size-fecundity relationship (r^2 = 0.12). A sample from Milford Haven, Wales (Free, 1994) was included despite the sample size being very small (n = 8) because the data exhibited a reasonable correlation for a power-fitted size–fecundity slope (r^2 = 0.62).

Results

Physical fecundity estimation

Among females collected from Falmouth, UK, CL ranged from 90 to 155 mm (mean = 110 mm, ± 1.9 mm), and estimated egg production ranged from 3,712 to 35,241 eggs individual^{-1}. The relationship between fecundity (F) and female size (CL) was described by $F = 0.0066CL^{3.10}$ using a power-fitted model (r^2 = 0.68, p < 0.001; Table 1), or by $F = 406.92CL - 29\,749$ using a linear-fitted model (r^2 = 0.77, p <0.001). Mean dry mass egg^{-1} ranged from 1.53 to 2.24 mg among females, but demonstrated no relationship with overall fecundity (linear fit; r^2 = 0.14, p <0.01). Mass egg^{-1} appeared to fit a natural logarithm

relationship with female size, as described by Agnalt (2008), although overall correlation of this model fit was weak (r^2 = 0.29, p <0.001) (see Supplementary material). Compared to the sample from Kvitsøy (K) and pooled Irish samples (I), mean dry mass egg^{-1} (mg) at Falmouth (F) was slightly higher at the lower distribution of female sizes (90 mm CL: K = 1.3; I = 1.4; F = 1.6), but was comparable at upper size limits (150 mm CL: K = 1.9; I = 1.9; F = 2.0) (Tully et al., 2001; Agnalt, 2008). Estimates suggest that fecundity among lobsters from Falmouth is fairly central within the range recorded for the species across northern Europe, despite the location lying at the southwest geographical extremity of all samples.

Table 1. Summary results of regional fecundity samples, including: study origin; sample region; sample size (*n*); central coordinates used for sample SST data and geographic factors in modelling, SST-derived mean temperature, and temperature range; *a* and *b* (F_{slope}) of the power-fitted relationship between fecundity and carapace length ($F = aCL^b$), with r^2 and associated *p*-values; and F_{mean}.

Study	Sample region	*n*	Lat.	Long.	SST mean (°C)	SST range (°C)	a	b (F_{slope})	r^2	p	F_{mean} (*n* eggs)
Ellis et al. (this study)	Falmouth, SW England	52	50° 8' 24"N	5° 1' 48"W	11.85	6.76	0.0066	3.08	0.68	<0.001	11,011
Tully et al. (2001)	Arranmore, NW Ireland	73	55° 0' 36"N	8° 30' 36"W	11.64	4.63	0.0042	3.18	0.81	<0.001	9,559
Tully et al. (2001)	Galway, W Ireland	144	53° 6' 36"N	9° 35' 60"W	12.41	5.31	0.0017	3.29	0.73	<0.001	9,353
Tully et al. (2001)	Cork, SW Ireland	70	51° 27' 36"N	9° 24' 0"W	12.87	5.98	0.0031	3.18	0.57	<0.001	8,947
Tully et al. (2001)	Rosslare, SE Ireland	111	52° 10' 12"N	6° 24' 0"W	12.13	7.40	0.0164	3.01	0.49	<0.001	10,105
Bennett & Howard (1987)	St Davids, SW Wales	80	51° 52' 12"N	5° 19' 48"W	11.10	7.13	0.0003	3.42	0.73	<0.001	9,466
Free (1994)	Milford Haven, SW Wales	8	51° 42' 0"N	5° 8' 24"W	11.75	7.19	0.0000	3.14	0.48	0.02	10,293
Free (1994)	Selsey, S England	76	50° 42' 36"N	0° 46' 48"W	12.94	7.81	0.1827	2.85	0.26	<0.001	11,622
Free (1994)	Bridlington, NE England	177	54° 4' 48"N	0° 10' 12"W	10.06	8.68	0.0344	2.84	0.59	<0.001	11,776
Roberts (1992)	Poole, S England	50	50° 40' 48"N	1° 58' 12"W	12.49	7.05	0.0114	3.03	0.53	<0.001	11,208
Agnalt (2008)	Kvitsøy, SW Norway	217	59° 3' 36"N	5° 26' 24"E	9.84	9.38	0.0047	3.11	0.85	<0.001	12,920

Drivers of fecundity variation

Table 1 shows SST and fecundity relationship results for each regional sample. North Sea sites at Kvitsøy and Bridlington had both the lowest mean temperatures (9.84 and 10.06°C, respectively) and highest temperature ranges (9.38 and 8.68°C). Mean temperature was highest at sites in the English Channel at Selsey (12.94°C) and Poole (12.49°C) and in the Northeast Atlantic off western Ireland at Cork (12.87°C) and Galway (12.41°C). Western Ireland also experienced the smallest temperature ranges, decreasing northwards from Cork (5.98°C) to Galway (5.31°C) and being lowest at Arranmore (4.63°C). Across all samples, F_{mean} corresponded to a female size of 102.8 mm CL. For the log power-fitted relationship, $\log(F) = \log(aCL)^b$, b (F_{slope}) was lowest for the samples from Bridlington (2.84) and Selsey (2.85), and was highest for the St Davids sample (3.42). F_{mean} ranged from 8947 eggs female^{-1} in Cork to 12,920 in Kvitsøy. For all North Sea and English Channel samples, F_{mean} exceeded 11,000 eggs female^{-1}, whereas it was below 10,300 eggs for all samples from the Irish Sea and western Ireland.

We found that increases in F_{mean} were strongly associated with increases in both (easterly) longitude and mean annual temperature range (Figure 2). Each variable had a high cumulative AIC weight (temperature range = 0.92; longitude = 0.89; Table 2), and a model-averaged effect size identifiably greater than zero, with 95% confidence intervals not overlapping zero (Figure 3). The influence of longitude and temperature range on fecundity also extends to females in other size classes. These variables also yielded identifiable positive effect sizes in linear mixed-effect models of fecundity at the current European Commission minimum landing size of 87 mm CL (data not presented). Latitude and mean temperature variables, and interactions of these factors, had no influence on fecundity variation, however. Modelled with F_{mean}, these variables had low cumulative model weightings (AIC weights <0.1) and 95% confidence intervals that spanned an effect-size of zero (Figure 3). We also demonstrated that variation in F_{slope} could not be attributed to any of the geographical or environmental variables investigated (Figure 3). No variable had an identifiable effect upon F_{slope}, with confidence intervals spanning zero effect-sizes and low cumulative weighting (AIC weights <0.4) for all model factors. Linear

regressions between variables showed a significant positive correlation between mean annual temperature range and longitude (Pearson's coefficient: r^2 = 0.90, p <0.001; Figure 4), and a significant negative relationship between latitude and mean temperature among regional fecundity samples (r^2 = –0.74, p <0.01).

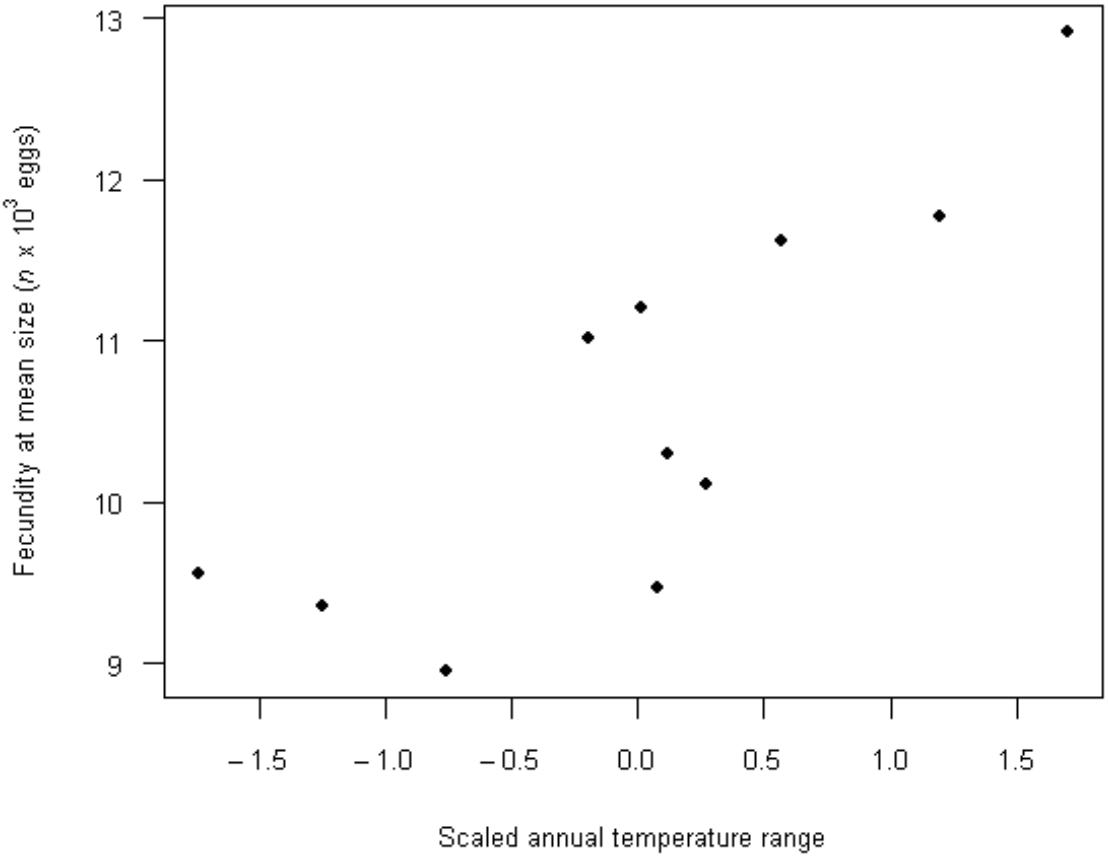

Figure 2. Average fecundity at the total mean female size against mean annual range in sea temperature. Plot of the relationship between F_{mean} and scaled temperature range, showing that increased F_{mean} was positively associated with increased range in annual temperature (r^2 = 0.83, p <0.002).

Table 2. Summary results of candidate linear mixed models, with measures of model likelihood and weighting to show the effect of geographical and environmental covariates on F_{mean}.

***F* parameter**	**Factors**	**Model variables**	**d.f.**	**logLik**	**AICc**	**Δ AIC**	**AIC weight**
F_{mean}	Geographical	Longitude*	3	20.966	–32.5	0.00	0.894
		Latitude + longitude	4	21.406	–28.1	4.36	0.101
		Latitude + longitude + latitude:longitude	5	22.075	–22.2	10.35	0.005
		Latitude	3	8.444	–7.5	25.04	0.000
	Environmental	Temperature range*	3	14.687	–19.9	0.00	0.915
		Mean temperature + temperature range	4	14.816	–15.0	4.98	0.076
		Mean temperature	3	9.712	–10.0	9.95	0.006
		Mean temperature + temperature range + mean temperature:temperature range	5	14.948	–7.9	12.05	0.002
F_{slope}	Geographical	Longitude	3	3.454	2.5	1.64	0.251
		Latitude + longitude	4	5.147	4.4	3.49	0.099
		Latitude	4	2.313	4.8	3.92	0.080
		Latitude + longitude + latitude:longitude	5	7.036	7.9	7.05	0.017
	Environmental	Temperature range	3	4.060	1.3	0.43	0.384
		Mean temperature	3	2.565	4.3	3.42	0.086
		Mean temperature + temperature range	4	4.680	5.3	4.43	0.052
		Mean temperature + temperature range + mean temperature:temperature range	5	4.997	12.0	11.13	0.002

Factors denoted * were deemed identifiable effects by model-averaging.

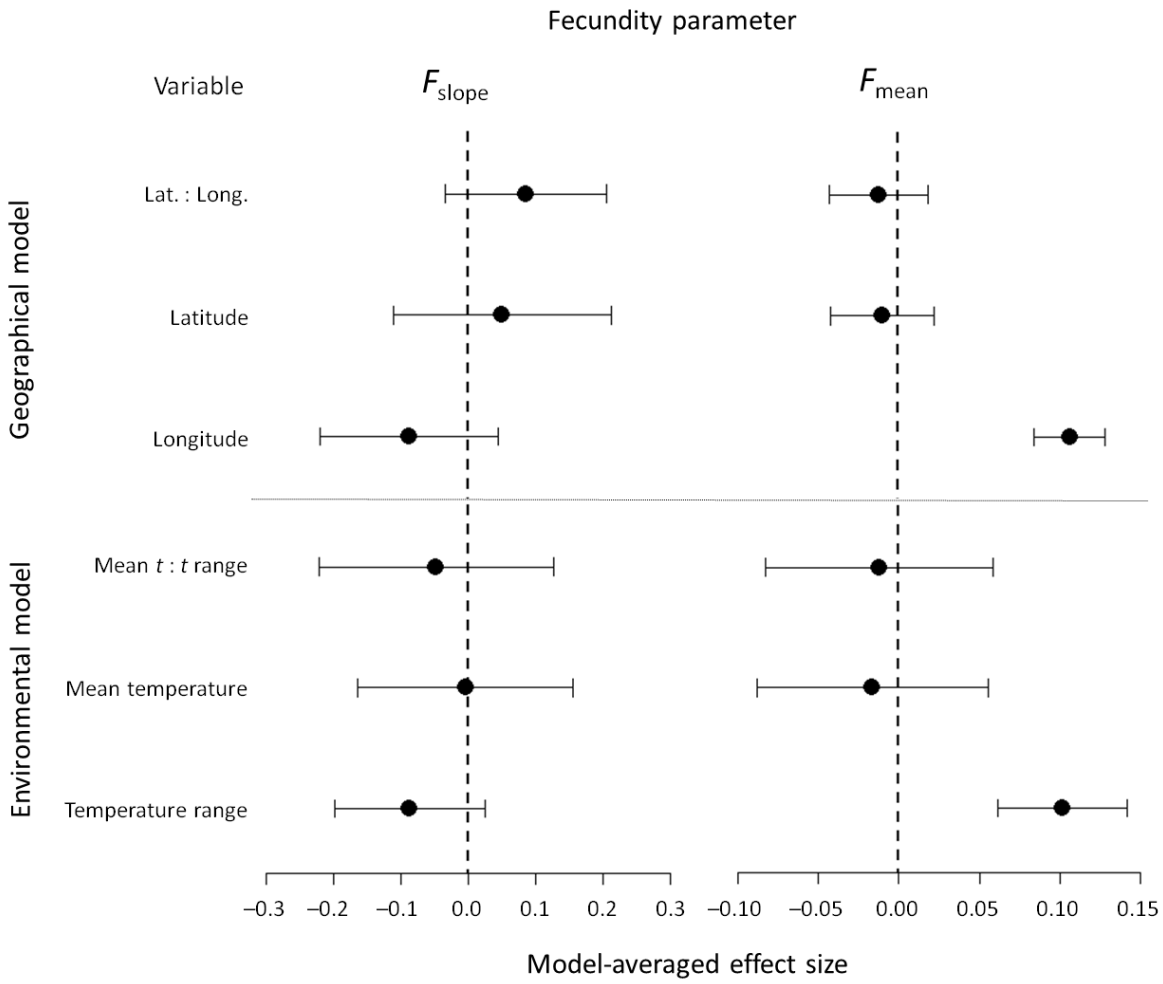

Figure 3. Model-averaged effect sizes. Model-averaged effect sizes of geographical and environmental variables modelled against the fecundity parameters F_{slope} and F_{mean}. Variables with effect-sizes that are identifiably different from zero have 95% confidence interval bars that do not overlap the model mean (dashed vertical line).

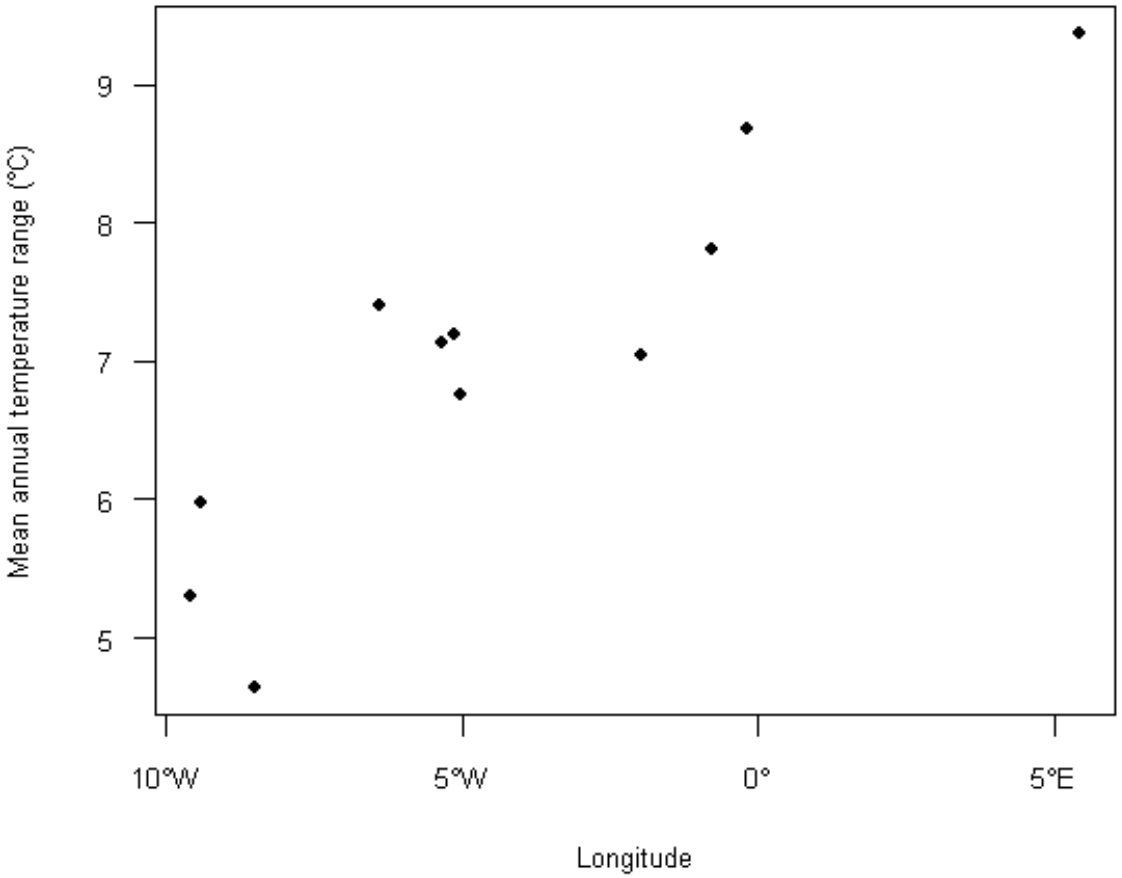

Figure 4. Longitude against mean annual range in sea temperature. Plot of the relationship between longitude and temperature range among regional fecundity samples (r^2 = 0.90, p <0.001).

Discussion

Knowledge of factors contributing to fecundity variation is vital to ensure that fishery management strategies are suitable for exploited species throughout their range (Lambert, 2008; Morgan, 2008). We have demonstrated geographical and environmental factors that correlate with fecundity variation in *H. gammarus* across a portion of its range which has accounted for over 75% of the species' recorded landings in recent years (Fisheries and Aquaculture Department, 2016a). Our results are an important indication that the observed spatial variation may reflect differences between the fecundity of putative populations, not simply study-level differences in investigative approach, and that environmental temperature is a driver contributing to variation in the production and/or retention of eggs in *H. gammarus*. In isolation, the new fecundity sample taken from Falmouth, the first such assessment in southwest

England, can contribute an important parameter of the reproductive capacity of *H. gammarus* in this important regional fishery.

Our most important findings are that fecundity at mean size improved with increasing range in annual temperature, along a gradient towards more easterly longitudes, and that longitude and temperature range were closely associated. The most obvious underlying driver linking gradients of longitude and temperature range in the area of this study is proximity to the North Atlantic Drift of the Gulf Stream. The North Atlantic Drift brings greater thermal stability to the coastal waters of the immediate Atlantic coast along western Europe than that experienced by more enclosed shelf sea areas. By example, among the three most northerly regional samples we surveyed, the mean annual range in sea temperature for the Northeast Atlantic at Arranmore was only 4.6°C, compared to 8.7°C around Bridlington and 9.4°C at Kvitsøy in the North Sea. Considering the strength of the associations we found between fecundity at mean female size and both longitude and temperature range, we propose that proximity to currents associated with the Gulf Stream contributes to the regulation of egg production and/or retention in *H. gammarus* across the northern part of the species' distribution.

In contrast to the relationship detected for *H. americanus* by Currie and Schneider's (2011) similar meta-analysis of spatial variation in fecundity, we found no evidence of the slope of size-specific fecundity increasing with decreased latitudinal gradient. Instead, we found that fecundity at mean size was increased among regions with high ranges in annual temperature, irrespective of mean temperature. This finding defies the expectation that mean temperature drives the reproductive investment of ectotherms (e.g. Ernsting & Isaaks, 2000; Thorsen et al., 2010; Tobin & Wright, 2011; Wright et al., 2011a). Currie and Schneider (2011) found that fecundity-at-size in *H. americanus* (in this case, 85mm CL lobsters) met this expectation, as it aligned closely to latitudinal gradient. However, the direction of this relationship was unexpected, with fecundity-at-size found to increase in higher latitudes (Currie & Schneider, 2011), suggesting that clutch size does not increase with increasing temperature in either *Homarus* species.

Rather than being a function of size, Currie and Schneider (2011) propose that *H. americanus* fecundity may be age-related, with fewer growing degree-days (e.g. Neuheimer & Taggart, 2007) at higher latitudes leading to smaller size at maturity and comparatively greater clutches at equivalent body sizes. Age-at-size validation methods remain too unreliable among crustaceans (Hartnoll, 2001) to evidence this, but the proposition is not supported by Currie and Schneider's (2011) own assertion of overall increases in size-specific fecundity slopes towards southerly latitudes, nor by our finding of a disconnect between fecundity at mean size and mean temperature in *H. gammarus*. A comparable pattern to that which we revealed is shown by sole (*S. solea*) populations from colder North Sea environments, whose earlier maturity and higher reproductive investment compared to conspecifics from warmer seas to the south and west has been attributed to counter-gradient environmental adaptation. This suggests that greater fecundity can arise among populations inhabiting colder regions to compensate for high mortality caused by winter sea temperatures (Conover, 1992; Mollet et al., 2013), and that similar pressures could be driving variation in egg production for *H. gammarus*. In most studies of fish, spatial and temporal trait adaptations associated with temperature variation have been attributed to phenotypic plasticity (Crozier & Hutchings, 2014), although evolutionary mechanisms are more commonly proposed to explain counter-gradient variations (Conover, 1992; Mollet et al., 2013). Compared to plastic traits, locally-adapted fecundity variation is less likely to be flexible to global climate change (Conover et al., 2009), and evidence of such adaptation to thermal gradients has already been established among *H. americanus* populations across the Atlantic, with larval growth and planktonic duration found to be comparatively shortened under local sea temperatures (Quinn et al., 2013).

Reported variation in size at the onset of maturity (SOM) also appears to support the suggestion that geographical and environmental factors may influence reproductive ecology atypically in *H. gammarus*. Female SOM has been estimated to be generally smaller in those samples farther from the mild Northeast Atlantic currents (Table 3), despite an expectation to positively align with mean temperature as a product of greater energy acquisition and growth

rate (e.g. Zuo et al. 2011, Green et al., 2014), as has been asserted for *H. americanus* (Little & Watson, 2003, 2005; Caputi et al., 2013). Physiological assessments found SOM to be smaller in Bridlington than at any location around Ireland (Free, 1994; Tully et al., 2001), and morphologically-determined SOM was lower in the Scottish North Sea than at the Hebridean Atlantic coast. In both scenarios, lobsters mature at smaller sizes in the area of greater temperature range, despite those areas experiencing lower overall mean temperatures. Assessing the relative contributions of environmental, demographic, and genotypic factors can be extremely challenging (Wright, 2013), but the alignment of multiple traits to gradients of temperature range is a strong indicator that reproductive variation in *H. gammarus* is driven by thermal environment.

Table 3. Size at the onset of maturity and average fecundity at total mean female size. Regional samples ranked via smallest SOM L_{P50} (the carapace length at which 50% of females are functionally mature), as physiologically-determined by Free *et al.* (1992) and Tully *et al.* (2001), with comparison to fecundity at the global mean female size (F_{mean}) as calculated in this study using raw data from Free (1994) and Tully *et al.* (2001).

Study	Sample region	SOM (CL, mm), (rank)	F_{mean} (n x10^3 eggs), (rank)
Free *et al.* (1992), Free (1994)	Selsey, S England	82 (1)	11.6 (2)
	Bridlington, NE England	90 (2)	11.8 (1)
Tully *et al.* (2001)	Galway, W Ireland	92 (3)	9.35 (5)
	Cork, SW Ireland	94 (4)	8.95 (6)
	Rosslare, SE Ireland	95 (5)	10.1 (3)
	Arranmore, NW Ireland	96 (6)	9.56 (4)

It is not possible to disentangle whether the observed spatial variation in *H. gammarus* fecundity arises as a result of differences in the production of eggs or in the retention of eggs after oviposition, or both. Agnalt (2008) measured fecundity soon after extrusion and again soon before hatch, and detected no egg loss across seven months among lobsters from Kvitsøy, whereas Latrouite *et al* (1984) estimated that 27% of eggs were lost during incubation off the northwest coast of France. Agnalt (2008) sourced lobsters stringently and argued that the egg loss observed by Latrouite *et al* (1984) could have arisen from handling and inappropriate storage, factors well known to downwardly bias subsequent egg counts. Nevertheless, most studies of *H. americanus* imply that

15% or more of eggs are lost during incubation (Wahle et al., 2013), and egg retention could exist as a result of thermal environment, so egg loss during incubation cannot be discounted as a mechanism of *H. gammarus* fecundity variability. Egg loss among communally-captive *H. gammarus* is dramatically reduced below a thermal tipping point of approximately 9°C (B. Marshall, National Lobster Hatchery, pers. comm.), with decreased metabolism and movement inhibiting behaviours and interactions which otherwise inhibit egg retention. It is also conceivable that the diversity and abundance of known fungal and nemertean pathogens of lobster eggs (e.g. Alderman & Polglase, 1986; Campbell & Brattey, 1986) is influenced by sea temperatures. However, speculative hypotheses that rate of egg loss may be improved in colder winters are tempered by the extended duration of the incubation period at lower temperatures (Charmantier & Mounet-Guillaume, 1992; Schmalenbach & Franke, 2010) and by our analysis of samples from Selsey and Poole, which also had high fecundity at mean size, but where high temperature ranges were driven by warm summers rather than cold winters.

Although there is a tendency for mass egg^{-1}, egg and larval size, and larval robustness to increase with female size (Tully et al., 2001; Agnalt, 2008; Moland et al., 2010), scant evidence has been found of any trade-off between quantity and quality of egg production in *H. gammarus*. Investment per egg in terms of dry mass appears consistent between samples from Ireland, Kvitsøy, and Falmouth and showed no discernible association to clutch size in our Falmouth sample. In the geographic range of this study, it is also unlikely that fecundity variation arises as a result of regional differences in spawning frequency, as a biennial reproductive cycle has been recorded for the majority of lobsters in both Norway and Ireland (Tully et al., 2001; Agnalt et al., 2007), although variation in spawning strategies is apparent in the genus and is poorly understood (Gendron & Ouellet, 2009). Fishing-induced mortality is another candidate driver of spatial variation in lobster fecundity. A response to selection pressures incurred via recruitment overfishing has been proposed to explain temporal fecundity increases in North Sea populations of cod (*G. morhua*), haddock (*Melangrammus aeglefinus*), and plaice (*Pleuronectes platessa*) (Yoneda & Wright, 2004; Rijnsdorp et al., 2005; Stares et al., 2007; Wright et

al., 2011b) and was also considered as a driver of temporal SOM variation in *H. americanus* (Landers et al., 2001). Among the samples we investigated, the highest fecundities at mean size were recorded from the post-collapse population at Kvitsøy (Agnalt et al., 1999) and the samples from Bridlington, Selsey, and Poole, which are from stocks in the east and south of England that experience heavier fishing pressure than those of Atlantic coasts towards the southwest (CEFAS, 2015). The status of stocks around Ireland and Wales are not known. The strong effects of longitude and temperature range that we identified suggest that any demographic pressure must also align closely with these gradients, although from the limited information available on current and historical fishing pressure, this does seem to be the case for *H. gammarus* in parts of northern Europe.

The confirmation and elucidation of geographical and/or environmental drivers of fecundity variation would be valuable to the management of reproductive potential in *H. gammarus* stocks, especially among unassessed regions in lieu of laborious manual quantifications (Currie & Schneider, 2011). Predictions facilitated via relationships we have demonstrated with temperature range may be a suitable method of fecundity estimation among unmeasured populations, although the associations we found between temperature and fecundity are not as categorical as those offered by Currie and Schneider (2011) for *H. americanus*. This may be an artefact of uncontrolled variation in the effective spatial ranges of the regional samples we analysed. Our findings would be strengthened by the standardized assessment of *H. gammarus* fecundity in other regions within the spatial range encompassed by this study, as well as in areas such as subarctic Norway, the Iberian peninsula, Morocco, and the Mediterranean to determine whether temperature range may be a driver of clutch size throughout the species' range. Repeat estimations in regions previously assessed could elucidate whether fecundity varies temporally as well as spatially, and provide further evidence that the recorded variation in lobster fecundity reflects population-level differences in the production and/or retention of eggs, rather than inherent bias between samples.

Conclusions

We show that the fecundity of European lobsters at mean female size correlates positively with easterly longitude and annual range in sea surface temperatures across the northern range of this species. Fecundity at mean size did not correlate with mean temperature or latitude, contradicting the widely assumed temperature dependence of ectotherms. We propose that the proximity of populations to stable Atlantic currents is the driver of this variation. With further validation, temperature-correlated fecundity predictions would provide a valuable tool in ensuring that conservation management is suited to the reproductive characteristics of lobster populations.

Supplementary Material

Table S1. Log-likelihood (log-Lik) and Akaike Information Criterion (AIC) results for different model fits to the size–fecundity relationship. The power-fitted model was used to describe the global *F*~CL relationship since it had the lowest values of log-Lik and AIC.

Model fit	log-Lik	AIC
Power; $\log(F) = \log(aCL)^b$	–85	217
Log-linear; $\log(F) = aCL + b$	–105	256
Linear; $F = aCL + b$	–9 959	19 964

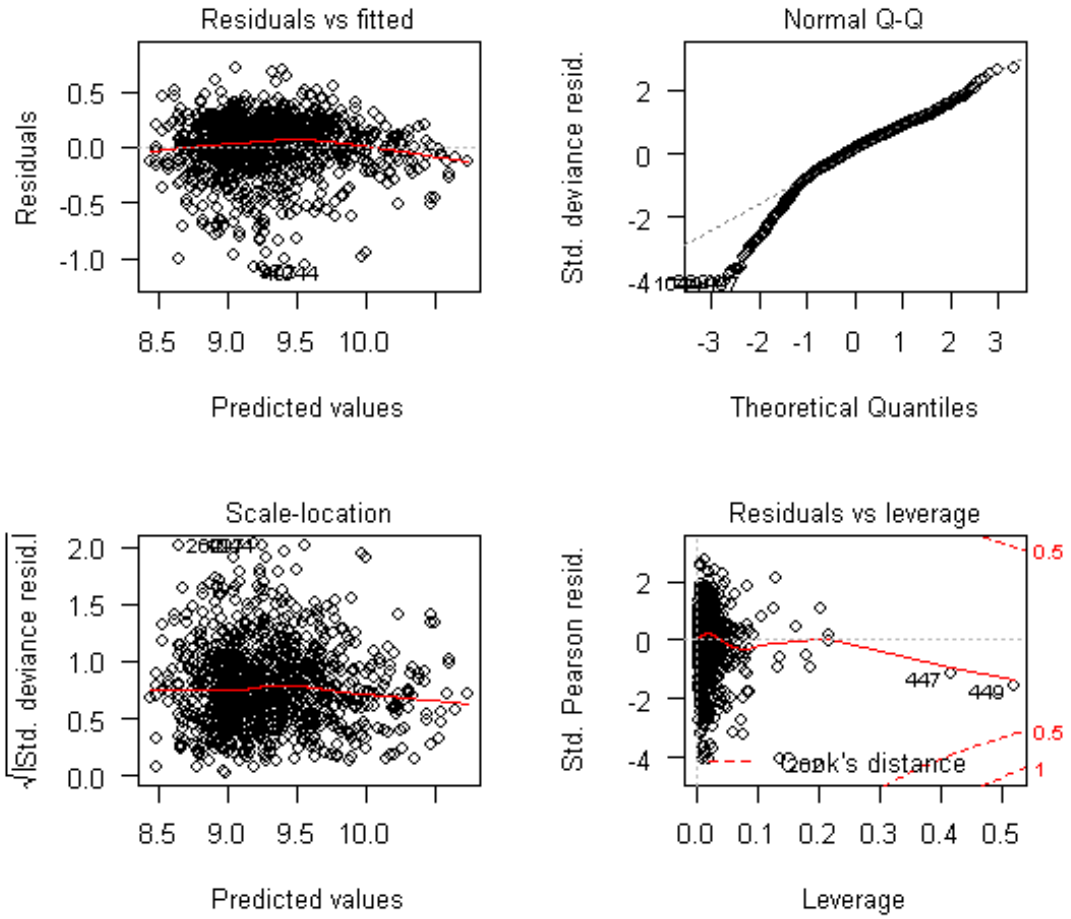

Figure S1. Model validation plots, demonstrating that fecundity data satisfied normal distribution and homoscedastic residuals after allometric transformation.

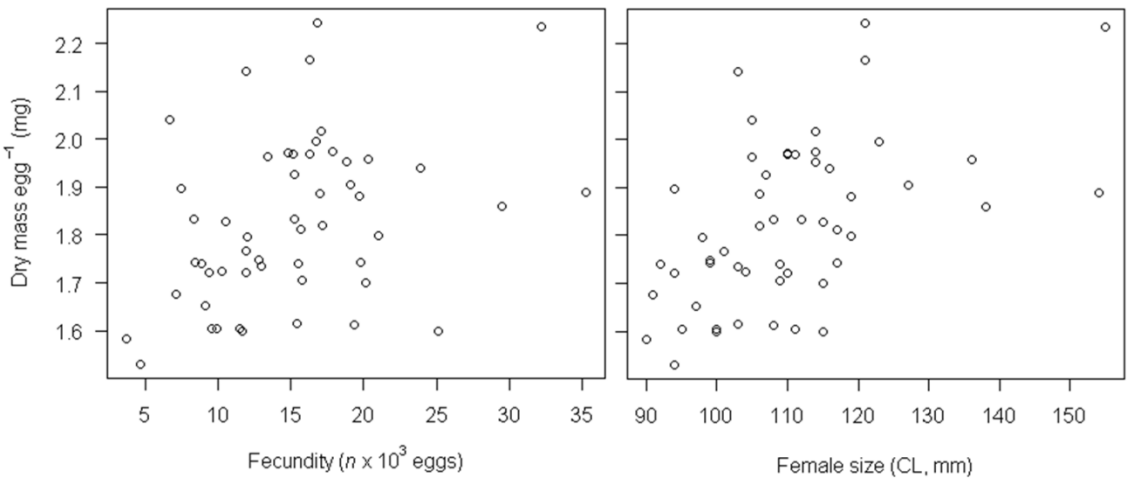

Figure S2. Fecundity and female size against mass per egg. Plots of the relationships among lobsters from Falmouth (n = 52) between dry mass egg^{-1} and fecundity, at left (linear fit; r^2 =0.14, p <0.01), and with female size, at right (logarithm fit; r^2 =0.29, p <0.001).

Chapter 4: Genotype reconstruction of paternity in European lobsters.

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Abstract

Decapod crustaceans exhibit considerable variation in fertilisation strategies, ranging from pervasive single paternity to the near-ubiquitous presence of multiple paternity, and such knowledge of mating systems and behaviour are required for the informed management of commercially-exploited marine fisheries. We used genetic markers to assess the paternity of individual broods in the European lobster, *Homarus gammarus*, a species for which paternity structure is unknown. Using 13 multiplexed microsatellite loci, three of which are newly described in this study, we genotyped 10 eggs from each of 34 females collected from an Atlantic peninsula in the south-western United Kingdom. Single reconstructed paternal genotypes explained all observed progeny genotypes in each of the 34 egg clutches, and each clutch was fertilised by a different male. Simulations indicated that the probability of detecting multiple paternity was in excess of 95% if secondary sires account for at least a quarter of the brood, and in excess of 99% where additional sire success was approximately equal. Our results show that multiple paternal fertilisations are either absent, unusual, or highly skewed in favour of a single male among *H. gammarus* in this area. Potential mechanisms upholding single paternal fertilisation are discussed, along with the prospective utility of parentage assignments in evaluations of hatchery stocking and other fishery conservation approaches in light of this finding.

Introduction

The reproductive behaviour and ecology of fished species can affect their vulnerability to population collapses, and their subsequent ability to recover (Rowe & Hutchings, 2003). Polyandry may arise in breeding females as a life history strategy in order to increase the genetic diversity or fitness of offspring (Jennions & Petrie, 2000; Avise et al., 2002), or where males are sperm limited (Wedell et al., 2002). Selective fishing may also influence the occurrence of polyandry, especially where mating strategies are dependent on age, size, or sex ratio (Rowe & Hutchings, 2003; Berkeley et al., 2004; van Doornik et al., 2008). As a result, information on the dynamics of female mating strategies is a vital component to the informed conservation management of exploited fisheries (Chevolot et al., 2007).

Clutch fertilisation in marine decapods varies between species and populations, from pervasive single paternity (e.g. snow crab – Urbani et al. 1998) to ubiquitous multiple paternity (e.g. squat lobsters – Bailie et al., 2011). Multiple sires have been detected within individual clutches in a variety of aquatic crustaceans (e.g. ghost shrimp – Bilodeau et al,. 2004; Norway lobster – Streiff et al., 2004; porcelain crab – Toonen, 2004; Dungeness crab – Jensen & Bentzen, 2012; rock shrimp – Bailie et al., 2014; freshwater crayfishes – Karhl et al., 2014; Pacific gooseneck barnacle – Plough et al., 2014). However, the frequency of polyandrous fertilisation remains unknown in the European lobster (*Homarus gammarus*), a high-value species exploited extensively throughout its range by trap fishing. The presence of multiple paternal fertilisations has been detected among individual egg clutches of the closely-related American lobster, *Homarus americanus* (Jones et al., 2003; Gosselin et al., 2005), with some evidence from the wild that increased fishing pressure disrupts the natural monandrous behaviour of some females via reductions in the abundance, size or post-copulatory mate-guarding ability of breeding males (Gosselin et al., 2005).

Despite supporting a highly lucrative fishery, information on the reproductive ecology of *H. gammarus* in the wild is scarce (André & Knutsen, 2010), and is often implied from that of the better-studied *H. americanus*. Female *H.*

americanus are thought to seek out and compete for males and usually moult during a period of shelter cohabitation, whereupon a spermatophore is deposited by the male into the seminal receptacle of the female (Gosselin et al., 2003; Wahle et al., 2013). The male attempts to prevent further insemination from competitors by guarding the female until both her shell and a sperm plug blocking the entrance to the seminal receptacle have hardened (Talbot & Helluy, 1995; Gosselin et al., 2003). Females vacate the male's shelter and usually store the spermatophore for approximately a year before spawning, whereupon it is released to externally fertilise the eggs during extrusion and oviposition (Aiken et al., 2004; Agnalt et al., 2007). Homarid eggs hatch following 9-11 months of development while stored ventrally along the female abdomen, at which point most mature females mate and moult again, forming a biennial reproductive cycle (Agnalt et al., 2007; Wahle et al., 2013). Occasionally females moult, mate and spawn annually (Agnalt et al., 2007), while large (>120 mm carapace length [CL]) females can go several years without moulting and may mate during intermoult if spermatophore reserves are insufficient to sire a brood (Waddy & Aiken, 1990).

It has long been established that female fecundity increases with increasing body size (e.g. Tully et al., 2001; Agnalt et al., 2007; Ellis et al., 2015b), and studies on the effects of male size in other lobster species show that ejaculate load is also size-specific and may be reduced by previous copulations (MacDiarmid & Butler, 1999; Gosselin et al., 2003). Where the abundance and mean size of males is reduced by fishing, it has been proposed that the population may become sperm limited, with the production of larvae restricted by a lack of available spermatophore with which to fertilise the maximum egg capability of breeding females (MacDiarmid & Butler, 1999). Such sperm limitation may cause females to seek additional copulations, with more than one spermatophore used to fertilise an egg clutch (Gosselin et al., 2003, 2005). Alongside sperm limitation, other hypotheses proposed to explain observed multiple paternity in marine invertebrates have included convenience polyandry (e.g. Saint-Marie et al., 1999; Thiel & Hinojosa, 2003; Panova et al., 2010) and enforced mating (e.g. Bailie et al., 2014). Where multiple paternity has been identified among marine crustaceans, considerable skews in fertilisation

success towards a single male have often been detected (e.g. Gosselin et al., 2005; Bailie et al., 2011, 2014; Plough et al., 2014). This has been proposed to result from various post-copulatory processes including spermatophore stratification (e.g. Sévigny & Sainte-Marie, 1996), cryptic female choice (e.g. Thiel & Hinojosa, 2003) and sperm competition, although the latter was ruled out for *H. americanus* because their sperm lack motility (Talbot & Helluy, 1995; Gosselin et al., 2005).

We investigated *H. gammarus* paternity around Cornwall, an Atlantic peninsula in south-western UK, where lobsters are intensively fished and are also the focus of stock enhancement by a local hatchery (National Lobster Hatchery, 2015). Because physical tags having proven largely ineffective in marking early-stage post-larval lobsters (e.g. Linnane & Mercer, 1998; Neenan et al., 2015; Ellis et al., 2015a), the hatchery is interested in pursuing genetic methods of parentage assignment that have allowed the successful identification of stocked finfish among admixed wild populations (e.g. Sekino et al., 2005; Jeong et al., 2007; Christie et al., 2012a). The tissue archiving requirements and general suitability of such an application are in part dependent on the number of sires contributing to individual clutches, adding to the need for information of lobster paternity in the region. By reconstructing male genotypes from clutches of fertilised eggs, we aimed to estimate the frequency of multiple paternity and thus elucidate the typical fertilisation scenario in lobsters from this important regional fishery.

Materials and methods

Ethics statement

Permission to obtain tissue samples from adult lobsters (for both paternity assays and population screening) were obtained from the Cornwall Inshore Fisheries Conservation Authority (IFCA), who regulate and manage the lobster fishery within coastal waters. Tissue samples were collected on board commercial vessels as part of regular fishing routines. The collection of tissue samples from adult lobsters from the Isles of Scilly did not require the permission of the Isles of Scilly IFCA since samples were obtained from animals

already landed to a merchant on the mainland. Eggs for paternity assays were collected from ovigerous females captured within the six nautical mile inshore jurisdiction of Cornwall IFCA, who provided written permission for both the sampling of eggs and the temporary landing of ovigerous lobsters, which is normally prohibited by a regional bylaw (Cornwall IFCA, 2015). The European lobster is categorised as being of Least Concern in the Red List of Threatened Species of the International Union for Conservation of Nature (Butler et al., 2015).

Sites and sampling

During March and April 2013, trap-caught ovigerous female lobsters were collected directly from selected inshore fishers temporarily permitted to land these animals by the regional fisheries management authority. Typically, the rocky Celtic Sea habitats to the north and far west support a greater abundance of lobster than the mixed substrates of the western English Channel along the southern coast (Davies, 2007). As such, lobsters were sourced from two sites in each area (four sites in total, separated by a minimum Euclidean distance of 55 km) to account for any spatial variation in paternity structure (Figure 1). Where possible, samples were taken immediately upon receipt of the lobsters, although occasionally they were stored in holding tanks for a maximum of 48 hours before sampling. Sampling consisted of the removal of a small piece of maternal tissue from the tip of a hindmost pleopod, and of ten eggs from the clutch (total clutch size is specific of female size and even region, though is typically 9-13,000 for mean-sized individuals of 103 mm CL; Ellis et al., 2015b). An egg was removed from both the base and the tip of the egg-mass from each of the five pairs of pleopods, giving a 10-offspring array per clutch. Egg sampling was structured in this way to maximise the likelihood of detecting multiple paternity and because some marine decapods (though not *H. americanus* – Gosselin et al., 2005) have demonstrated spatial segregation of multiple paternal fertilisations (Bilodeau et al., 2004; Bailie et al., 2011). Twelve females were sampled from each of two Celtic Sea and English Channel locations, although insufficient DNA yields from undeveloped eggs later reduced these sample sizes. As such, 340 eggs from 34 females were genotyped successfully (Figure 1). Female carapace length (CL) was measured using a

Vernier caliper and rounded down to the nearest whole millimetre, as per Agnalt *et al* (1999). The assessment of a wide range of female sizes is important given the expectation that the frequency of multiple paternity may vary with female size, particularly if caused by sperm-limitation (MacDiarmid & Butler, 1999; Gosselin et al., 2003).

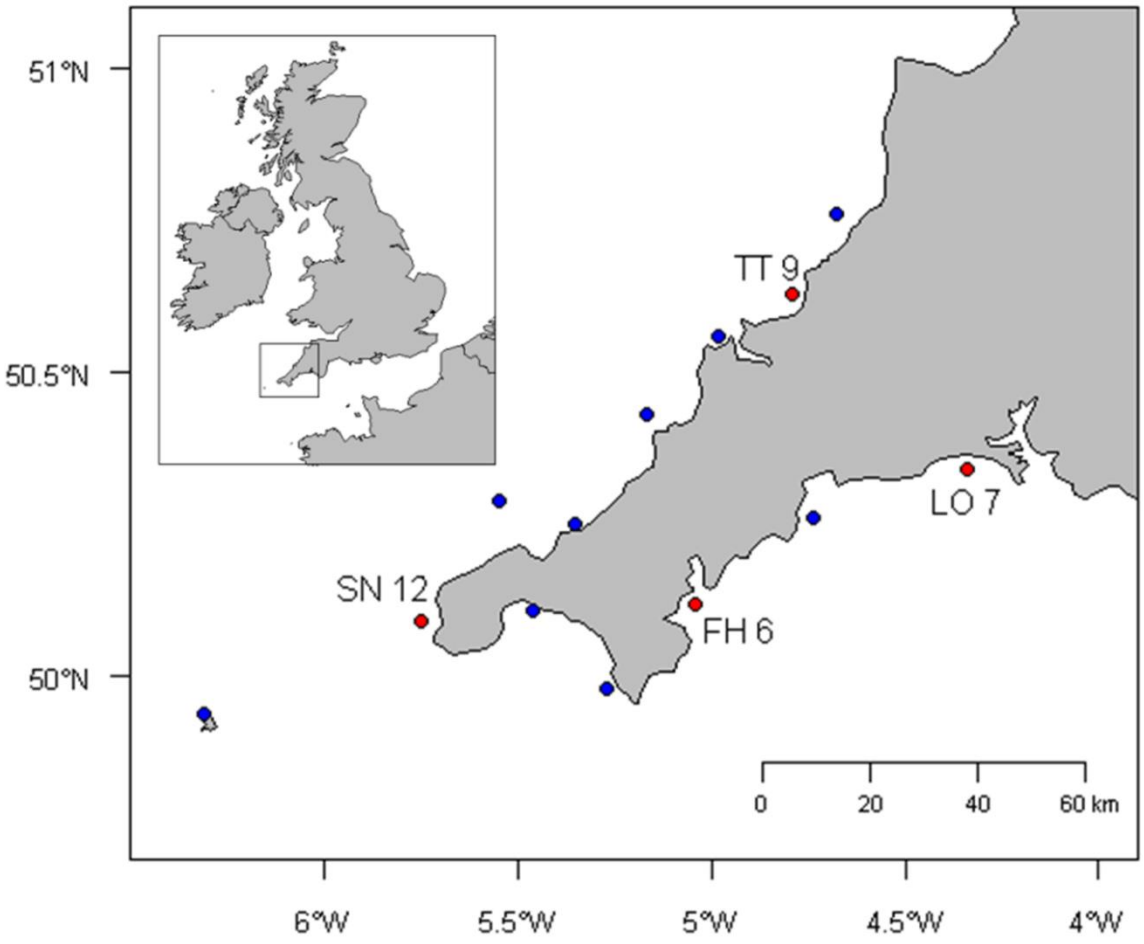

Figure 1. Map of sample sites. Map of the Cornwall peninsula showing the location of sampling sites. Red points denote the paternity sample sites Tintagel (TT), Sennen (SN), Falmouth (FH) and Looe (LO), with sample sizes denoting the number of clutches successfully tested. These four sites, and nine additional sites denoted by blue points, were each used to sample 24 individuals to provide accurate estimates of regional allele frequencies. Position relative to the UK, Ireland and continental Europe is inset.

Microsatellite genotyping

Genotyping of tissue samples was carried out using 15 microsatellite loci; 12 previously published (André & Knutsen, 2010), and the three newly characterised loci (see Text S1 for development process). Maternal DNA was extracted from individual pleopod tissues and progeny DNA from whole eggs

using the Wizard® SV 96 Genomic DNA Purification System (Promega). Primer oligonucleotides were synthesized by Eurofins Genomics (Eurofins Genomics), with forward primers 5'-tagged with one of four fluorescent sequencing dyes; FAM, ATTO 550, ATTO 565 and Yakima Yellow. The Multiplex PCR Kit (Qiagen) was used to allow the amplification of all loci across four multiplexes (See Table 1 for multiplex organisation). PCR volumes of 8 µl were prepared in the following reaction mix: 4 µl Multiplex PCR Mix; forward and reverse primers at 0.48 – 1.33 µM (Multiplex 1, 0.88 µM, apart from HGD106, 0.48 µM; Multiplex 2, 1.00 µM; Multiplexes 3 and 4, 1.33 µM); and 2 µl template DNA (20-50 ng). PCR was conducted in a Techne Prime Elite 96 thermocycler (Bibby Scientific Ltd.), with an initial denaturation (94^{o}C, 3 min), then 35 cycles of denaturation (94^{o}C, 40 s), annealing (55^{o}C, 40 s) and extension (72^{o}C, 30 s), before a final extension (72^{o}C, 4 min). Fragment analysis was carried out for the 312 samples using an ABI 3130 Genetic Analyser (Applied Biosystems Inc.). Alleles were automatically sized against Genescan™ 500 LIZ™ size standard (Applied Biosystems Inc.) using Geneious 6.1 software (Biomatters Ltd.), before also being checked manually and rescored where necessary.

While some studies have previously pooled eggs from each pleopod region or the whole clutch into single extractions, we elected to genotype eggs individually. Pooling progeny genotypes can allow the detection of multiple paternity while boosting the number of progeny screened and the sample size of females per unit effort, but such an approach can significantly underestimate the true number of sires (Bailie et al., 2011) and provides no way of estimating fertilisation skew. To prevent genotyping errors overestimating the occurrence of multiple paternity, any progeny genotype that did not support a single paternal contribution (i.e. where three or more alleles were recorded at a locus) was retested in single-locus PCR (using Qiagen Taq PCR Master Mix in place of Multiplex PCR Mix) and controlled fragment analysis procedures. The software FreeNA (Chapuis & Estoup, 2007) was used to estimate the frequency of null alleles from regional population genotype data of 312 individuals (see Text S1 for sampling details).

Statistical analysis

Probabilities of detecting multiple paternal contributions (PrDM) were quantified by the software *PrDM* (Neff & Pitcher, 2002). Using regional population allele frequencies (from 312 individuals – see Text S1 for sampling details), *PrDM* used Monte Carlo simulations to calculate PrDM under various scenarios of skew between the fertilisation contributions of multiple males; two males in ratios of 50:50, 60:40, 70:30, 80:20 and 90:10, and three males in ratios of 34:33:33, 50:25:25, 60:20:20, 70:15:15, 80:10:10 and 90:5:5. The software GERUD 2.0 (Jones, 2005) was used to estimate the exclusion probabilities (the probability that they exclude an unrelated individual from a putative pedigree; Dodds et al., 1996) of individual loci to enable loci to be ranked by power to assign parentage. GERUD 2.0 was used to reconstruct the minimum number of possible paternal genotypes, which were also independently assembled manually from progeny genotypes. Because GERUD 2.0 only reconstructs the minimum number of unknown parental contributions that can explain the progeny genotypes, two-allele genotypes are presumed to be heterozygotes. Although unlikely given the number of markers used, it is therefore possible that two males displaying only homozygote or shared alleles would be reconstructed as a single male. As such, total heterozygosity calculations and heterozygote excess tests were carried out on pooled parental genotypes using GENEPOP 4.2 software (Raymond & Rousset, 1995). The presence of heterozygote excess or significantly increased heterozygosity compared to known maternal genotypes could suggest an underestimation of the number of males contributing to reconstructed paternal genotypes.

Results

Egg DNA yields and female sizes

All eggs in intermediate and later stages of development (as evidenced by brown and red colouration) yielded suitable quantities of DNA for downstream analysis. However, 3 of 24 Celtic Sea females and 11 of 24 English Channel females possessed eggs that were either unfertilised (Johnson et al., 2011) or in early stages of development (as evidenced by black and/or dark green

colouration) from which DNA yields were insufficient to allow successful genotyping, reducing the actual sample sizes to 21 and 13 respectively. Of those females providing successful progeny arrays, size (CL) ranged from 94-155 mm (n_{Total} = 34, mean CL = 113.5 mm, SE ±2.31), with English Channel individuals (mean CL = 117.9 mm, SE ±4.26) tending to be slightly larger than those from Celtic Sea sites (mean = 110.7 mm, SE ±2.56).

Genotyping and marker power

Maternal and progeny samples that amplified effectively were screened at all 15 loci, however two loci were dropped from the analysis upon the detection of null alleles, which are known to introduce substantial errors in empirical assessments of parentage (Dakin & Avise, 2004; Hoffman & Amos, 2005; Morrissey & Wilson, 2005). In this case, null alleles appear to have caused mismatches between maternal and progeny genotypes, or progeny genotypes to suggest three paternal alleles at the loci HGA8 and HGC129 (in 11 and four occasions among 68 parents, respectively). FreeNA confirmed null alleles at frequencies of 0.11 for HGA8 and 0.04 for HGC129. Null allele frequencies were zero for all other loci except HGC103 and HGD111, for which negligible frequencies of 0.02 were estimated. Because of this, only the remaining 13 markers were used in the determination of potential paternal genotypes and PrDM. The exclusion probabilities of these individual loci ranged from 0.21 to 0.73 when using ten progeny arrays and a known maternal genotype (Table 1). Note that this probability is not a measurement of the likelihood of individual loci successfully detecting multiple paternity or determining the number of sires, but of their likelihood to correctly exclude unrelated males from potential parentage via genotypic mismatch (e.g. when surveying paternal candidates). As such it is indicative of the relative power provided by each locus. The three most powerful loci were HGC120, HGC131b and HGD110.

Table 1. Loci exclusion probabilities. Table ranking loci via individual exclusion probabilities, assuming an assay of 10 progeny genotypes and deriving allele frequencies from a regional population survey (see Text S1 for sampling details). * = loci which were removed from paternity analyses due to the presence of null alleles; as such these are ranked last and their exclusion probabilities will be inaccurate.

Rank	Locus	Multiplex	Exclusion Probability	
			Maternal genotype known	Neither parental genotype known
1	HGC120	4	0.732	0.575
2	HGC131b	4	0.662	0.491
3	HGD110	4	0.611	0.435
4	HGC111	3	0.494	0.314
5	HGB6	2	0.483	0.308
6	HGD106	1	0.481	0.301
7	HGC103	2	0.476	0.304
8	HGB4	1	0.430	0.251
9	HGC118	1	0.378	0.201
10	HGD111	3	0.350	0.186
11	HGD129	2	0.347	0.179
12	HGD117	1	0.320	0.178
13	HGC6	2	0.212	0.071
14	HGA8[a]	1	*0.647*	*0.473*
15	HGC129[a]	3	*0.543*	*0.363*

Loci are ranked via individual exclusion probabilities, assuming an assay of 10 progeny genotypes and deriving allele frequencies from a regional population survey (see Text S1 for sampling details). [a]Loci which were removed from paternity analyses due to the presence of null alleles; as such these are ranked last and their exclusion probabilities (italicised) will be inaccurate

Probability of detecting multiple paternity

With 10 progeny genotyped at 13 loci, the probability of detecting a secondary paternal contribution where one was present exceeded 0.99 assuming equal fertilisation contributions (Figure 2). The confidence threshold for the detection of additional males dropped below 95% only when the paternal contribution of secondary sires accounted for 25% or fewer of the progeny. If the paternal contribution had been highly skewed in favour of a primary male in this way, then more than 10 progeny genotypes would have been required to retain a 95% confidence level in PrDM (Figure 2). In scenarios where secondary contributions were split between two males (three sires in total), PrDM effectively remained unchanged, although for some scenarios, one or two fewer

progeny genotypes could still yield PrDM >0.95 (Supplementary Material, Table S3). Estimates of PrDM based on genotyping at only the three most polymorphic loci (all amplified within Multiplex 4) were almost as powerful as those attained by all 13 loci. PrDM was <0.95 at a lower paternal skew (70:30 as opposed to 75:25), but was only decreased by 0.002 - 0.037 under the fertilisation scenarios investigated.

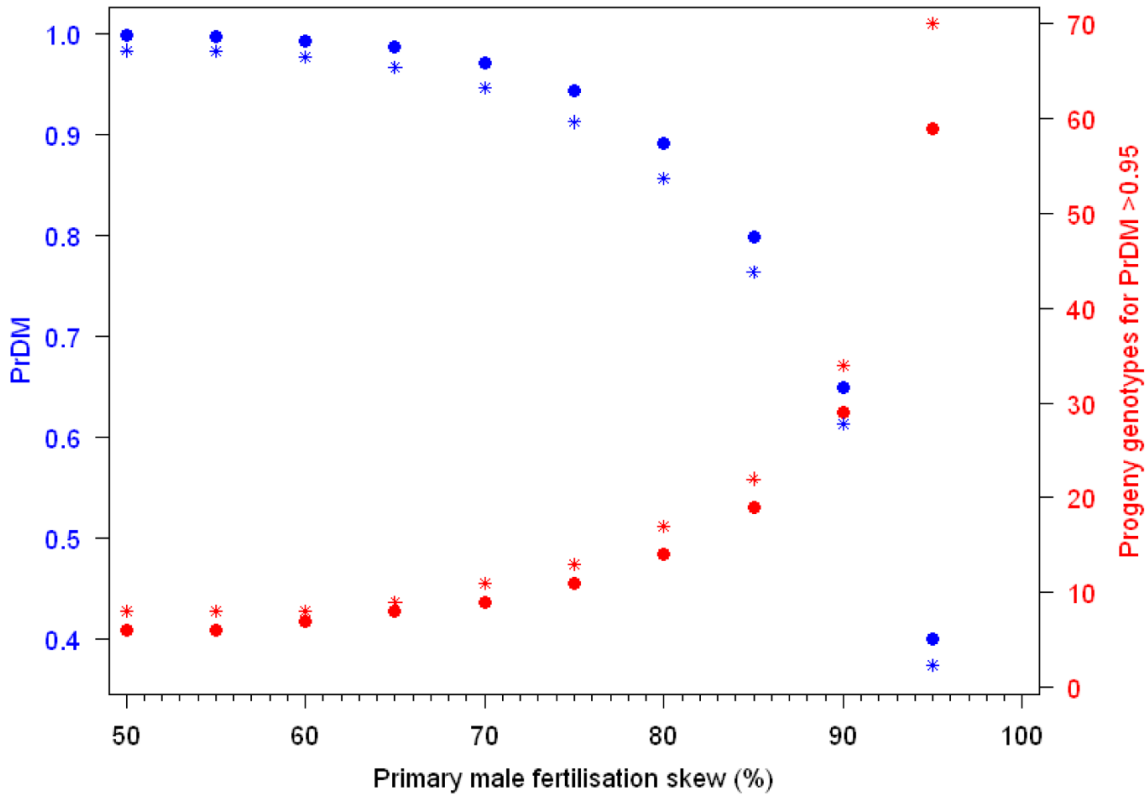

Figure 2. PrDM with skewed male fertilisation success. Variation in PrDM from 10 progeny genotypes (blue axis and data points) and the number of progeny genotypes required to achieve a 95% confidence level in PrDM (red axis and data points) under various scenarios of male fertilisation skew. Round points infer progeny genotyping at all 13 loci, while starred points infer progeny genotyping at only the three most informative loci (all amplified within Multiplex 4).

Paternal reconstruction

Reconstructions of paternal genotypes by GERUD 2.0 showed that single male genotypes explained all of the 34 progeny arrays. Where a clutch is sired by a single father, an array of ten offspring should give 99.9% power to reconstruct the paternal genotype (power = $100*(1-(0.5^{N.offspring}))$). Of the candidate paternal genotypes, 28 were able to be reconstructed in full at all 13 loci. For six reconstructed paternal genotypes, it was not possible for GERUD 2.0 to resolve

the paternal genotype at all 13 loci; four reconstructions were unable to determine paternal genotype at one locus and two more were unresolved at two loci. In these instances, both maternal and paternal genotypes were heterozygous and the paternal genotype possessed one allele that was shared with a maternal allele, but the progeny array contained no homozygotes to determine which allele was shared. On such occasions, GERUD 2.0 simply returned multiple single-sire genotypes that could explain the progeny array which were ranked in order of likelihood according to Mendelian segregation probability. All reconstructed male genotypes differed at multiple loci; no paternal genotype matched those provided by any other progeny array, so the clutches of all 34 females appeared to have been fertilised by 34 separate males. Total heterozygosity of reconstructed paternal genotypes was 0.68, while known maternal genotypes had a total heterozygosity of 0.69. A test for heterozygous excess among reconstructed paternal genotypes was non-significant ($p = 0.50$) and comparable to that obtained for known maternal genotypes ($p = 0.49$). Twelve allele scores (1.6%) were altered after genotyping was repeated. Had the original scores been analysed, it would have led to four incidences of multiple paternity (all with 1/10 progeny supporting a second sire).

Discussion

Unlike many other genetic studies on aquatic crustaceans (e.g. Bilodeau et al,. 2004; Streiff et al., 2004; Toonen, 2004; Gosselin et al., 2005; Bailie et al., 2011, 2014; Jensen & Bentzen, 2012; Karhl et al., 2014; Plough et al., 2014), our investigation found no evidence for multiple paternal fertilisations of individual *H. gammarus* broods. The loci employed ensured the statistical power to detect additional paternal fertilisations was consistently high, exceeding 99% when assuming approximately equal male representation among the progeny, and exceeding 95% wherever secondary males accounted for at least a quarter of the brood. This power to detect secondary sires is greater than that reported by Bailie *et al* (2011), which failed to reach 95% at any fertilisation skew when genotyping up to 86 galatheid squat lobster progeny at only two or three microsatellites, and is commensurate with that of Gosselin *et al* (2005) for *H. americanus* at equal (50:50) skews, but not at extreme (90:10) skews due to our

genotyping fewer eggs. The power to detect secondary paternal genotypes with low progeny representation is important since multiply-sired crustacean broods often show high levels of paternal skew, with Bailie *et al* (2011) estimating that secondary paternal fertilisations composed 14% or fewer of the majority of galatheid broods. Due to the statistical power of our method falling outside of 95% confidence limits at high paternal fertilisation skews, it is possible that multiple paternity was present but undetected in *H. gammarus* broods we assessed. It is unlikely, however; most (64%) multiply-sired broods identified by Gosselin *et al* (2005) exhibited secondary fertilisation contributions at ratios where detection probability would have exceeded 95% in our study. Even applying the least frequent rate of detection in a sub-population (11%) and the maximum skew (90:10) found among multiply-sired *H. americanus* clutches (Gosselin et al., 2005), we would still anticipate at least three cases of multiple paternity among our *H. gammarus* samples (two from Celtic Sea sites and one from English Channel sites), of which our power of detection (65%) would have been expected to overlook only one. Overall, our results suggest that multiple paternity is likely to be absent, or rare and highly skewed in favour of a dominant male, among *H. gammarus* in this geographical region.

While the reconstruction of paternal genotypes was conservative in that it provides the minimum number of males required to explain the observed progeny genotypes, it appears to be have been accurate in confirming single paternity. Overall heterozygosity of reconstructed paternal genotypes was equal to that of all maternal individuals, and showed no evidence of heterozygous excess, suggesting no underestimation of the number of sires represented among paternal reconstructions. Alongside reconstructing sire contributions from individual egg genotypes, some studies have inferred multiple paternity via significant departures of progeny genotypes from Mendelian expectations of allele frequencies (e.g. Bailie et al., 2011). However, this method was not considered for our analysis because it was deemed potentially ambiguous and unlikely to prove informative given the size of the progeny array per brood, and because the possibility of missing additional paternal alleles across 13 loci was remote.

The prevalence of single paternity among individual *H. gammarus* broods suggests that either (i) all females copulated only with a single male; or (ii) females copulated with more than one male, but fertilisation was attained by only a single male. In *H. americanus*, regular monandrous mating appears to be maintained by both female choice (female preference for the protection and/or spermatophore of dominant males; Cowan & Atema, 1990) and male competition (male efforts to prevent rival inseminations prior to the formation of a sperm plug; Gosselin et al., 2005). Clear evidence of female choice has also been observed in *H. gammarus* (Debuse et al., 2003), so the same processes may well occur in both species. Where polyandry was found in *H. americanus*, Gosselin *et al* (2005) proposed that female choice and/or male competition could have been altered by effects of fisheries-induced sex ratio imbalance, which may have included sperm limitation. However, male and female abundance and size distributions are approximately equal in *H. gammarus* around Cornwall (Davies, 2007; CEFAS, 2015), which may serve to maintain the ubiquity of monandrous mating. Male density affects the frequency of multiple paternity in many species (e.g. house mice, Dean et al., 2006; European earwig, Sandrin et al., 2015), and if the proportion of breeding males were driving variation in the occurrence of multiple paternity in lobsters, the frequency of multiply-sired clutches could follow a Gaussian distribution; both even sex ratios and extreme male depletion would be expected to lead to single paternity, with multiple paternity most frequent in an intermediate state of partial male depletion. For example, Levitan (2004) found that male density explained a normally-distributed dynamic in the fertilisation success of female Red sea urchins. Even if female lobsters were inseminated by multiple males, spermatophore stratification may ensure last-male precedence upon fertilisation, as is the case in Snow crabs (Urbani et al., 1998).

Potential mechanisms preserving single paternity in Cornwall may be weakened or absent in other *H. gammarus* stocks, however. Further assessments of paternity would be particularly valuable in stocks recovering from collapse (e.g. Norway – Agnalt et al., 1999, 2004, 2007), of limited size distribution (e.g. NE England – CEFAS, 2015), of high abundance (e.g. Lundy, UK – Hoskin et al., 2011; Wooton et al., 2012; Davies et al., 2015) and in the absence of fishing

(e.g. Lundy, UK; Flødevigen, Bolærne and Kvernskjær in Scandinavia – Huserbråten et al., 2013). If destabilised population demography were found to affect the frequency of multiple paternity, such data could be a useful reference point as to the health of lobster fisheries. Although *Homarus* species are presumed to be polygynous (Wahle et al., 2013), we found no evidence of any male fertilising multiple clutches, despite some females within individual sample sites being captured in close proximity (i.e. traps approximately 100 m apart). Sex-biased conservation measures may result in sperm limitation (e.g. MacDiarmid & Butler, 1999), so knowledge on paternity and the fertilisation success of individual males would benefit fishery managers in ensuring conservation legislation safeguards recruitment.

The results of PrDM simulations suggest that a different sampling regime to that which we employed would enhance power to detect multiple paternity at highly uneven skews. Genotyping 10 eggs per clutch at 13 loci amplified in four multiplexes (40 PCR reactions) gave us an estimated 65% power to detect additional males contributing just 10% of fertilisations. However, PrDM was only slightly reduced by using only the three most informative loci, which can be multiplexed together. As such, the attainment of >95% power to detect secondary males in a 90:10 fertilisation skew would have been possible with a progeny array of 34 eggs per clutch, each genotyped in a single PCR reaction (34 PCR reactions). Although this would require more DNA extractions, it may be a preferable option in future studies of parentage using these microsatellites, assuming those loci are similarly diverse elsewhere. Especially where population allele frequencies are readily available, *a priori* analysis of PrDM would be advisable to determine the most efficient sampling regime and marker panel. Further attempts to genotype *H. gammarus* eggs would also be advised to avoid clutches in early phases of development to ensure only fertilised eggs are sampled and that DNA yields are sufficient for downstream analysis.

Our findings of high allelic diversity and single paternal fertilisations in this population of *H. gammarus* bodes well for the potential utility of genetic markers in parentage assignments (Bernatchez & Duchesne, 2000) to enable evaluations of fisheries conservation measures, and particularly hatchery stocking. As a result of the recent collapses seen in some stocks and the

increased fishing pressures on others, attempts have been made in a variety of European locations, including Cornwall (National Lobster Hatchery, 2015), to enhance the productivity and sustainability of *H. gammarus* fisheries via the release of cultured juveniles (Bannister & Addison, 1998; Agnalt et al., 1999, 2004; Schmalenbach et al., 2011; Ellis et al., 2015a). Genetic tagging, the establishment hatchery origin via multi-locus assignment of parentage, has important advantages over existing tagging options for juvenile lobsters, such as sub-lethal sampling and no restrictions on the body size of released individuals, as well as providing data for the assessment of genetic impacts on the wild target stock (Ellis et al., 2015a). Hatcheries sourcing ovigerous lobsters from the wild may genotype maternal tissues directly, but paternal genotype(s) must be deduced from a sample of eggs or larvae in order to establish all possible progeny genotypes (Ellis et al., 2015a). Since single paternity appears to be the regular mode of fertilisation in this region, the resolution of parentage may be achieved by genotyping many fewer progeny than would be required were multiple paternity frequent. As a result, the compilation of the anticipated genotypes of released lobsters, a necessary step before surveying the wild population, would be more affordable. The development of a genetic tagging approach may become a crucial tool with which to assess and compare different *H. gammarus* conservation strategies, particularly in light of the scarcity of methods with which to monitor recruitment and the performance of wild larvae and juveniles (e.g. Mercer et al., 2001; Wahle et al., 2013; Ellis et al., 2015a).

Conclusions

Multi-locus genotyping proved a powerful tool in the assessment of paternity in *H. gammarus*, and provided evidence only of singly-sired clutches in an important regional population. Multiple paternity was not detected, indicating it is likely to be either absent, or irregular and highly skewed in favour of a single male. The detection of only single paternity among *H. gammarus* may reflect demographic stability in sex-ratios across a wide size distribution in this region. The development of additional microsatellite markers provides greater power for further studies of parentage and population genetics in *H. gammarus*. The prospects of their potential utility in evaluations of hatchery stocking and other

fishery conservation measures in Cornwall are increased by the establishment of single paternity as the dominant method of fertilisation.

Data availability: Multi-locus progeny arrays with paternal reconstructions, and microsatellite genotypes of 312 individuals, (13 spatial samples from Cornwall, UK, each of 24 individuals), used to in the calculation of regional allele frequencies, tests of HWE, linkage and null alleles, and in the development and characterisation of the novel loci HGD110, HGD117 and HGD129, are freely available online from the Dryad Digital Repository at http://dx.doi.org/10.5061/dryad.v176m.2

Supplementary material

Text S1 – Description of microsatellite development.

To improve analytical power, novel loci were developed to complement the species-specific microsatellite panel already publicly available. To characterise new loci, eight tetra-repeat microsatellites, isolated from partial genomic libraries, were used to design primer pairs as described by André & Knutsen (2010). Preliminary marker tests were conducted by analysing 12 individuals (none included in paternity assays), four from each of three of the study sites; Tintagel, Sennen and Looe. Of these eight loci, five either failed to amplify (HGC106), appeared to be monomorphic (HGC121), or presented significant difficulties in scoring alleles consistently (HGA5, HGC107, and HGD121) (Table S1). Further, comprehensive screening was conducted for the three loci that amplified reliably and were polymorphic (HGD110, HGD117 and HGD129). Comprehensive screening involved the analysis of 312 individuals; 24 from each of 13 geographic samples (including the four paternity sample sites; see Figure 1 in the main paper for locations) spanning 230 km of coastal waters from Looe (the south-eastern-most paternity sample site) to Boscastle (beyond the north-eastern-most paternity sample site) and west to the Isles of Scilly (offshore from the western-most paternity sample site). These samples were genotyped at the novel loci, as well as the existing 12 loci (André & Knutsen, 2010) to enable checks for linkage disequilibrium.

DNA extraction, PCR amplification and fragment analysis of loci followed the protocols listed in the Microsatellite Genotyping section in the main paper. Taq PCR Master Mix (Qiagen) used to amplify loci instead of Multiplex PCR Mix. Population differentiation among geographic samples was checked by G-tests in the web-based GENEPOP 4.2 software (Raymond & Rousset, 1995), to justify pooling samples as a single unit for the characterisation of novel loci, testing for null alleles, and the estimation of allele frequencies. Across all 15 loci, significant genic differentiation was detected among the 13 spatial samples, but not after the removal of HGA8 and HGC129, loci later found to be affected by null alleles. A G-test for overall population differentiation was then non-

significant (p = 0.07), and only four of 91 sample pairs showed significant differentiation (p < 0.05), as expected by chance alone.

These genotypes were also tested in GENEPOP 4.2 for heterozygosity, linkage disequilibrium and deviation from Hardy-Weinberg expectations. All tests of linkage disequilibrium were non-significant after this threshold was adjusted to account for multiple tests (Benjamini & Hochberg, 1995). No deviation from Hardy-Weinberg expectations were detected via the exact probability test (p = 0.30; Haldane, 1954) or U-test of global heterozygote excess (p = 0.50; Rousset & Raymond, 1995). For the newly-developed loci HGD110, HGD117 and HGD129, genotyping of the 312 individuals from Cornwall revealed that the number of alleles ranged from 10 to 11 and the observed heterozygosity was 0.56 to 0.82 (Table S2). The likelihood of null alleles being present was estimated in the software FreeNA (Chapuis & Estoup, 2007), which did not detect any failed amplification among alleles (estimated frequencies of null alleles were <0.0001 for all loci).

Table S1. Primer sequences of tested loci. Table featuring primer sequences of novel loci tested and cause of discard where development was not achieved.

Locus	Primer	Primer sequence (5'-3')	Developed / Reason undeveloped
HGD110	HGD110F	ACGGATGGATGGATAGGTAG	Developed
	HGD110R	ATTCTCTGGCAGGTCAAGAC	
HGD117	HGD117F	GCCTACTCTCTCCTTCCTTC	Developed
	HGD117R	ACCTGTCTATCGTTCTGTTTG	
HGD129	HGD129F	CCGTGCTGAAAGGGTTAT	Developed
	HGD129R	CAAACTATTCGTCCACAAAGTC	
HGA5	HGA5F	GGTGTCCAGCAAACAATATAGG	Difficulty in consistent scoring
	HGA5R	ACCTGCACTTGTACCCACAC	
HGD121	HGD121F	AGCAGATGTAACCGAGGTAGT	Difficulty in consistent scoring
	HGD121R	GAATGAAGCACCATAACACAG	
HGC107	HGC107F	CTCTGCTCTTTCTGGTGTTG	Difficulty in consistent scoring
	HGC107R	GTCGGCACTAAACTCATCAC	
HGC121	HGC121F	TCAACCTTTCCAGACAAGTGA	Appeared monomorphic
	HGC121R	AGGAACGTAGACCCGTACAGAG	
HGC106	HGC106F	GATCGAACTCAGGTCCAC	Failed to amplify
	HGC106R	TTTGTGTGTGTATGTGTG	

Table S2. Characterisation of three novel microsatellite loci. Genetic diversity information: N_A = number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; H-W = p-values for deviation from Hardy-Weinberg equilibrium as evidenced by exact test (*p*) and U-test of heterozygote excess (H_{ex}).

GenBank accession number	Locus	Primer sequence (5'-3')	Repeat motif	Size range (bp)	N_A	H_E	H_O	H-W	
								P	H_{ex}
KT240103	**HGD110**	F: ACGGATGGATGGATAGGTAG R: ATTCTCTGGCAGGTCAAGAC	(AGAT)8	176-220	11	0.799	0.824	0.563	0.201
KT240104	**HGD117**	F: GCCTACTCTCTCCTTCCTTC R: CCTGTCTATCGTTCTGTTTG	(ATAG)7	254-302	10	0.574	0.574	0.116	0.195
KT240105	**HGD129**	F: CCGTGCTGAAAGGGTTAT R: CAAACTATTCGTCCACAAAGTC	(AGAT)11	234-290	10	0.563	0.564	0.837	0.640

Table S3. Estimates of PrDM at various paternity scenarios. Table shows calculations of the probability of detecting multiple paternal contributions (PrDM) and the number of egg genotypes required to achieve a 95% confidence level in PrDM. Values reflect various scenarios of numbers of sires and their fertilisation skew, and are calculated for all 13 loci (as used in this study) and the three most polymorphic loci (all from Multiplex 4). Predictions used allele frequencies obtained from a survey of 312 individuals in the south-western United Kingdom.

Paternal skew – two sires (Primary male : Secondary male)		**50:50**	**60:40**	**70:30**	**80:20**	**90:10**
13 loci (4 multiplexes)	**PrDM with 10 eggs**	0.998	0.993	0.970	0.891	0.649
	***n* eggs for PrDM >0.95**	6	7	9	14	29
3 loci (1 multiplex)	**PrDM with 10 eggs**	0.983	0.976	0.946	0.856	0.612
	***n* eggs for PrDM >0.95**	8	8	11	17	34

Paternal skew – three sires (Primary male : Secondary males)		**34:33:33**	**50:25:25**	**60:20:20**	**70:15:15**	**80:10:10**	**90:5:5**
13 loci (4 mplxs)	**PrDM with 10 eggs**	1.000	0.999	0.994	0.971	0.890	0.648
	***n* eggs for PrDM >0.95**	5	5	6	9	14	29
3 loci (1 mplx)	**PrDM with 10 eggs**	0.998	0.996	0.986	0.955	0.862	0.616
	***n* eggs for PrDM >0.95**	6	6	8	10	16	32

Table S4. Progeny arrays of 34 clutches, in which surveyed maternal (MAT) and embryo (1-5, b&t) genotypes were used to reconstruct the paternal (PAT) genotype(s) at 15 loci, two of which (A8 and C129) were omitted from subsequent paternity analysis due to non-amplifying alleles.

Tintagel 1: TT103a	Locus	D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120		*Loci dropped due to null alleles*	A8		C129	
	Allele	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
	Mat	**164**	**164**	**280**	**284**	**216**	**224**	**262**	**278**	**232**	**244**	**186**	**190**	**258**	**270**	**289**	**289**	**174**	**178**	**235**	**275**	**192**	**212**	**255**	**255**	**297**	**297**		**302**	**306**	**299**	**299**
	1b	164	164	284	288	224	232	278	278	232	240	182	186	250	258	289	289	174	186	275	275	188	192	255	259	297	297		306	306	295	299
	1t	156	164	284	288	216	232	278	278	236	244	186	186	258	266	289	289	174	174	235	275	196	212	255	259	297	297		278	306	299	303
	2b	164	164	284	288	216	232	278	278	232	240	182	190	250	258	289	289	174	178	275	275	196	212	255	259	297	297		278	306	295	299
	2t	164	164	280	296	192	216	262	274	232	240	182	190	266	270	289	289	174	186	275	275	196	212	255	259	297	297		306	306	299	303
	3b	156	164	280	288	192	216	274	278	240	244	182	186	250	270	289	289	174	178	235	275	196	212	255	267	297	297		302	306	295	299
	3t	164	164	280	288	216	232	262	274	232	240	186	186	250	270	289	289	178	186	235	275	192	196	255	259	289	297		278	302	299	303
	4b	164	164	280	296	216	232	278	278	232	240	186	190	266	270	289	289	174	178	275	275	196	212	255	259	289	297		278	306	295	299
	4t	156	164	284	288	192	224	262	274	240	244	182	190	250	258	289	289	174	186	275	275	192	196	255	259	289	297		278	302	299	303
	5b	156	164	284	296	224	232	262	278	240	244	182	186	258	266	289	289	174	174	235	275	188	212	255	259	297	297		278	302	295	299
	5t	164	164	284	296	192	216	262	278	232	240	182	186	258	266	289	289	174	174	235	275	188	192	255	267	289	297		278	306	295	299
	Pat	**156**	**164**	**288**	**296**	**192**	**232**	**274**	**278**	**236**	**240**	**182**	**186**	**250**	**266**	**289**	**289**	**174**	**186**	**275**	**275**	**188**	**196**	**259**	**267**	**289**	**297**		**278**	**306**	**295**	**303**

Tintagel 2: TT105	Locus	D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120		*Loci dropped due to null alleles*	A8		C129	
	Allele	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
	Mat	**156**	**164**	**284**	**284**	**192**	**212**	**274**	**278**	**240**	**244**	**182**	**190**	**258**	**266**	**277**	**289**	**182**	**186**	**239**	**275**	**196**	**200**	**251**	**263**	**249**	**293**		**294**	**310**	**299**	**299**
	1b	156	164	276	284	192	236	274	278	232	244	182	190	266	266	289	289	178	186	239	275	196	200	263	267	249	281		294	310	299	299
	1t	156	156	276	284	192	224	274	278	240	244	182	182	266	266	289	293	178	182	239	275	192	196	251	267	293	293		294	310	299	299
	2b	156	156	276	284	212	224	274	274	232	240	182	182	266	266	289	289	178	182	235	239	196	200	263	263	281	293		306	310	299	299
	2t	156	156	284	284	212	224	274	274	232	244	182	182	258	266	289	293	174	182	235	275	196	200	263	267	249	293		310	310	295	299
	3b	156	156	276	284	212	236	274	274	240	244	182	182	258	266	289	289	174	186	239	275	192	200	263	267	249	281		310	310	295	299
	3t	156	156	284	284	192	236	274	278	240	240	182	190	266	266	277	293	174	186	235	239	196	200	263	267	249	293		306	310	299	299
	4b	156	164	276	284	212	224	274	278	240	244	182	182	266	266	289	293	178	182	235	275	192	196	263	267	249	281		294	310	299	299
	4t	156	156	284	284	192	224	274	278	240	244	182	182	266	266	289	293	178	182	239	275	192	200	263	263	249	293		294	306	295	299
	5b	156	156	284	284	212	224	274	274	240	240	182	182	258	266	289	293	178	186	235	275	192	196	263	267	249	293		294	310	295	299
	5t	156	164	284	284	192	236	274	278	240	240	182	182	258	266	289	293	174	182	235	275	200	200	263	267	281	293		294	306	295	299
	Pat	**156**	**156**	**276**	**284**	**224**	**236**	**274**	**274**	**232**	**240**	**182**	**182**	**266**	**266**	**289**	**293**	**174**	**178**	**235**	**239/ 275**	**192**	**200**	**263**	**267**	**281**	**293**		**306**	**310**	**295**	**299**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Tintagel 3: TT106	**Mat**	**160**	**160**	**284**	**292**	**232**	**232**	**274**	**274**	**240**	**244**	**182**	**182**	**270**	**270**	**285**	**285**	**162**	**174**	**239**	**239**	**188**	**200**	**255**	**263**	**297**	**297**	*Loci dropped due to null alleles*	**298**	**306**	**295**	**303**
	1b	156	160	276	284	232	236	274	278	240	240	182	186	258	270	285	285	162	182	239	239	188	188	255	263	297	303		298	306	295	303
	1t	156	160	276	292	212	232	274	278	240	244	182	186	258	270	285	285	174	182	239	239	188	188	251	263	297	303		298	306	303	303
	2b	156	160	280	284	212	232	274	278	240	240	182	182	258	270	285	289	162	182	239	239	188	188	251	255	297	303		306	306	303	303
	2t	156	160	276	292	232	236	274	278	240	240	182	182	258	270	285	285	174	182	239	239	188	192	251	255	297	303		298	306	303	303
	3b	156	160	280	292	232	236	274	278	244	244	182	182	258	270	285	289	162	182	239	239	192	200	263	263	297	303		298	306	303	303
	3t	156	160	276	292	212	232	274	278	240	240	182	186	258	270	285	289	162	182	239	239	188	188	251	263	297	303		298	306	303	303
	4b	156	160	280	284	212	232	274	278	240	244	182	186	258	270	285	285	174	182	239	239	188	192	251	255	297	303		298	306	303	303
	4t	156	160	280	284	232	236	274	278	240	244	182	182	258	270	285	289	162	182	239	239	192	200	251	255	297	303		298	306	295	303
	5b	156	160	276	284	212	232	274	278	240	244	182	182	258	270	285	289	174	182	239	239	188	192	263	263	297	303		298	306	295	303
	5t	156	160	276	292	232	236	274	278	244	244	182	186	258	270	285	285	174	182	239	239	192	200	255	263	297	303		298	306	295	303
	Pat	**156**	**156**	**276**	**280**	**212**	**236**	**278**	**278**	**240**	**244**	**182**	**186**	**258**	**258**	**285**	**289**	**182**	**182**	**239**	**239**	**188**	**192**	**251**	**263**	**303**	**303**		**306**	**306**	**303**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Tintagel 4: TT114	**Mat**	**156**	**164**	**280**	**284**	**212**	**212**	**274**	**274**	**244**	**244**	**174**	**186**	**258**	**270**	**289**	**289**	**178**	**186**	**239**	**251**	**188**	**200**	**263**	**267**	**297**	**297**	*Loci dropped due to null alleles*	**294**	**314**	**291**	**291**
	1b	152	164	276	284	192	212	274	274	244	244	162	174	258	270	289	289	186	186	239	239	200	200	247	263	285	297		294	302	291	307
	1t	152	164	284	284	192	212	274	274	244	244	162	174	270	270	289	289	178	186	239	239	188	200	247	267	289	297		294	302	291	307
	2b	152	156	276	280	192	212	274	274	244	244	150	186	258	258	289	289	178	186	239	239	200	200	263	267	289	297		294	302	291	307
	2t	156	156	276	280	212	212	274	274	240	244	162	174	258	270	289	289	186	186	239	239	188	200	247	267	289	297		314	314	291	307
	3b	156	164	276	284	192	212	274	274	240	244	162	186	258	258	289	289	178	186	239	251	188	200	247	267	289	297		302	314	299	299
	3t	152	156	284	284	192	212	274	274	240	244	150	186	258	258	289	289	186	186	239	239	188	200	247	263	285	297		294	302	307	307
	4b	156	164	284	284	192	212	274	274	240	244	162	174	270	270	289	289	186	186	239	251	200	200	247	263	285	297		294	294	299	299
	4t	156	164	280	284	192	212	274	274	240	244	150	186	258	270	289	289	186	186	239	251	188	200	247	267	289	297		302	314	291	307
	5b	152	156	280	284	192	212	274	274	244	244	150	174	258	270	289	289	186	186	239	251	188	200	263	267	289	297		314	314	307	307
	5t	152	156	284	284	192	212	274	274	240	244	150	174	258	270	289	289	186	186	239	239	188	200	247	263	289	297		294	294	299	299
	Pat	**152**	**156**	**276**	**284**	**192**	**212**	**274**	**274**	**240**	**244**	**150**	**162**	**258**	**270**	**289**	**289**	**186**	**186**	**239**	**239**	**200**	**200**	**247**	**263**	**285**	**299**		**302**	**Pat null?**	**299/ 307**	**Mat null?**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Tintagel 5: TT103b	**Mat**	**152**	**156**	**280**	**284**	**212**	**224**	**274**	**274**	**240**	**240**	**178**	**182**	**258**	**258**	**285**	**289**	**174**	**186**	**239**	**239**	**188**	**200**	**251**	**267**	**285**	**297**	*Loci dropped due to null alleles*	**274**	**310**	**299**	**303**
	1b	152	156	280	292	224	224	274	278	240	240	182	182	258	258	289	289	174	186	239	239	192	200	259	267	285	285		306	310	303	303
	1t	152	156	280	284	212	224	274	278	240	240	178	182	258	258	285	289	186	186	239	239	200	200	251	267	285	287		274	306	299	303
	2b	152	160	280	284	212	212	274	278	240	240	178	182	258	258	289	289	186	186	239	239	192	200	251	267	285	287		274	306	303	303
	2t	156	160	280	280	212	212	274	278	240	240	182	182	258	258	285	289	174	186	239	239	188	192	267	267	285	287		274	306	299	303
	3b	152	152	280	292	212	224	274	278	240	240	182	182	258	258	285	289	174	186	239	239	188	192	259	267	285	297		274	306	299	299
	3t	152	156	284	292	212	224	274	278	240	240	182	182	258	258	285	289	174	186	239	239	192	200	267	267	285	285		306	310	299	303
	4b	156	160	280	280	224	224	274	278	240	240	182	182	258	258	285	289	186	186	239	239	188	200	267	267	287	297		306	310	303	303
	4t	152	160	280	292	212	224	274	278	240	240	178	182	258	258	289	289	174	186	239	239	188	192	251	259	285	287		306	310	299	299
	5b	152	152	280	280	212	224	274	278	240	240	178	182	258	258	289	289	174	186	239	239	188	200	251	259	287	297		274	306	299	299
	5t	152	160	284	292	212	212	274	278	240	240	182	182	258	258	289	289	186	186	239	239	200	200	259	267	285	297		274	306	299	303
	Pat	**152**	**160**	**280**	**292**	**212**	**224**	**278**	**278**	**240**	**240**	**182**	**182**	**258**	**258**	**289**	**289**	**186**	**186**	**239**	**239**	**192**	**200**	**259**	**267**	**285**	**287**		**306**	**306**	**299**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Tintagel 6: TT110	**Mat**	**160**	**164**	**284**	**284**	**216**	**224**	**274**	**274**	**240**	**244**	**182**	**190**	**258**	**290**	**289**	**289**	**186**	**186**	**239**	**239**	**184**	**216**	**263**	**275**	**285**	**297**	*Loci dropped due to null alleles*	**302**	**306**	**299**	**303**
	1b	152	160	284	284	212	216	274	274	240	244	182	190	254	258	289	293	186	186	239	275	184	200	263	267	293	297		278	302	299	303
	1t	152	164	284	284	212	216	274	274	240	244	186	190	254	290	289	289	178	186	239	275	200	216	263	267	297	303		278	302	303	303
	2b	156	160	284	284	212	224	274	274	240	240	186	190	290	290	289	289	186	186	239	239	200	216	267	275	293	297		278	302	299	299
	2t	152	160	284	284	216	224	274	274	240	244	190	190	254	290	289	289	186	186	239	239	200	216	267	275	285	303		278	302	303	303
	3b	156	160	284	284	216	224	274	274	240	240	182	186	254	290	289	289	186	186	239	275	200	216	263	267	297	303		278	306	299	303
	3t	152	160	284	284	212	224	274	274	232	240	186	190	254	290	289	289	186	186	239	275	200	216	263	267	297	303		278	302	299	303
	4b	156	160	284	284	216	224	274	274	240	240	182	186	254	290	289	289	178	186	239	239	184	200	267	275	297	303		278	302	303	303
	4t	152	160	284	284	212	216	274	274	232	244	186	190	254	258	289	289	186	186	239	275	200	216	263	267	293	297		278	306	299	303
	5b	156	160	284	284	216	216	274	274	232	244	186	190	254	258	289	289	178	186	239	275	184	200	267	275	285	303		278	302	299	299
	5t	156	160	284	284	216	224	274	274	232	240	182	186	254	290	289	289	186	186	239	275	200	216	267	275	297	303		278	306	299	299
	Pat	**152**	**156**	**284**	**284**	**212**	**216**	**274**	**274**	**232**	**240**	**186**	**190**	**254**	**290**	**289**	**293**	**178**	**186**	**239**	**275**	**200**	**200**	**267**	**267**	**293**	**303**		**278**	**278**	**299**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Tintagel 7: TT111	**Mat**	**156**	**164**	**284**	**292**	**212**	**212**	**274**	**274**	**240**	**244**	**182**	**182**	**258**	**270**	**289**	**289**	**182**	**186**	**239**	**267**	**180**	**216**	**239**	**263**	**285**	**297**	*Loci dropped due to null alleles*	**274**	**314**	**295**	**303**
	1b	156	164	284	292	192	212	274	274	240	240	182	186	258	258	289	289	186	186	239	239	180	208	263	267	285	301		274	274	295	303
	1t	156	156	284	284	192	212	274	274	240	244	182	190	258	270	289	289	186	186	239	275	208	216	239	267	297	301		274	274	295	295
	2b	156	156	284	284	192	212	274	274	244	244	182	186	258	270	289	289	182	186	239	239	180	200	235	263	297	301		302	314	295	303
	2t	156	156	284	284	192	212	274	274	240	240	182	190	258	270	289	289	186	186	267	275	180	200	235	239	285	297		274	274	303	303
	3b	156	164	284	284	192	212	274	274	244	244	182	190	258	270	289	289	182	186	239	275	180	200	235	263	297	301		274	274	295	303
	3t	156	156	284	284	192	212	274	274	240	240	182	186	258	270	289	289	186	186	239	239	208	216	235	239	297	301		302	314	295	303
	4b	156	164	284	292	192	212	274	274	240	244	182	190	258	258	289	289	182	186	267	275	208	216	263	267	297	297		302	314	295	295
	4t	156	164	284	284	192	212	274	274	240	244	182	186	258	270	289	289	186	186	239	275	180	200	239	267	285	297		274	274	295	303
	5b	156	164	284	284	192	212	274	274	240	240	182	186	258	258	289	289	182	186	267	275	208	216	263	267	297	297		274	274	303	303
	5t	156	164	284	284	192	212	274	274	240	240	182	190	258	270	289	289	186	186	239	267	208	216	239	267	297	297		274	274	295	295
	Pat	**156**	**156**	**284**	**284**	**192**	**192**	**274**	**274**	**240**	**240**	**186**	**190**	**258**	**258**	**289**	**289**	**186**	**186**	**239**	**275**	**200**	**208**	**235**	**267**	**297**	**301**		**274**	**302**	**295**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Tintagel 8: TT97	**Mat**	**156**	**168**	**284**	**284**	**212**	**212**	**278**	**278**	**232**	**248**	**186**	**190**	**258**	**270**	**289**	**289**	**174**	**186**	**239**	**239**	**200**	**216**	**255**	**267**	**283**	**297**	*Loci dropped due to null alleles*	**302**	**306**	**303**	**307**
	1b	168	168	284	284	212	212	274	278	244	248	182	186	258	270	289	289	174	186	235	239	216	216	255	267	283	285		302	302	295	307
	1t	156	168	284	284	212	212	274	278	232	244	186	190	258	266	285	289	174	186	235	239	200	204	255	263	283	285		302	306	295	303
	2b	148	168	284	284	212	212	274	278	232	232	190	190	266	270	285	289	186	186	235	239	204	216	267	267	297	297		302	314	295	303
	2t	156	168	284	284	212	212	274	278	232	232	190	190	258	270	285	289	174	186	239	239	200	204	267	267	283	285		302	314	295	303
	3b	148	156	284	284	212	212	274	278	244	248	186	190	266	270	289	289	174	186	235	239	204	216	267	267	285	297		306	314	295	307
	3t	156	168	284	284	212	212	274	278	232	232	182	186	258	266	285	289	174	186	239	239	200	204	255	263	283	297		302	314	295	307
	4b	148	156	284	284	212	212	274	278	232	248	182	190	258	258	289	289	186	186	235	239	200	204	255	263	285	297		302	302	295	303
	4t	168	168	284	284	212	212	274	278	232	248	182	186	258	258	285	289	174	174	239	239	216	216	255	263	283	297		302	306	295	303
	5b	148	168	284	284	212	212	274	278	232	244	182	186	258	266	289	289	186	186	239	239	200	216	263	267	297	297		302	302	295	307
	5t	148	168	284	284	212	212	274	278	232	244	182	186	258	258	285	289	174	186	239	239	204	216	255	267	283	297		302	302	295	303
	Pat	**148**	**168**	**284**	**284**	**212**	**212**	**274**	**274**	**232**	**244**	**182**	**190**	**258**	**266**	**285**	**289**	**174**	**186**	**235**	**239**	**204**	**216**	**263**	**267**	**285**	**297**		**302**	**314**	**295**	**295**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Tintagel 9: TT94	**Mat**	**156**	**164**	**276**	**284**	**212**	**212**	**274**	**278**	**240**	**244**	**162**	**182**	**258**	**258**	**289**	**289**	**170**	**174**	**239**	**239**	**196**	**200**	**263**	**267**	**297**	**303**	*Loci dropped due to null alleles*	**274**	**310**	**179**	**303**
	1b	156	164	284	284	212	212	274	278	244	244	162	182	258	262	289	289	170	174	239	275	196	204	263	263	283	303		274	274	179	179
	1t	156	156	280	284	212	236	274	278	232	240	182	182	258	258	289	289	170	174	239	275	196	196	263	263	293	297		274	274	179	303
	2b	152	164	276	284	212	212	278	278	232	244	162	182	258	262	289	289	170	170	239	275	200	204	263	267	283	303		310	310	179	179
	2t	152	164	284	284	212	236	262	274	244	244	162	182	258	258	289	289	174	174	239	239	196	200	263	263	283	303		274	274	179	179
	3b	152	156	276	284	212	236	274	278	232	244	162	182	258	262	289	289	170	174	239	239	196	200	263	263	283	297		274	310	179	179
	3t	156	156	284	284	212	236	262	278	244	244	162	182	258	262	289	289	170	174	239	275	200	204	263	263	283	297		274	274	179	179
	4b	152	164	284	284	212	212	262	274	232	244	182	182	258	262	289	289	170	170	239	239	200	204	263	267	283	303		274	274	179	303
	4t	156	156	280	284	212	236	262	274	232	240	182	182	258	258	289	289	174	174	239	275	200	204	263	263	293	303		310	310	179	303
	5b	156	156	280	284	212	236	274	278	232	244	162	182	258	258	289	289	170	174	239	275	196	204	263	267	283	297		274	310	179	303
	5t	152	156	284	284	212	236	262	274	232	240	182	182	258	262	289	289	170	170	239	239	196	204	263	267	293	297		310	310	179	179
	Pat	**156**	**152**	**280**	**284**	**212**	**236**	**278**	**262**	**232**	**244**	**182**	**182**	**258**	**262**	**289**	**289**	**170**	**174**	**239**	**275**	**196**	**204**	**263**	**263**	**283**	**293**		**274**	**310**	**179**	**179**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 1: SN110a	**Mat**	**156**	**164**	**284**	**284**	**232**	**232**	**274**	**278**	**232**	**240**	**182**	**186**	**250**	**290**	**289**	**289**	**174**	**178**	**239**	**239**	**188**	**216**	**263**	**263**	**279**	**285**	*Loci dropped due to null alleles*	**306**	**310**	**295**	**299**
	1b	156	164	284	284	212	232	274	278	232	240	162	182	250	258	289	289	174	178	199	239	188	216	255	263	279	283		302	310	295	303
	1t	156	156	284	284	212	232	274	274	240	244	182	182	258	290	289	289	178	178	239	275	188	188	239	263	279	283		306	306	299	299
	2b	156	164	284	284	224	232	274	274	240	244	162	186	250	258	289	289	178	178	239	275	192	216	255	263	279	297		310	310	299	303
	2t	156	156	284	284	224	232	274	278	240	244	182	182	258	290	289	289	174	186	199	239	188	192	255	263	285	297		310	310	299	299
	3b	156	156	284	284	224	232	274	274	232	240	182	186	258	290	289	289	178	178	199	239	188	216	239	263	279	283		310	310	299	303
	3t	156	156	284	284	224	232	274	278	232	232	182	186	258	290	289	289	178	178	199	239	188	192	239	263	279	297		310	310	295	303
	4b	156	164	284	284	212	232	274	274	232	244	182	186	250	258	289	289	178	186	199	239	188	216	239	263	279	297		302	310	299	303
	4t	156	164	276	284	212	232	274	274	240	244	182	182	250	266	289	289	174	186	239	275	188	192	255	263	285	297		302	306	299	303
	5b	156	164	276	284	224	232	274	274	232	232	182	186	266	290	289	289	174	186	199	239	188	216	255	263	283	285		306	306	299	303
	5t	156	156	284	284	224	232	274	274	232	232	182	186	250	258	289	289	178	178	239	275	192	216	255	263	279	283		302	306	299	299
	Pat	**156**	**156**	**276**	**284**	**212**	**224**	**274**	**274**	**232**	**244**	**162**	**182**	**258**	**266**	**289**	**289**	**178**	**186**	**199**	**275**	**188**	**192**	**239**	**255**	**283**	**297**		**302**	**Pat null?**	**299**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 2: SN110b	**Mat**	**140**	**156**	**284**	**284**	**224**	**224**	**274**	**278**	**232**	**244**	**162**	**182**	**258**	**258**	**289**	**289**	**182**	**186**	**275**	**275**	**192**	**200**	**263**	**267**	**297**	**297**	*Loci dropped due to null alleles*	**306**	**306**	**303**	**303**
	1b	152	156	284	296	212	224	278	278	240	244	162	182	258	258	285	289	186	186	239	275	192	212	263	267	281	297		306	306	299	303
	1t	156	156	284	284	212	224	278	278	224	232	182	182	258	258	285	289	186	186	239	275	192	212	251	267	297	297		306	306	299	303
	2b	152	156	284	296	212	224	274	278	224	244	162	182	258	258	289	289	186	186	239	275	196	200	251	263	281	297		306	306	299	303
	2t	140	156	284	284	212	224	274	278	232	240	162	182	258	258	289	289	178	186	239	275	196	200	267	267	297	297		306	306	303	307
	3b	140	156	284	284	212	224	274	274	240	244	162	182	258	258	289	289	178	186	239	275	192	212	267	267	281	297		306	306	303	307
	3t	156	156	284	284	212	224	274	274	224	232	162	182	258	258	289	289	182	186	239	275	192	196	251	267	297	297		306	306	303	307
	4b	156	156	284	284	212	224	274	278	232	240	162	182	258	258	285	289	182	186	239	275	196	200	267	267	297	297		306	306	303	307
	4t	152	156	284	284	212	224	274	278	240	244	162	182	258	258	289	289	178	182	239	275	196	200	251	263	281	297		306	306	299	303
	5b	152	156	284	296	212	224	274	278	224	244	182	182	258	258	285	289	178	182	235	275	192	212	267	267	297	297		306	306	299	303
	5t	152	156	284	284	212	224	274	278	224	232	182	182	258	258	289	289	186	186	239	275	200	212	263	267	281	297		306	306	299	303
	Pat	**152**	**156**	**284**	**296**	**212**	**212**	**274**	**278**	**224**	**240**	**182**	**182**	**258**	**258**	**285**	**289**	**178**	**186**	**235**	**239**	**196**	**212**	**251**	**267**	**281**	**297**		**306**	**306**	**299**	**307**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 3: SN134	**Mat**	**156**	**164**	**284**	**284**	**212**	**212**	**274**	**278**	**240**	**244**	**186**	**190**	**258**	**258**	**285**	**289**	**186**	**186**	**199**	**239**	**188**	**188**	**259**	**267**	**275**	**303**	*Loci dropped due to null alleles*	**306**	**310**	**299**	**299**
	1b	156	156	284	284	212	224	274	274	240	244	190	190	258	258	285	289	182	186	239	239	188	188	267	267	289	303		306	306	299	307
	1t	156	156	276	284	212	224	274	274	240	244	186	190	258	258	285	293	182	186	239	239	188	192	267	267	275	289		306	306	299	303
	2b	156	164	284	284	212	224	274	278	240	240	190	190	258	258	289	293	182	186	199	239	188	188	267	267	297	303		310	310	299	307
	2t	156	156	284	284	212	224	274	278	240	244	190	190	258	258	285	289	178	186	239	239	188	192	267	267	275	297		302	310	299	303
	3b	156	156	276	284	212	224	274	274	240	244	186	190	258	258	285	289	182	186	199	239	188	192	259	267	297	303		306	306	299	307
	3t	156	156	284	284	212	224	274	278	240	244	186	190	258	258	285	285	178	186	239	239	188	192	259	267	275	289		310	310	299	303
	4b	156	164	276	284	212	224	278	278	240	244	190	190	258	258	285	289	182	186	239	239	188	188	267	267	289	303		302	306	299	303
	4t	156	164	276	284	212	224	278	278	240	240	190	190	258	258	289	293	178	186	199	239	188	192	259	267	297	303		302	306	299	303
	5b	156	156	284	284	212	224	274	278	240	244	182	186	258	258	285	293	178	186	199	239	188	192	267	267	289	303		302	306	299	303
	5t	156	156	276	284	212	224	274	278	244	244	186	190	258	258	285	293	182	186	199	239	188	188	259	267	275	297		306	306	299	303
	Pat	**156**	**156**	**276**	**284**	**224**	**224**	**274**	**278**	**240**	**240**	**182**	**190**	**258**	**258**	**285**	**293**	**178**	**182**	**239**	**239**	**188**	**192**	**267**	**267**	**289**	**297**		**302**	**Pat null?**	**303**	**307**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 4: SN101	**Mat**	**152**	**160**	**284**	**292**	**212**	**224**	**262**	**274**	**240**	**240**	**182**	**186**	**258**	**290**	**289**	**289**	**170**	**178**	**239**	**239**	**188**	**188**	**255**	**267**	**297**	**303**	*Loci dropped due to null alleles*	**302**	**302**	**303**	**303**
	1b	156	160	280	292	216	224	262	274	240	244	182	186	234	258	289	289	170	178	239	275	188	192	251	267	297	297		306	306	303	307
	1t	152	156	280	284	216	224	262	274	240	240	182	186	234	258	289	289	170	178	239	239	188	192	251	267	297	303		302	302	303	307
	2b	156	160	280	284	212	232	274	274	240	240	182	186	258	290	289	289	178	178	239	275	188	192	267	267	297	297		306	306	303	303
	2t	156	160	280	292	216	224	262	278	240	244	182	186	258	258	289	289	170	174	239	239	188	192	267	267	297	303		306	306	303	303
	3b	152	156	280	284	212	232	274	278	240	240	182	186	258	290	289	289	174	178	239	275	188	192	251	255	297	303		306	306	303	307
	3t	156	160	280	284	224	232	262	274	240	244	182	182	234	290	289	289	174	178	239	239	188	192	255	267	297	297		302	302	303	307
	4b	152	156	280	284	216	224	262	274	240	244	182	186	258	290	289	289	170	174	239	275	188	192	251	267	297	303		302	302	303	307
	4t	152	156	280	292	212	232	274	278	240	240	186	186	234	258	289	289	174	178	239	239	188	192	267	267	297	303		306	306	303	303
	5b	156	160	280	292	224	232	262	274	240	240	182	182	234	258	289	289	174	178	239	275	188	192	267	267	297	297		306	306	303	303
	5t	156	160	280	284	212	232	274	274	240	244	182	186	258	290	289	289	170	174	239	275	188	192	251	255	297	303		306	306	303	303
	Pat	**156**	**156**	**280**	**280**	**216**	**232**	**274**	**278**	**240**	**244**	**182**	**186**	**234**	**258**	**289**	**289**	**174**	**178**	**239**	**275**	**192**	**192**	**251**	**267**	**297**	**297**		**306**	**Mat null?**	**303**	**307**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 5: SN97	**Mat**	**152**	**156**	**284**	**284**	**212**	**212**	**274**	**274**	**240**	**244**	**190**	**190**	**258**	**258**	**289**	**289**	**186**	**186**	**239**	**275**	**184**	**192**	**255**	**267**	**293**	**305**	*Loci dropped due to null alleles*	**294**	**310**	**303**	**303**
	1b	156	156	284	284	212	236	274	274	244	244	186	190	258	258	281	289	186	186	239	275	184	192	267	267	301	305		278	310	303	315
	1t	152	156	284	284	212	236	274	274	240	240	186	190	258	270	281	289	186	186	239	251	192	200	255	267	293	301		294	310	315	315
	2b	152	156	284	284	212	236	274	274	240	244	162	190	258	270	289	289	174	186	251	275	184	200	267	267	301	305		294	310	303	303
	2t	152	156	284	284	212	236	274	274	244	244	186	190	258	270	281	289	186	186	239	239	192	200	267	267	293	305		278	310	303	303
	3b	152	156	284	284	212	224	274	274	244	244	186	190	258	270	289	289	186	186	239	251	184	192	255	267	293	293		310	310	303	303
	3t	152	156	284	284	212	236	274	274	240	244	186	190	258	258	281	289	186	186	251	275	184	200	267	267	293	293		310	310	303	303
	4b	152	156	284	284	212	224	274	274	240	244	186	190	258	258	281	289	174	186	239	275	184	192	255	267	301	305		278	294	315	315
	4t	152	156	284	284	212	236	274	274	240	240	162	190	258	258	289	289	174	186	239	275	192	200	267	267	301	305		310	310	303	303
	5b	152	156	284	284	212	224	274	274	240	240	162	190	258	270	289	289	186	186	239	239	184	200	267	267	293	301		278	310	315	315
	5t	156	156	284	284	212	236	274	274	240	244	162	190	258	258	281	289	174	186	251	275	184	192	267	267	293	293		310	310	303	315
	Pat	**156**	**156**	**284**	**284**	**224**	**236**	**274**	**274**	**240**	**244**	**162**	**186**	**258**	**270**	**281**	**289**	**174**	**186**	**239**	**251**	**184/ 192**	**200**	**267**	**267**	**293**	**301**		**278**	**310**	**303/ 315**	**Mat null?**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 6: SN95	**Mat**	**156**	**160**	**284**	**292**	**212**	**212**	**278**	**278**	**232**	**240**	**174**	**182**	**258**	**266**	**289**	**289**	**174**	**178**	**239**	**275**	**200**	**208**	**259**	**267**	**289**	**297**	*Loci dropped due to null alleles*	**274**	**306**	**299**	**303**
	1b	156	156	292	292	212	212	278	278	232	240	182	182	258	258	289	289	174	186	239	275	192	208	255	259	297	297		274	302	299	299
	1t	156	160	292	292	212	212	278	278	232	240	182	182	258	258	289	289	178	186	239	239	192	208	255	259	297	297		274	302	299	303
	2b	156	156	280	284	212	212	278	278	232	240	182	182	258	266	289	289	174	178	239	275	192	200	255	267	297	297		302	306	303	303
	2t	156	156	284	292	212	212	278	278	240	248	174	182	258	266	289	289	174	186	239	239	192	208	255	259	289	297		274	302	299	303
	3b	156	160	280	292	212	212	278	278	240	248	182	182	258	258	289	289	174	186	239	275	192	208	259	263	289	297		302	306	299	299
	3t	156	160	292	292	212	212	274	278	240	240	174	182	258	258	289	289	174	174	239	275	192	208	255	259	289	297		274	302	303	303
	4b	156	156	292	292	212	212	274	278	240	248	182	182	258	258	289	289	174	174	239	275	192	200	255	267	297	297		302	306	299	299
	4t	156	160	280	292	212	212	274	278	232	248	174	182	258	258	289	289	174	178	239	239	188	208	259	263	297	301		274	302	299	299
	5b	156	156	280	284	212	212	274	278	232	240	182	182	258	266	289	289	174	174	239	275	188	200	255	259	289	297		302	306	299	299
	5t	156	160	280	284	212	212	278	278	240	240	182	182	258	266	289	289	174	186	239	275	188	208	255	267	289	301		274	302	303	303
	Pat	**156**	**156**	**280**	**292**	**212**	**212**	**274**	**278**	**240**	**248**	**182**	**182**	**258**	**258**	**289**	**289**	**174**	**186**	**239**	**239**	**188**	**192**	**255**	**263**	**297**	**301**		**302**	**302**	**299**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 7: SN129	**Mat**	**144**	**152**	**284**	**284**	**212**	**212**	**274**	**274**	**240**	**240**	**182**	**186**	**258**	**266**	**289**	**293**	**178**	**186**	**239**	**275**	**188**	**192**	**267**	**267**	**285**	**297**	*Loci dropped due to null alleles*	**306**	**306**	**303**	**303**
	1b	152	164	276	284	212	232	274	274	240	244	182	186	258	258	289	289	178	186	239	275	192	192	251	267	285	297		306	314	303	303
	1t	152	156	284	284	212	216	274	274	240	240	186	186	258	266	289	289	186	186	275	275	192	216	251	267	297	297		306	306	303	303
	2b	152	164	284	284	212	232	274	274	240	240	182	186	258	266	289	289	178	186	275	275	188	192	267	267	285	297		306	306	263	303
	2t	144	156	276	284	212	232	274	274	240	240	182	186	258	258	289	293	186	186	239	275	188	216	267	267	297	297		306	306	263	303
	3b	152	156	284	284	212	216	274	274	240	244	182	186	258	266	289	289	186	186	239	275	192	192	251	267	285	297		306	306	263	303
	3t	144	164	276	284	212	216	274	274	240	244	182	182	258	258	289	289	186	186	275	275	192	192	267	267	285	297		306	314	303	303
	4b	152	156	284	284	212	216	274	274	240	240	182	186	258	258	289	293	186	186	239	275	192	192	251	267	297	297		306	306	303	303
	4t	152	164	276	284	212	216	274	274	240	240	186	186	258	266	289	293	178	186	275	275	188	216	251	267	297	297		306	306	263	303
	5b	144	164	284	284	212	216	274	274	240	240	182	182	258	258	289	293	178	186	275	275	188	216	267	267	285	297		306	314	263	303
	5t	144	156	276	284	212	216	274	274	240	244	186	186	258	266	289	289	178	186	275	275	192	192	251	267	297	297		306	314	303	303
	Pat	**156**	**164**	**276**	**284**	**216**	**232**	**274**	**274**	**240**	**244**	**182**	**186**	**258**	**258**	**289**	**289**	**186**	**186**	**275**	**275**	**192**	**216**	**251**	**267**	**297**	**297**		**306**	**314**	**263**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 8: SN117	**Mat**	**156**	**156**	**276**	**292**	**192**	**224**	**274**	**278**	**232**	**240**	**182**	**182**	**258**	**266**	**289**	**289**	**186**	**186**	**239**	**275**	**192**	**200**	**243**	**263**	**281**	**287**	*Loci dropped due to null alleles*	**306**	**306**	**179**	**303**
	1b	156	156	284	292	192	224	274	274	232	244	150	182	266	266	289	289	186	186	239	239	188	192	263	275	279	287		306	310	179	299
	1t	156	156	276	284	212	224	274	274	240	244	182	182	258	266	289	289	186	186	239	275	188	192	251	263	287	297		278	306	179	295
	2b	156	156	276	284	212	224	274	274	240	244	150	182	266	266	289	289	186	186	239	275	188	192	243	251	279	281		306	310	295	303
	2t	156	156	276	292	192	192	274	274	240	244	182	182	258	258	289	289	186	186	239	275	192	216	263	275	287	297		306	310	179	295
	3b	156	156	276	292	192	192	274	278	240	244	150	182	258	258	289	289	186	186	239	239	200	216	243	275	281	297		278	306	295	303
	3t	156	156	276	276	192	224	274	278	232	244	150	182	258	266	289	289	186	186	239	275	188	200	263	275	281	297		306	310	295	303
	4b	156	156	284	292	192	192	274	274	240	244	150	182	258	266	289	289	186	186	239	275	192	216	263	275	287	297		278	306	299	303
	4t	156	156	276	292	192	224	274	278	240	244	182	182	258	258	289	289	186	186	239	275	192	216	243	275	279	281		306	310	299	303
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	5t	156	156	276	292	192	192	274	278	232	244	182	182	258	258	289	289	186	186	239	239	192	216	243	251	279	287		278	306	295	303
	Pat	**156**	**156**	**276**	**284**	**192**	**212**	**274**	**274**	**244**	**244**	**150**	**182**	**258**	**266**	**289**	**289**	**186**	**186**	**239**	**239**	**188**	**216**	**251**	**275**	**279**	**297**		**278**	**310**	**295**	**299**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 9: SN118	**Mat**	**156**	**164**	**284**	**284**	**232**	**236**	**274**	**278**	**240**	**240**	**186**	**186**	**258**	**290**	**289**	**289**	**170**	**186**	**239**	**239**	**192**	**200**	**239**	**255**	**293**	**303**	*Loci dropped due to null alleles*	**306**	**306**	**303**	**303**
	1b	164	164	284	292	216	232	274	278	240	240	182	186	258	290	289	289	170	186	239	267	188	200	239	267	293	301		306	310	299	303
	1t	156	156	284	292	216	232	274	278	240	240	182	186	258	290	289	289	170	186	239	267	200	216	255	267	301	303		306	310	299	303
	2b	156	156	276	284	216	236	274	278	240	240	186	186	290	290	289	289	178	186	239	267	188	192	239	267	293	293		306	310	299	303
	2t	164	164	284	292	216	236	278	278	240	240	182	186	258	258	289	289	178	186	239	239	188	192	255	267	301	303		306	310	303	303
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	3t	156	164	284	292	212	232	274	278	232	240	182	186	258	290	289	289	186	186	239	239	188	192	239	251	293	301		306	310	299	303
	4b	156	164	276	284	212	236	274	274	240	240	182	186	290	290	289	289	186	186	239	239	192	216	251	255	301	303		306	310	303	303
	4t	156	164	284	292	212	232	274	278	240	240	182	186	258	290	289	289	170	186	239	267	188	192	239	251	293	301		306	306	303	303
	5b	156	164	276	284	216	232	274	274	240	240	182	186	290	290	289	289	178	186	239	267	188	200	239	267	301	303		306	306	299	303
	5t	164	164	276	284	216	232	274	274	232	240	182	186	290	290	289	289	186	186	239	267	188	192	251	255	293	303		306	310	303	303
	Pat	**156**	**164**	**276**	**292**	**212**	**216**	**274**	**278**	**232**	**240**	**182**	**186**	**258**	**290**	**289**	**289**	**178**	**186**	**239**	**267**	**188**	**216**	**251**	**267**	**293**	**301**		**306**	**310**	**299**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 10: SN130	**Mat**	**156**	**160**	**276**	**284**	**212**	**224**	**274**	**278**	**240**	**244**	**162**	**182**	**258**	**266**	**277**	**289**	**182**	**186**	**239**	**243**	**196**	**200**	**247**	**247**	**281**	**285**	*Loci dropped due to null alleles*	**306**	**310**	**303**	**307**
	1b	156	164	276	280	212	232	262	274	240	244	182	186	258	266	289	289	182	186	239	243	188	200	247	255	281	285		310	310	303	307
	1t	156	164	280	284	212	212	262	274	240	244	162	186	258	258	289	289	174	186	239	275	188	200	247	251	281	281		310	314	303	307
	2b	152	156	272	284	212	224	274	278	240	244	182	186	258	262	277	289	182	186	239	239	188	196	247	251	281	281		310	310	303	307
	2t	152	156	272	284	212	212	262	278	240	244	182	186	258	262	277	289	182	182	243	275	188	200	247	251	281	285		306	310	307	307
	3b	152	156	276	280	212	224	274	278	240	244	162	186	262	266	289	289	182	186	239	243	188	196	247	255	281	281		306	314	303	307
	3t	156	164	272	276	224	232	262	274	244	244	182	186	262	266	277	289	174	186	239	239	188	200	247	255	281	285		310	314	303	303
	4b	152	156	280	284	212	232	274	278	240	240	162	186	258	258	277	289	174	182	243	275	188	196	247	255	281	281		306	314	307	307
	4t	160	164	276	280	212	224	274	274	240	244	182	186	262	266	289	289	174	186	239	243	188	196	247	251	281	285		306	314	303	307
	5b	160	164	280	284	224	232	274	278	244	244	182	186	258	258	289	289	182	186	239	275	188	196	247	251	281	281		310	310	307	307
	5t	152	160	272	276	224	232	274	278	240	244	182	186	262	266	277	289	174	182	239	243	188	200	247	255	281	281		310	310	307	307
	Pat	**152**	**164**	**272**	**280**	**212**	**232**	**262**	**274**	**240**	**244**	**186**	**186**	**258**	**262**	**289**	**289**	**174**	**182**	**239**	**275**	**188**	**188**	**251**	**255**	**281**	**281**		**310**	**314**	**303**	**307**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 11: SN116	**Mat**	**156**	**164**	**276**	**284**	**232**	**236**	**274**	**278**	**236**	**244**	**182**	**186**	**258**	**266**	**289**	**293**	**182**	**186**	**275**	**275**	**188**	**200**	**239**	**251**	**285**	**297**	*Loci dropped due to null alleles*	**306**	**306**	**303**	**303**
	1b	152	164	276	276	212	232	254	278	244	248	162	182	258	266	289	293	186	186	271	275	188	200	251	271	285	293		306	314	303	303
	1t	152	156	276	284	212	236	254	278	236	248	182	182	258	258	289	289	182	186	275	275	200	200	239	271	293	297		302	306	303	303
	2b	152	156	276	276	212	236	274	274	232	236	182	182	258	266	273	289	182	186	275	275	184	200	239	267	293	297		302	302	303	303
	2t	152	164	276	284	212	236	254	274	236	248	182	182	258	258	273	289	182	186	275	275	188	200	239	267	293	297		302	302	303	303
	3b	156	156	276	276	212	232	274	278	236	248	182	182	258	266	289	293	182	182	275	275	184	200	251	267	293	297		306	314	303	303
	3t	156	164	276	284	212	236	254	274	244	248	182	186	258	258	289	289	182	186	275	275	184	200	251	267	285	293		306	314	303	303
	4b	152	164	276	276	212	236	274	274	232	236	182	186	258	266	289	289	182	182	275	275	184	200	251	267	285	301		302	306	303	303
	4t	152	164	276	284	212	236	274	274	236	248	162	182	258	258	289	293	182	182	275	275	184	200	239	267	285	293		314	314	303	303
	5b	152	164	276	276	212	236	274	278	236	248	162	186	258	266	289	293	182	182	275	275	188	200	239	271	293	297		314	314	303	303
	5t	152	164	276	276	212	236	274	278	236	248	162	186	258	258	289	289	186	186	275	275	200	200	251	271	293	297		302	302	303	303
	Pat	**152**	**156**	**276**	**276**	**212**	**212**	**254**	**274**	**232**	**248**	**162**	**182**	**258**	**258**	**273**	**289**	**182**	**186**	**271**	**275**	**184**	**200**	**267**	**271**	**293**	**301**		**302/ 314**	**Mat null?**	**303**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Sennen 12: SN125	**Mat**	**156**	**164**	**284**	**288**	**212**	**232**	**274**	**278**	**240**	**244**	**174**	**174**	**262**	**270**	**289**	**289**	**174**	**186**	**239**	**239**	**188**	**188**	**243**	**267**	**273**	**297**	*Loci dropped due to null alleles*	**306**	**314**	**295**	**303**
	1b	164	164	276	288	212	232	274	278	240	244	174	186	262	266	285	289	174	174	239	239	188	192	243	243	297	301		306	314	295	303
	1t	156	156	284	288	212	232	274	278	240	240	174	186	262	266	289	293	174	174	239	239	188	192	239	267	297	305		306	314	291	303
	2b	156	156	284	288	212	232	274	278	240	240	174	186	262	266	285	289	174	174	239	239	188	188	243	243	273	305		306	306	295	303
	2t	156	164	276	288	212	232	274	278	240	240	174	186	262	266	289	293	174	174	239	239	188	192	243	243	297	305		306	306	291	295
	3b	156	164	284	284	212	232	274	274	240	244	174	186	266	270	289	293	174	174	239	239	188	188	243	243	273	305		314	314	295	303
	3t	156	156	284	284	212	212	274	278	240	240	174	186	266	270	289	293	174	174	239	239	188	192	243	243	297	305		306	314	291	303
	4b	164	164	284	288	212	232	274	274	240	240	174	186	262	266	285	289	174	174	239	239	188	188	243	243	273	301		314	314	303	303
	4t	156	156	276	288	168	212	270	274	240	244	174	186	262	266	289	293	174	174	239	239	188	192	243	243	297	305		306	314	303	303
	5b	164	164	284	284	212	212	274	278	240	244	174	186	266	270	285	289	174	174	239	239	188	188	239	243	273	301		306	306	295	303
	5t	156	164	284	288	212	212	274	278	240	244	174	186	262	266	289	293	174	174	239	239	188	192	239	243	297	305		306	314	291	303
	Pat	**156**	**164**	**276**	**284**	**168**	**212**	**270**	**274**	**240**	**240**	**186**	**186**	**266**	**266**	**285**	**293**	**174**	**174**	**239**	**239**	**188**	**192**	**239**	**243**	**301**	**305**		**306**	**314**	**291**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Falmouth 1: FH96	**Mat**	**156**	**168**	**276**	**280**	**212**	**232**	**274**	**274**	**236**	**240**	**182**	**186**	**258**	**266**	**281**	**289**	**178**	**178**	**239**	**275**	**200**	**208**	**243**	**251**	**289**	**297**	*Loci dropped due to null alleles*	**298**	**298**	**307**	**386**
	1b	148	168	280	284	212	232	274	274	240	240	186	186	258	266	289	289	178	186	239	239	200	216	243	247	289	297		302	302	299	386
	1t	148	168	280	284	212	232	274	274	236	236	186	186	258	266	281	289	178	178	239	239	192	208	251	267	297	297		298	306	299	307
	2b	148	168	276	280	192	212	274	274	236	236	186	186	258	266	289	289	178	186	239	275	192	208	243	267	287	297		302	302	299	307
	2t	156	168	280	284	212	232	274	274	236	236	186	186	258	266	289	289	178	178	239	275	208	216	247	251	287	297		302	302	299	307
	3b	156	156	276	284	212	232	274	274	236	240	182	186	258	266	281	289	178	178	239	239	192	208	251	267	297	297		306	306	299	386
	3t	156	168	276	280	192	212	274	274	240	240	182	186	258	266	281	289	178	186	239	239	192	200	243	247	297	297		306	306	299	386
	4b	156	168	276	284	212	232	274	274	236	240	186	186	266	266	289	289	178	186	239	275	208	216	247	251	297	297		306	306	299	386
	4t	156	156	280	284	192	232	274	274	240	240	186	186	258	266	289	289	178	178	239	239	192	200	243	247	287	297		298	302	299	386
	5b	156	156	276	284	192	232	274	274	236	240	186	186	258	266	289	289	178	178	239	239	192	208	243	267	289	297		298	302	299	386
	5t	148	156	276	284	192	212	274	274	236	240	182	186	266	266	289	289	178	178	239	239	192	208	243	267	287	289		302	302	299	386
	Pat	**148**	**156**	**276/ 280**	**284**	**192**	**212/ 232**	**274**	**274**	**236**	**240**	**186**	**186**	**266**	**266**	**289**	**289**	**178**	**186**	**239**	**239**	**192**	**216**	**247**	**267**	**287**	**297**		**302/ 306**	**Mat null?**	**299**	**299**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Falmouth 2: FH119	**Mat**	**152**	**156**	**280**	**284**	**224**	**232**	**262**	**274**	**232**	**244**	**182**	**186**	**258**	**262**	**289**	**289**	**182**	**186**	**267**	**275**	**188**	**188**	**255**	**267**	**291**	**297**	*Loci dropped due to null alleles*	**306**	**306**	**295**	**303**
	1b	152	164	284	284	224	232	274	274	244	244	186	186	262	266	289	293	174	186	239	267	188	200	255	267	285	297		306	306	295	295
	1t	152	168	276	280	212	224	262	274	232	244	174	186	258	266	289	289	174	186	239	275	188	216	267	267	285	297		306	306	295	303
	2b	152	168	276	284	232	232	262	274	232	244	186	186	262	266	289	289	186	186	239	275	188	216	255	267	285	297		278	278	295	295
	2t	156	168	276	280	232	232	262	274	232	244	182	186	258	266	289	293	186	186	239	267	188	200	255	267	285	297		306	306	295	295
	3b	152	168	280	284	224	232	274	274	232	244	186	186	258	258	289	293	174	182	239	275	188	200	267	267	285	297		278	278	295	303
	3t	152	164	280	284	212	224	262	274	244	244	186	186	258	266	289	289	174	182	251	267	188	216	267	267	285	291		278	306	295	303
	4b	156	164	280	284	212	232	274	274	244	244	186	186	258	258	289	289	182	186	239	267	188	200	255	267	285	291		278	278	295	303
	4t	156	164	284	284	232	232	274	274	232	244	174	182	262	266	289	293	174	182	239	275	188	216	267	267	285	297		278	306	295	303
	5b	152	164	276	280	212	224	262	274	244	244	182	186	258	266	289	293	174	186	239	267	188	216	267	267	285	297		278	278	295	303
	5t	152	168	284	284	212	224	262	274	232	244	174	186	258	262	289	289	174	186	251	275	188	216	267	267	285	291		306	306	295	303
	Pat	**164**	**168**	**276**	**284**	**212**	**232**	**274**	**274**	**244**	**244**	**174**	**186**	**258**	**266**	**289**	**293**	**174**	**186**	**239**	**251**	**200**	**216**	**267**	**267**	**285**	**285**		**278**	**Mat null?**	**295**	**295**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Falmouth 3: FH99	**Mat**	**156**	**156**	**284**	**284**	**212**	**232**	**274**	**274**	**244**	**244**	**186**	**186**	**258**	**258**	**289**	**289**	**174**	**186**	**235**	**239**	**192**	**192**	**259**	**267**	**293**	**305**	*Loci dropped due to null alleles*	**278**	**310**	**291**	**299**
	1b	152	156	276	284	212	212	274	278	240	244	162	186	250	258	281	289	174	178	239	239	188	192	255	259	297	305		278	306	299	303
	1t	152	156	284	284	212	212	274	278	240	244	162	186	258	258	289	289	186	186	239	239	188	192	255	267	293	297		278	302	295	299
	2b	156	164	284	284	212	212	274	278	232	244	186	186	258	258	289	289	174	178	239	239	188	192	259	267	285	305		302	310	295	299
	2t	156	164	276	284	212	212	274	278	232	244	186	186	250	258	281	289	178	186	239	239	192	192	259	259	293	297		306	310	295	299
	3b	152	156	284	284	212	232	274	278	232	244	162	186	258	258	281	289	186	186	239	239	192	192	259	259	297	305		278	306	299	303
	3t	156	164	276	284	212	232	274	274	232	244	162	186	250	258	281	289	174	186	235	239	188	192	255	259	285	305		306	310	291	303
	4b	156	164	284	284	212	232	274	274	240	244	162	186	258	258	281	289	186	186	239	239	188	192	255	267	285	293		278	306	295	299
	4t	156	164	276	284	212	232	274	278	240	244	162	186	250	258	281	289	186	186	235	239	192	192	259	267	297	305		278	306	291	303
	5b	156	164	284	284	212	232	274	278	232	244	162	186	258	258	281	289	186	186	235	239	188	192	259	259	285	293		278	302	291	303
	5t	156	164	276	284	232	232	274	274	240	244	162	186	250	258	281	289	178	186	235	239	188	192	259	259	293	297		306	310	295	299
	Pat	**152**	**156**	**276**	**284**	**212**	**232**	**274**	**278**	**232**	**240**	**162**	**186**	**250**	**258**	**281**	**289**	**178**	**186**	**239**	**239**	**188**	**192**	**255**	**259**	**285**	**297**		**302**	**306**	**295**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Falmouth 4: FH118	**Mat**	**156**	**156**	**284**	**284**	**224**	**232**	**274**	**278**	**240**	**244**	**186**	**186**	**250**	**266**	**289**	**293**	**174**	**186**	**239**	**239**	**204**	**216**	**251**	**267**	**281**	**297**	*Loci dropped due to null alleles*	**278**	**306**	**295**	**307**
	1b	152	156	284	284	212	232	274	274	240	244	174	186	266	266	289	293	186	186	239	239	204	216	251	267	297	297		286	306	295	303
	1t	152	156	284	284	212	232	274	274	244	244	186	186	250	266	289	289	186	186	239	239	204	216	243	251	297	297		306	306	295	303
	2b	152	156	284	284	212	224	274	274	240	244	186	186	266	266	289	293	178	186	239	239	216	216	243	267	279	297		286	306	295	303
	2t	152	156	284	284	224	224	274	278	232	240	186	186	250	266	289	293	186	186	239	239	216	216	267	267	279	281		278	286	295	303
	3b	152	156	284	284	212	232	274	274	244	244	186	186	266	266	289	293	174	178	239	275	204	216	267	267	279	281		286	306	295	295
	3t	156	156	284	284	212	232	274	274	232	240	174	186	250	266	289	293	174	178	239	275	200	216	251	267	297	297		278	306	295	303
	4b	152	156	284	284	224	224	274	274	232	240	174	186	266	266	289	289	178	186	239	275	200	204	243	267	279	281		306	306	295	303
	4t	156	156	284	284	212	224	274	274	240	244	186	186	250	266	289	293	174	186	239	239	200	204	243	267	279	297		278	286	295	295
	5b	156	156	284	284	212	232	274	278	232	244	186	186	250	266	289	293	178	186	239	239	204	216	243	267	281	297		278	306	303	307
	5t	152	156	284	284	212	224	274	278	240	244	186	186	266	266	289	293	178	186	239	275	216	216	267	267	279	297		286	306	303	307
	Pat	**152**	**156**	**284**	**284**	**212**	**224**	**274**	**274**	**232**	**244**	**174**	**186**	**266**	**266**	**289**	**289**	**178**	**186**	**239**	**275**	**200**	**216**	**243**	**267**	**279**	**297**		**286**	**306**	**295**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Falmouth 5: FH126	**Mat**	**152**	**156**	**284**	**284**	**212**	**232**	**262**	**278**	**232**	**240**	**178**	**186**	**234**	**262**	**285**	**289**	**174**	**186**	**275**	**275**	**188**	**200**	**263**	**267**	**275**	**281**	*Loci dropped due to null alleles*	**298**	**302**	**307**	**307**
	1b	152	156	280	284	212	232	262	274	240	240	178	186	234	266	285	289	174	182	199	275	188	216	263	267	275	281		298	298	307	311
	1t	152	152	280	284	212	224	262	274	240	240	186	186	262	266	289	289	174	182	239	275	188	216	263	267	281	293		298	298	307	311
	2b	152	156	280	284	212	232	262	274	232	240	178	186	262	266	281	285	174	174	199	275	188	200	255	263	275	293		298	306	303	307
	2t	152	156	284	284	212	224	262	274	232	240	186	186	258	262	289	289	174	174	239	275	200	216	255	263	281	293		302	306	303	307
	3b	152	152	280	284	212	212	274	278	240	240	178	186	234	266	285	289	182	186	239	275	188	216	255	267	281	293		302	306	303	303
	3t	152	156	280	284	212	212	274	278	240	244	186	186	234	266	281	285	174	174	199	275	200	216	255	263	281	293		298	306	303	303
	4b	152	156	284	284	224	232	274	278	240	244	178	186	234	258	281	289	174	174	239	275	188	216	263	267	281	281		298	302	311	311
	4t	152	156	280	284	212	212	274	278	240	240	186	186	234	266	281	289	174	186	199	275	200	216	255	263	281	293		302	306	311	311
	5b	152	156	280	284	224	232	274	278	240	240	186	186	234	266	281	289	182	186	239	275	188	200	255	263	275	281		302	306	303	303
	5t	152	156	284	284	224	232	262	274	240	240	178	186	234	258	281	289	174	182	239	275	200	216	255	263	281	293		302	306	303	307
	Pat	**152**	**152**	**280**	**284**	**212**	**224**	**274**	**274**	**240**	**244**	**186**	**186**	**258**	**266**	**281**	**289**	**174**	**182**	**199**	**239**	**188/ 200**	**216**	**255**	**263/ 267**	**281**	**293**		**298**	**306**	**303/ 311**	**Mat null?**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Looe 1: LO107	**Mat**	**156**	**160**	**284**	**288**	**232**	**232**	**274**	**302**	**232**	**244**	**186**	**186**	**258**	**258**	**289**	**289**	**178**	**186**	**239**	**275**	**188**	**188**	**263**	**267**	**285**	**297**	*Loci dropped due to null alleles*	**302**	**302**	**295**	**303**
	1b	156	160	284	288	212	232	278	302	232	240	186	186	258	270	289	289	182	186	239	239	188	188	263	267	297	297		278	302	299	303
	1t	152	160	284	284	212	232	262	302	232	244	186	186	258	258	289	289	178	182	239	239	188	188	263	263	297	297		302	306	299	303
	2b	156	156	284	284	212	232	274	278	232	244	186	186	258	258	289	289	186	186	239	239	188	188	251	267	285	289		306	306	299	303
	2t	156	156	284	284	212	232	262	274	244	244	162	186	258	258	289	289	182	186	239	239	188	188	251	267	285	297		278	302	295	299
	3b	156	156	284	288	212	232	274	278	232	244	186	186	258	258	289	289	186	186	239	239	188	188	263	263	297	297		278	302	299	303
	3t	152	160	284	288	212	232	262	302	232	240	186	186	258	270	289	289	186	186	239	275	188	188	263	263	297	297		306	306	299	303
	4b	156	160	284	288	212	232	262	274	240	244	162	186	258	258	289	289	178	186	239	275	188	188	263	263	289	297		302	306	295	303
	4t	156	160	284	288	212	232	262	302	244	244	186	186	258	258	289	289	178	182	239	275	188	188	263	267	297	297		278	302	295	299
	5b	152	156	284	288	212	232	278	302	232	244	162	186	258	258	289	289	178	186	239	275	188	188	251	263	285	289		278	302	295	295
	5t	156	160	284	288	212	232	278	302	244	244	186	186	258	258	289	289	182	186	239	239	188	188	251	267	297	297		278	278	295	303
	Pat	**152**	**156**	**284**	**284**	**212**	**212**	**262**	**278**	**240**	**244**	**162**	**186**	**258**	**270**	**289**	**289**	**182**	**186**	**239**	**239**	**188**	**188**	**251**	**263**	**289**	**297**		**278/ 306**	**Mat null?**	**295**	**299**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Looe 2: LO128	**Mat**	**152**	**164**	**280**	**284**	**168**	**224**	**274**	**274**	**240**	**244**	**182**	**182**	**258**	**266**	**289**	**289**	**178**	**186**	**239**	**239**	**188**	**208**	**267**	**271**	**283**	**285**	*Loci dropped due to null alleles*	**294**	**306**	**303**	**311**
	1b	156	164	280	284	168	236	274	274	240	244	182	186	258	266	289	289	174	178	239	239	188	196	263	267	283	297		306	306	303	303
	1t	152	156	284	284	224	236	266	274	240	244	174	182	258	258	289	289	178	178	239	239	196	208	243	267	285	297		294	310	303	303
	2b	156	164	284	284	224	232	274	274	240	240	182	186	258	258	289	289	178	186	239	239	188	208	243	271	283	297		294	306	303	303
	2t	152	156	280	284	224	232	266	274	240	240	174	182	258	266	289	289	174	186	239	239	188	196	243	267	283	297		306	306	303	303
	3b	152	156	284	284	168	236	274	274	240	248	174	182	258	266	289	289	178	186	239	239	196	208	263	271	283	297		306	310	303	303
	3t	156	164	280	280	168	236	266	274	240	248	182	186	258	266	289	289	178	186	239	239	188	208	263	271	285	297		306	306	303	303
	4b	156	164	280	284	168	236	274	274	244	248	174	182	258	266	289	289	178	178	239	239	188	208	243	271	285	297		306	306	303	311
	4t	156	164	280	284	224	236	266	274	244	248	174	182	258	258	289	289	174	178	239	239	188	196	243	271	283	297		306	310	303	311
	5b	156	164	284	284	168	236	274	274	240	240	174	182	258	266	289	289	178	178	239	239	188	208	263	271	283	297		294	306	303	311
	5t	152	156	280	284	168	232	274	274	244	248	174	182	258	258	289	289	178	186	239	239	188	208	263	267	283	297		294	306	303	311
	Pat	**156**	**156**	**280**	**284**	**232**	**236**	**266**	**274**	**240**	**248**	**174**	**186**	**258**	**258**	**289**	**289**	**174**	**178**	**239**	**239**	**196**	**188/ 208**	**243**	**263**	**297**	**297**		**306**	**310**	**303**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Looe 3: LO127	**Mat**	**152**	**156**	**264**	**292**	**212**	**224**	**262**	**274**	**240**	**244**	**174**	**182**	**258**	**258**	**289**	**289**	**182**	**182**	**239**	**239**	**196**	**216**	**259**	**267**	**297**	**297**	*Loci dropped due to null alleles*	**278**	**306**	**295**	**366**
	1b	152	156	264	284	212	212	262	278	240	244	182	190	258	258	289	289	174	182	239	275	188	196	259	267	285	297		306	314	299	366
	1t	156	156	284	292	212	224	262	274	232	244	182	186	258	258	273	289	182	182	239	239	188	216	267	267	297	297		278	278	295	366
	2b	156	156	284	292	212	212	262	278	240	244	182	190	258	258	289	289	174	182	239	275	188	196	247	259	285	297		278	278	295	295
	2t	156	156	264	284	212	212	274	278	240	244	174	186	258	258	289	289	174	182	239	275	188	196	247	259	285	297		306	306	299	366
	3b	152	156	264	280	212	224	262	274	232	244	174	190	258	258	289	289	174	182	239	239	188	216	267	267	285	297		306	314	295	366
	3t	152	156	284	292	212	212	262	262	232	240	174	186	258	258	273	289	182	182	239	275	188	196	247	267	285	297		306	314	299	366
	4b	156	156	280	292	212	224	262	274	232	240	174	186	258	258	273	289	182	182	239	239	188	196	247	259	297	297		306	306	295	295
	4t	156	156	264	284	212	224	262	274	240	244	182	186	258	258	289	289	182	182	239	239	188	216	247	259	285	297		278	314	295	295
	5b	156	156	264	280	212	212	262	262	232	240	174	186	258	258	273	289	182	182	239	275	188	216	247	267	297	297		306	314	295	299
	5t	152	156	264	280	212	212	274	278	232	244	174	186	258	258	289	289	174	182	239	275	188	196	247	259	285	297		306	306	295	299
	Pat	**156**	**156**	**280**	**284**	**212**	**212**	**262**	**278**	**232**	**240/ 244**	**186**	**190**	**258**	**258**	**273**	**289**	**174**	**182**	**239**	**275**	**188**	**188**	**247**	**267**	**285**	**297**		**314**	**Pat null?**	**295**	**299**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Looe 4: LO115	**Mat**	**156**	**156**	**284**	**292**	**224**	**232**	**274**	**278**	**240**	**248**	**186**	**190**	**258**	**266**	**289**	**289**	**178**	**182**	**239**	**251**	**200**	**216**	**259**	**267**	**285**	**297**	*Loci dropped due to null alleles*	**306**	**306**	**299**	**303**
	1b	148	156	276	292	232	236	262	274	232	240	186	190	258	258	285	289	182	186	239	275	204	216	251	267	297	297		306	306	299	303
	1t	148	156	280	284	212	224	262	278	232	248	182	190	258	258	289	289	178	186	239	275	192	216	251	267	285	293		274	306	303	307
	2b	156	156	280	284	212	224	274	274	244	248	182	190	258	266	289	289	174	182	243	251	192	200	251	259	297	297		306	306	299	307
	2t	148	156	280	284	224	236	274	274	232	248	182	190	258	266	289	289	182	186	239	243	192	200	255	259	285	293		274	306	299	307
	3b	148	156	280	292	212	232	274	274	232	248	182	190	258	266	289	289	182	186	239	275	204	216	255	259	285	297		274	306	299	307
	3t	156	156	276	292	212	224	262	274	232	240	182	186	258	258	289	289	178	186	251	275	192	216	255	259	285	297		306	306	299	307
	4b	148	156	276	292	212	224	274	274	232	240	186	186	258	258	285	289	182	186	251	275	204	216	255	267	285	293		306	306	299	303
	4t	156	156	280	292	232	236	274	274	240	244	186	190	258	258	285	289	174	178	243	251	200	204	255	259	285	293		306	306	299	307
	5b	148	156	280	292	212	224	262	278	232	248	186	186	258	258	285	289	182	186	239	275	204	216	255	267	285	297		274	306	299	303
	5t	156	156	276	284	212	232	262	274	232	248	186	190	258	266	285	289	182	186	239	275	204	216	251	267	285	293		274	306	299	307
	Pat	**148**	**156**	**276**	**280**	**212**	**236**	**262**	**274**	**232**	**244**	**182**	**186**	**258**	**258**	**285**	**289**	**174**	**186**	**243**	**275**	**192**	**204**	**251**	**255**	**293**	**297**		**274**	**306**	**299/ 303**	**307**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Looe 5: LO155	**Mat**	**152**	**156**	**284**	**284**	**212**	**232**	**274**	**274**	**240**	**244**	**174**	**182**	**258**	**258**	**285**	**289**	**186**	**186**	**239**	**267**	**184**	**188**	**251**	**251**	**297**	**305**	*Loci dropped due to null alleles*	**306**	**314**	**299**	**307**
	1b	152	152	280	284	212	232	274	278	240	244	174	182	258	258	285	289	186	186	239	267	188	192	251	255	297	305		306	306	303	307
	1t	152	156	284	284	212	212	274	278	240	240	174	186	250	258	285	289	178	186	239	239	188	192	251	255	297	305		306	306	299	307
	2b	152	156	280	284	212	212	274	278	232	240	174	182	258	258	285	289	178	186	239	267	188	192	251	255	297	297		306	306	303	307
	2t	152	156	284	284	212	232	274	278	232	240	174	186	250	258	285	289	186	186	239	239	184	192	251	267	297	305		314	314	307	307
	3b	152	152	284	284	212	232	274	274	232	244	174	186	250	258	285	289	178	186	239	239	188	192	251	255	297	297		306	306	299	307
	3t	152	156	280	284	212	232	274	278	232	244	174	186	258	258	289	289	186	186	239	239	184	192	251	255	297	297		314	314	299	307
	4b	152	156	280	284	212	212	274	278	232	244	174	182	258	258	285	289	178	186	239	267	188	200	251	255	297	305		306	306	303	307
	4t	156	156	284	284	212	232	274	278	232	240	174	182	250	258	285	289	178	186	239	267	188	200	251	255	297	305		314	314	307	307
	5b	152	156	284	284	212	232	274	278	240	240	174	186	258	258	289	289	178	186	239	267	184	192	251	267	297	297		314	314	307	307
	5t	156	156	284	284	212	232	274	278	240	244	174	182	250	258	289	289	178	186	239	267	184	200	251	267	297	297		306	306	307	307
	Pat	**152**	**156**	**280**	**284**	**212**	**212**	**274**	**278**	**232**	**240**	**174**	**186**	**250**	**258**	**289**	**289**	**178**	**186**	**239**	**239**	**192**	**200**	**255**	**267**	**297**	**297**		**306/ 314/**	**2Pat null?**	**303**	**307**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Looe 6: LO103	**Mat**	**152**	**160**	**284**	**284**	**232**	**232**	**274**	**278**	**244**	**244**	**182**	**186**	**258**	**270**	**289**	**289**	**178**	**186**	**239**	**243**	**200**	**208**	**251**	**255**	**285**	**289**	*Loci dropped due to null alleles*	**302**	**310**	**295**	**295**
	1b	156	160	284	284	212	232	274	274	244	244	162	186	258	270	289	289	178	186	235	243	200	208	251	267	285	305		278	302	295	303
	1t	152	164	284	284	212	232	274	274	240	244	182	186	258	258	289	289	174	178	239	239	208	216	251	251	285	305		278	302	295	303
	2b	160	164	284	284	224	232	274	278	240	244	162	182	258	270	285	289	178	178	235	243	200	200	251	251	285	289		302	310	295	303
	2t	152	164	284	284	212	232	274	278	240	244	162	182	258	270	289	289	174	186	235	239	200	208	251	255	285	285		278	310	295	303
	3b	152	156	284	284	224	232	274	278	240	244	162	186	258	270	285	289	178	186	235	243	200	208	251	255	285	289		302	310	295	303
	3t	156	160	284	284	212	232	274	278	244	244	162	182	258	270	285	289	178	186	239	243	200	216	255	267	289	305		302	310	303	303
	4b	156	160	284	284	212	232	274	274	240	244	182	186	258	258	285	289	178	186	239	239	200	200	251	255	285	305		302	302	303	303
	4t	152	164	284	284	224	232	274	274	244	244	182	182	258	270	289	289	178	186	239	243	200	216	255	267	285	305		302	310	303	303
	5b	152	156	284	284	212	232	274	278	240	244	162	186	258	258	285	289	178	186	235	239	200	208	251	267	289	305		302	310	303	303
	5t	160	164	284	284	224	232	274	274	244	244	162	182	258	258	289	289	178	186	239	243	200	200	255	267	285	285		302	302	303	303
	Pat	**156**	**164**	**284**	**284**	**212**	**224**	**274**	**274**	**240**	**244**	**162**	**182**	**258**	**258**	**285**	**289**	**174**	**178**	**235**	**239**	**200**	**216**	**251**	**267**	**285**	**305**		**278**	**302**	**303/ 303**	**Mat null?**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Looe 7: LO119	**Mat**	**144**	**152**	**280**	**292**	**216**	**232**	**258**	**274**	**244**	**248**	**182**	**186**	**250**	**266**	**289**	**289**	**174**	**186**	**239**	**239**	**192**	**200**	**255**	**275**	**285**	**297**	*Loci dropped due to null alleles*	**310**	**314**	**303**	**307**
	1b	152	152	280	284	212	216	274	274	232	248	182	186	258	266	289	289	174	186	239	239	192	200	259	275	285	297		294	314	303	307
	1t	152	156	284	292	212	232	258	274	244	244	182	186	250	270	273	289	174	186	239	239	188	200	255	267	297	301		310	310	303	307
	2b	144	152	280	284	212	232	258	302	232	248	182	186	258	266	273	289	186	186	239	239	192	192	255	259	285	285		310	310	295	307
	2t	144	156	284	292	212	232	274	274	232	248	182	186	250	258	273	289	174	186	239	239	188	192	255	259	285	301		294	314	303	303
	3b	152	152	280	284	212	216	274	274	244	248	182	186	266	270	289	289	186	186	239	239	192	200	267	275	285	301		314	314	303	303
	3t	152	152	280	284	212	216	258	302	244	248	186	186	258	266	289	289	174	186	239	239	192	192	255	267	285	297		294	314	303	303
	4b	144	156	280	284	212	216	258	302	244	248	182	182	266	270	289	289	186	186	239	239	192	192	259	275	297	301		294	314	303	303
	4t	144	152	280	284	212	232	274	274	244	244	182	186	258	266	289	289	174	186	239	239	192	192	259	275	285	297		294	314	303	303
	5b	152	152	280	284	212	232	258	302	244	248	186	186	266	270	289	289	186	186	239	239	188	200	255	259	285	297		294	314	303	303
	5t	144	152	280	284	212	232	258	274	232	244	186	186	258	266	273	289	174	186	239	239	192	192	259	275	285	301		314	314	295	303
	Pat	**152**	**156**	**284**	**284**	**212**	**212**	**274**	**302**	**232**	**244**	**182**	**186**	**258**	**270**	**273**	**289**	**186**	**186**	**239**	**239**	**188**	**192**	**259**	**267**	**285**	**301**		**294**	**Pat null?**	**295**	**303**

Locus		D106		C118		B4		D117		C103		B6		C129		C6		C111		D111		D110		C131b		C120			A8		C129	
Allele		1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2		1	2	1	2
Looe 8: LO121	**Mat**	**156**	**164**	**284**	**284**	**212**	**212**	**262**	**274**	**240**	**244**	**162**	**186**	**258**	**270**	**289**	**289**	**182**	**182**	**239**	**239**	**192**	**196**	**263**	**267**	**285**	**293**	*Loci dropped due to null alleles*	**286**	**306**	**303**	**311**
	1b	144	164	284	284	212	212	274	274	232	244	162	186	258	270	289	289	182	186	239	239	192	208	251	267	293	301		306	306	303	303
	1t	156	164	284	284	212	212	262	274	240	240	182	186	258	270	289	289	182	186	239	239	188	196	263	267	293	301		290	306	299	311
	2b	156	156	284	284	212	212	262	274	240	244	162	182	258	258	289	289	178	182	239	239	196	208	267	267	285	301		290	306	299	303
	2t	156	156	284	284	212	212	274	274	232	240	182	186	258	258	289	289	178	182	239	251	188	192	251	267	285	301		306	306	299	303
	3b	156	164	284	284	212	212	262	274	240	240	186	186	258	258	289	289	178	182	239	239	192	208	251	263	285	301		286	306	299	311
	3t	156	156	284	284	212	212	274	274	240	244	182	186	258	258	289	289	182	186	239	251	196	208	251	263	285	301		286	306	303	311
	4b	144	164	284	284	212	212	262	274	240	244	186	186	270	270	289	289	182	186	239	251	188	196	263	267	285	301		306	306	299	303
	4t	144	164	284	284	212	212	262	262	240	244	186	186	258	270	289	289	178	182	239	251	196	208	251	267	293	301		290	306	303	311
	5b	156	164	284	284	212	212	262	262	232	240	162	182	258	258	273	289	178	182	239	239	196	208	251	263	293	301		286	290	299	311
	5t	144	156	284	284	212	212	274	274	232	240	182	186	258	270	273	289	178	182	239	251	192	208	267	267	285	301		286	306	303	303
	Pat	**144**	**156**	**284**	**284**	**212**	**212**	**262**	**274**	**232**	**240**	**182**	**186**	**258**	**270**	**273**	**289**	**178**	**186**	**239**	**251**	**188**	**208**	**251**	**267**	**301**	**301**		**290**	**306**	**299**	**303**

Chapter 5: European lobster population genetic structure and implications for fisheries management and hatchery stocking

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Abstract

The European lobster (*Homarus gammarus*) is a marine crustacean highly-valued for human consumption, but its populations are threatened by fishery overexploitation across its range. The species' larval stages are planktonic, suggesting high levels of dispersal among populations. The potential threats of overexploitation and erosion of population structure due to hatchery stocking releases or inadvertent introductions make it important to understand the genetic structuring of populations across multiple geographic scales. Here we study lobster population structure at a fine scale in Cornwall, south-western UK, where a hatchery stocking operation introduces cultured individuals into the wild stock, and at a broader European level, in order to compare the spatial scale of hatchery releases with that of population connectivity. Microsatellite genotypes of 24 individuals from each of 13 locations in Cornwall showed no fine-scale population genetic structure across distances of up to ~230 km. Significant differentiation and isolation by distance were detected at a broader scale, using 300 additional individuals comprising a further 15 samples across Europe. Signals of genetic heterogeneity were evident between an Atlantic cluster and samples from Sweden. Connectivity is high within the Atlantic and Swedish clusters, although evidence for isolation by distance and a transitional zone within the eastern North Sea suggest that direct gene exchange between these stocks is limited and fits a stepping-stone model. We conclude that hatchery-reared lobsters should not be released where broodstock are sourced from distant localities, but find no evidence that the release of hatchery stock throughout Cornwall exceeds the geographic scale of natural connectivity.

Introduction

It was once commonly assumed that extensive dispersal capabilities of larval or adult life-stages would effectively maintain genetic panmixis among widely distributed marine species (Hellberg, 2009). However, molecular studies of marine populations have shown that although regional or range-wide panmixia is prevalent in some species (e.g. orange roughy – White et al., 2009; Norway lobster – Pampoulie et al., 2011; Atlantic wolfish – Pampoulie et al., 2012; unicornfish – Horne & van Herwerden, 2013; snow crab – Albrecht et al., 2014), other species, including those which are highly mobile or continuously distributed, exhibit extensive subpopulation divergence, even at very modest spatial scales (e.g. Atlantic cod – Knutsen et al., 2003, 2011; Jorde et al., 2007: Berg et al., 2015; ghost shrimp – Bilodeau et al., 2005; sticklebacks – Shikano et al., 2010; Shimada et al., 2011; Bruneaux et al., 2013; European spiny lobster – Babbucci et al., 2010; Atlantic herring – Lamichhaney et al., 2012; Limborg et al., 2012; Teacher et al., 2013a; northern shrimp – Knutsen et al., 2015; Jorde et al., 2015). The identification of spatial genetic heterogeneity provides an indication of the extent and limitations of intraspecific connectivity, and such information is vital for the conservation of threatened species and the sustainable management of populations pressured by fishing (Avise, 1992; Waples et al., 2008; Reiss, 2009; Allendorf et al., 2010). Where the spatial boundaries of biological populations are unknown or mismatched to those of management units, conservation initiatives may inadequately protect discrete stocks, making fisheries vulnerable to localised depletion or collapse (Kenchington, 2003; Waples et al., 2008; Reiss, 2009).

The European lobster (*Homarus gammarus* L.) is a decapod crustacean inhabiting the coastal shelf seas of the eastern North Atlantic which has been the subject of hatchery stocking in recent decades (Ellis et al., 2015a). The lobster ranges from Arctic Norway to Morocco, including the semi-enclosed seas of the Mediterranean and the Kattegat, up to a limit of the Black Sea and Baltic Sea respectively, where reduced salinity appears to inhibit settlement (Jørstad et al., 2004a; Triantafyllidis et al., 2005). Homarid lobsters perform key roles in maintaining biodiverse coastal seas by predating macro-algal grazers (Mann & Breen, 1972; Breen & Mann, 1976), and the species' considerable

market value makes it highly prized by commercial and recreational fishers, who generally target it using baited pots. Stocks are therefore of great importance to inshore ecosystems and the traditional fishing communities they support (Ellis et al., 2015a). However, overexploitation during the twentieth century led to severe and enduring stock depletions across some regions, including Scandinavia and the Mediterranean (Dow, 1980; Agnalt et al., 1999; Fisheries and Aquaculture Department, 2016a). This has encouraged the rearing of *H. gammarus* larvae in aquaria-based hatcheries to produce juvenile lobsters, whose release has been an attempt to sustain and supplement those fisheries which remain productive (e.g. Bannister & Howard, 1991; Burton, 1993; Bannister et al., 1994; Cook, 1995; Browne & Mercer, 1998), and to restore yields in those which have been heavily depleted (e.g. Agnalt et al., 1999, 2004; Schmalenbach et al., 2011).

Supportive breeding and rear-and-release programs aim to enhance the abundance of wild stock, and therefore the sustainability of fishery harvest. However, the admixture of hatchery stock can compromise wild populations; rearing in artificial environments can promote traits that are maladapted to the wild and may be introduced to natural stock (Gharret & Smoker, 1991; Araki et al., 2007b, 2008; Christie et al., 2012a, Lorenzen et al., 2012), while increased kin survival among cultured individuals may reduce the effective size of the targeted stock (Ryman & Laikre, 1991; Hindar et al., 1991; Laikre et al., 2010; Christie et al., 2012b). Compared to fisheries conservation strategies which demand the immediate release of demographically important natural stock following capture (i.e. undersized, ovigerous or v-notched individuals), hatchery stocking also has the potential to disrupt the structuring of intra-specific genetic diversity (Ward, 2006; Lorenzen et al., 2010).

Lobster hatcheries typically source ovigerous broodstock from the wild, across the spatial ranges covered by local fishers, and rear their larvae communally in cohorts based on the date of hatch (Ellis et al., 2015a). Hatchery release batches typically consist of several cohorts, so released individuals may be redistributed beyond the spatial extent over which they would naturally disperse. This can erode population structure, reducing diversity in the wild gene pool and inhibiting the evolutionary adaptability of stocks, compromising their

conservation (Kenchington et al., 2003; Ward, 2006). In some marine and anadromous fish, natural population structure has been eroded or disrupted as a result of inappropriate implementation of hatchery stocking, causing the loss of genetic diversity (Ruzzante et al., 2001; Ayllon et al., 2006; Blanco-Gonzalez et al., 2015). Knowledge of population structure is therefore vital to ensure released stock is genetically compatible with natural stock (Ward, 2006; Poćwierz-Kotus et al., 2015), and that unintended genetic impacts of hatchery stocking on the admixed population can be monitored (Koskinen et al., 2002).

Clearly, ensuring that released stock does not erode existing population structure is an important consideration in the implementation of hatchery stocking of *H. gammarus*, though it has often been overlooked (Ellis et al., 2015a). Basic assessments of regional genetic diversity have accompanied *H. gammarus* stocking in Kvitsøy, Norway (Jørstad & Farestveit, 1999; Agnalt et al., 1999, 2004) and Helgoland, Germany (Ulrich et al., 2001; Schmalenbach et al., 2011). However, the methods used – allozymes and RAPD, respectively – have been largely superseded and have been found to fail to detect weak but important genetic structure in other species (Saunders et al., 1986; Burton, 1994; Lougheed et al., 2000; Sunnucks, 2000; Selkoe & Toonen, 2006). Elsewhere, lobster stocking has occurred without any knowledge of contemporary or fine-scale population structure. Lorenzen *et al* (2010) advocate that local adaptation be assumed to exist at scales of tens of kilometres where population structure is unassessed, but historic and current lobster stocking ventures have frequently sourced broodstock and released juveniles more widely.

Several discrete *H. gammarus* subpopulations have been proposed in recent years via evidence of genetic (e.g. Triantafyllidis et al., 2005) and oceanographic (e.g. Øresland & Ulmestrand, 2013) isolation or trait variation (Ellis et al., 2015b), with extensive differentiation apparent between stocks as close as 142 km apart (Jørstad et al., 2004a). We aimed to investigate the fine-scale genetic structure of a putatively panmictic lobster population around the Atlantic peninsula of Cornwall in south-western UK, where a regional stock enhancement project collects ovigerous females to rear mixed batches of juveniles for wild release throughout a ~250 km section of coastal waters (Ellis

et al. 2015a). Releases commenced in 2002, with an average of 12,500 juveniles released annually up to 2013 (Ellis et al., 2015a). Even at optimistic projections of survival to fishery recruitment based on restorative restocking rather than supplemental stock enhancement (e.g. 30-40% – Schmalenbach et al., 2011), such numbers equate to fewer than 0.25% of the ~2,000,000 lobsters comprising the regional stock (CEFAS, 2015) and so are unlikely to have had detectable effects on population genetics. However, release numbers have recently surpassed 50,000 p.a., and further technical advancements are likely to continue this trend of increased output. As such, it is important to gain understanding of whether rearing and release strategies may engender a short-term enhancement of stock abundance at the unintended cost of loss of genetic structure among regional lobster populations. To evaluate this, we use 14 microsatellite loci to estimate the gene flow among lobsters from 13 geographic samples throughout Cornwall and nearby offshore islands. We further assess the genetic characteristics of an additional 15 geographic samples collected throughout Europe.

Materials and methods

Ethics statement

Permission to obtain lobster tissue samples from Cornwall and the Isles of Scilly were obtained from the fishery regulators and managers; the Inshore Fisheries Conservation Authorities (IFCA) of Cornwall and Scilly within coastal waters (<6 nmi.), and the Marine Management Organisation (MMO) within offshore waters (>6 nmi.). Samples from these locations were collected in situ on board commercial vessels as part of regular fishing routines, with permission granted to allow the temporary holding (for sampling) of individuals normally in breach of regional bylaws (e.g. ovigerous females and sub-legal sizes; Cornwall IFCA, 2015). Elsewhere, lobster tissue samples or extracted DNA were obtained from landed individuals comprising the legal catch, requiring only the permission of the owning merchants. All tissue sampling was sub-lethal and involved no endangered or protected species; the European lobster is categorised as being of Least Concern in the Red List of Threatened Species of the International Union for Conservation of Nature (Butler et al., 2015).

Sample collection

Samples from around mainland Cornwall, UK (Fig. 1, Table 1), were obtained during 2013, collected on board commercial potting vessels to enable fine-scale spatial data resolution. A tissue sample (pleopod clip) and a log on the custom-built sampling app DORIS (Teacher et al., 2013) was taken for each lobster (including pre-recruits). DORIS recorded each lobster's capture location via GPS, and logged a photograph to determine sex, and carapace length (CL, mm). Tissue samples, extracted DNA or genotype data from lobsters in other areas (Figure 1, Table 1) were provided with only approximate region and date of capture and, apart from the Isles of Scilly area, no size or sex information. The total number of lobsters was 612, including 312 fine-scale samples from Cornwall and pre-published data for 192 samples from western Sweden (Huserbråten et al., 2013).

Microsatellite genotyping

DNA from individual samples was extracted using the Wizard® SV 96 Genomic DNA Purification System (Promega, Madison, WI, USA). Individuals were each screened at 15 microsatellites – 12 from André & Knutsen (2010) and three from Ellis *et al* (2015c) – except 88/96 samples from both Lysekil (Gullmarfjord) and Strömstad (Singlefjord) in Sweden for which genotype data published by Huserbråten *et al* (2013) was supplied for only the 12 loci of André & Knutsen (2010). Primers were synthesized by Eurofins Genomics (Eurofins Genomics, Ebersberg, Germany), with forward primers 5'-tagged with one of four fluorescent sequencing dyes; FAM, ATTO 550, ATTO 565 and Yakima Yellow. Loci were amplified in four optimised multiplex reactions as per Ellis *et al* (2015c): (1) HGA8, HGB4, HGC118, HGD106 & HGD117; (2) HGB6, HGC6, HGC103 & HGD129; (3) HGC111, HGC129 & HGD111; and (4) HGC120, HGC131b & HGD110.

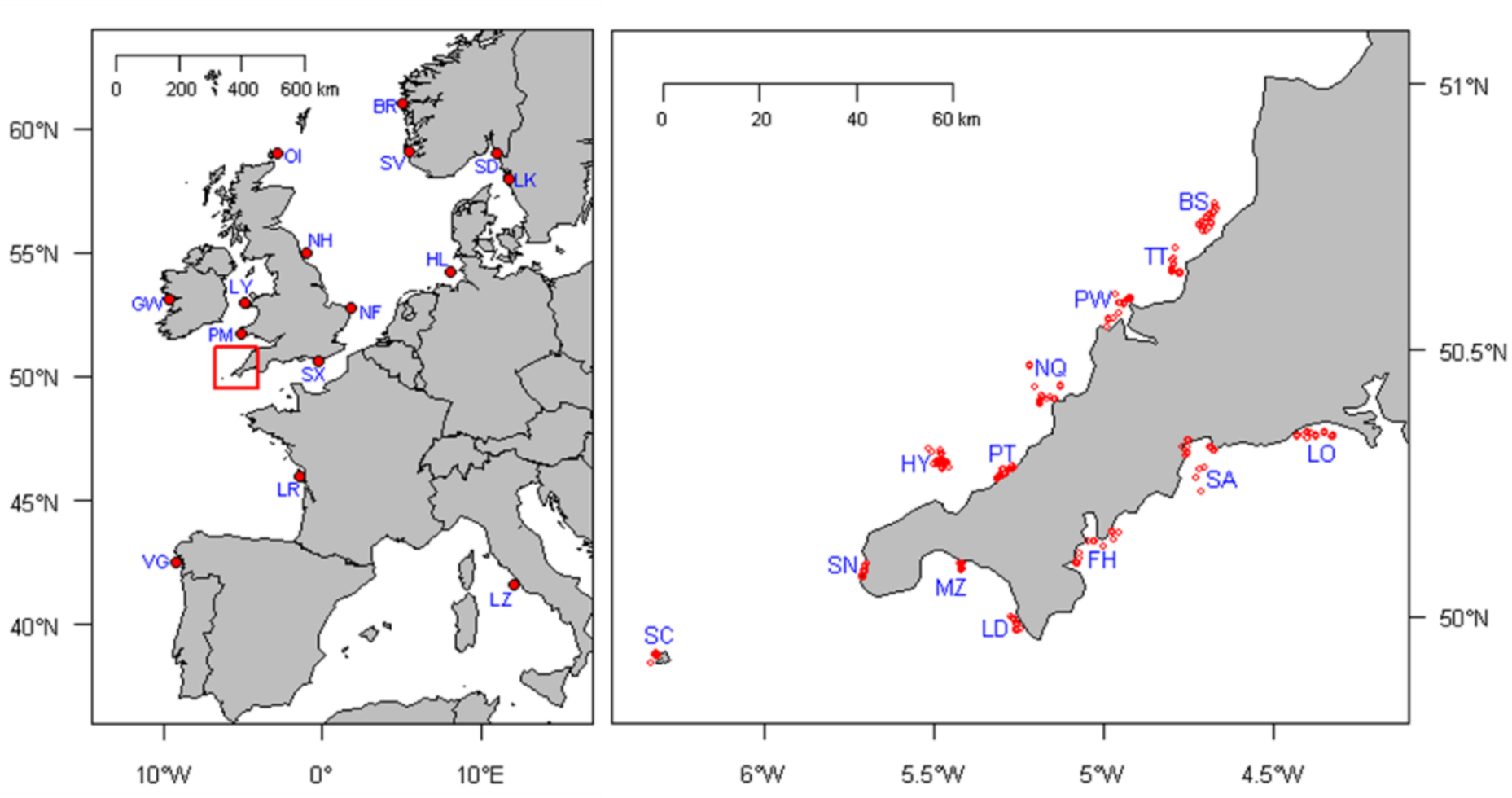

Figure 1. Map of sampling locations. Sample locations around Europe (left), in which inset red area designates locations of fine-scale sampling around Cornwall, U.K. (right). For lettered keys and sample information, see Table 1.

The Multiplex PCR Kit (Qiagen, Hilden, Germany) was used to prepare 8 µl PCR volumes in the following reaction mix: 4 µl Multiplex Master Mix; forward and reverse primers diluted to 0.15 µM with Qiagen nuclease free water; and 2 µl template DNA (20-50 ng). PCR was conducted in a Techne Prime Elite 96 thermocycler (Bibby Scientific Ltd., Stone, Staffs., UK), with an initial denaturation (94^{o}C, 3 min), then 35 cycles of denaturation (94^{o}C, 40 s), annealing (55^{o}C, 40 s) and extension (72^{o}C, 30 s), before a final extension (72^{o}C, 4 min).

Fragment analysis was carried out using an ABI 3130 Genetic Analyser capillary sequencer (Applied Biosystems Inc., Carlsbad, USA.). Alleles were automatically sized against Genescan™ 500 LIZ™ size standard (Applied Biosystems Inc., Carlsbad, USA.) using Geneious 6.1 software (Biomatters Ltd., Auckland, NZ), before being checked manually and re-scored where necessary. Ambiguous or non-amplifying loci were retested in single-locus PCR and fragment analysis procedures, with Multiplex Master Mix replaced by Taq Master Mix from the Taq PCR Kit (Qiagen, Hilden, Germany). To estimate genotyping error, PCR, fragment analysis and allele scoring were independently repeated for a sub-sample of individuals (n = 43; 7% of the total samples). The data provided by Huserbråten *et al* (2013) was calibrated by genotyping and analysing a sub-sample of eight individuals from each of the two Swedish samples, with allele scores of remaining individuals being adjusted in accordance with the rest of the dataset where necessary.

Statistical analysis

Deviations from Hardy-Weinberg equilibrium (HWE), both within geographic samples (across all loci) and at each locus (across all geographic samples), were tested using the inbreeding coefficient F_{IS} (Weir & Cockerham, 1984) to check for heterozygote deficiency. Significance of HWE exact probability tests were implemented in the web-based GENEPOP 4.2 software (Raymond & Rousset, 1995a), which was also used to carry out log-likelihood tests of linkage disequilibrium (LD) among loci globally and for each sample, and to calculate allele frequencies. The software FreeNA (Chapuis & Estoup, 2007) was used to check the likelihood of null alleles being present, while the program LOSITAN

(Antao et al., 2008) was used to detect selection on loci via the Fdist method (Beaumont & Nichols, 1996) assuming the mean sample size (n = 22). To measure basic genetic diversity, per-sample allelic richness (A_R) was calculated using the software FSTAT 2.9.3.2 (Goudet, 2001), while the observed (H_O) and expected (H_E) heterozygosity were calculated in ARLEQUIN 3.5 (Excoffier & Lischer, 2010), both per-locus and per-sample.

Global and pairwise measures of the fixation index F_{ST} (θ – Weir & Cockerham, 1984) were calculated in FSTAT, with standard error obtained by jackknifing over loci and global 95% confidence intervals by 15,000 bootstraps over loci. Global and pairwise p-values were obtained via G tests (P_G) of 50,000 and >7,500 permutations respectively, conducted in FSTAT (Goudet et al., 1996; Goudet, 2001). Because they weight results according to the polymorphism of loci, G tests are a more accurate (Petit et al., 2001) and conservative (Ryman et al., 2006) measure of significance for multi-locus data with low levels differentiation (Goudet, 2001). Nevertheless, p-values were also estimated by Fisher's (P_{Fish}) exact test (Raymond & Rousset, 1995b) in GENEPOP to allow comparison with analysis of power, for which the method used only estimates significance via P_{Fish} (Ryman & Palm, 2006). An adjusted significance threshold for pairwise F_{ST} p-values was calculated using Benjamini & Yekutieli's (2001) modified false discovery rate (FDR) method, which better controls Type I (α) error than the original FDR method of Benjamini & Hochberg (1995) without the loss of power to distinguish meaningful genetic differentiation that occurs with the overly conservative Bonferroni correction (Narum, 2006). FSTAT was also used to provide per-locus measures of F_{ST} and standard error, with p-values estimated by Fisher's exact test (P_{Fish}) in GENEPOP. The R (R Core Team, 2012) package DEMEtics (Gerlach et al., 2010) was used to provide global and per-locus estimates of actual differentiation D (Jost, 2008), along with 95% confidence intervals and p-values from 1,000 bootstrapped G tests (P_G) (Goudet et al., 1996). D provides a more logical and consistent measure of allelic differentiation than F_{ST} under many scenarios (Jost, 2009), including where multi-locus genotypes are based on highly polymorphic microsatellites (Jost, 2008).

Minimum Euclidean oceanic distances between geographic samples (obtained using Free Map Tools, 2015) were regressed against pairwise F_{ST} in a Mantel test of isolation-by-distance (IBD; Wright, 1943) with 10,000 permutations using the ISOLDE function in GENEPOP (Rousset, 1997). Effective population size (N_e) was estimated for each geographical sample using the LD method (Waples, 2006; Waples & Do, 2008) by NeEstimator 2.01 (Do et al., 2014), although because N_e estimation assumes closed, non-continuous populations (Waples & England, 2011; Neel et al., 2013), results are generally unreliable when the spatial definition of populations and other demographic parameters are not already established (Wang, 2005; Neel et al., 2013).

The Bayesian software STRUCTURE 2.3.4 (Pritchard et al., 2000) was used to infer population clusters. Model runs featured a burn-in of 400,000 followed by 800,000 Monte Carlo Markov chain (MCMC) steps, for an assumed number of clusters (K) of 2-10 with correlated allele frequencies, simulated for 5 iterations each. The model sets allowing population admixture and the inclusion of *a priori* location data (LOCPRIOR) were utilised, which improve the detection of weak structuring in open populations (Hubisz et al., 2009). The LOCPRIOR setting effectively informs the model of which individuals constitute each spatial sample (i.e. basic sample groupings, rather than explicit data on spatial position or relative distances), and instead of an assumption that all possible partitions of K are equally likely, the clustering algorithm is therefore able to assert greater weight to assignments which correlate with sample groupings (Hubisz *et al.*, 2009). This improves the detection of population divergence but does not infer it when it is absent, since algorithms ignore the designation of samples where no correlations exist with genotype clusters (Hubisz et al., 2009).

Repeat runs of STRUCTURE were implemented without population admixture or LOCPRIOR settings, and without acquired Swedish genotype data to test the effect these had on the optimisation of K. Individual loci were also tested in models with 400,000 MCMC steps and a burn-in of 200,000, with three iterations for K = 2. Any locus showing evidence for population structure in one or more of these iterations was additionally run for a total of 5 iterations per cluster assuming K = 2-10. STRUCTURE outputs were post-processed in the web versions of STRUCTURE HARVESTER (Earl & von Holdt, 2012), which

estimated the optimal number of K using Evanno's delta-K method (Evanno et al., 2005), and CLUMPAK (Kopelman et al., 2015) which formed a convergence between iterations for each value of K. An analysis of molecular variance (AMOVA) was conducted in ARLEQUIN 3.5 (Excoffier & Lischer, 2010) to compare the proportion of genetic variation attributable to regional grouping, samples and individuals. The analysis used >16,000 permutations and was weighted by locus to account for missing data (Excoffier & Lischer, 2010), with the two sample groups reflecting cluster assignment as inferred by STRUCTURE, but significance tests were ignored since these are biased by both the confounding effects of IBD (Meirmans, 2012) and by circularity when sample grouping is defined by cluster results (Meirmans, 2015).

Analysis of power

It is important to assess the power of genetic data when using molecular markers to infer the spatial structuring of populations (Putman & Carbone, 2014). POWSIM 4.1 (Ryman & Palm, 2006; Ryman et al., 2006) was used to estimate the probability of Type I (α) error (a rejection of the H_0 of genetic homogeneity when it is true) and the power of the loci to detect heterogeneity according to the sampling design used. POWSIM estimates α error rate and power as the proportion of random sub-samples which show statistically significant ($p < 0.05$) genetic differentiation after a base population, simulated from allele frequencies, undergoes genetic drift for a specified number of generations. The power deficit (1-β) is the probability of Type II (β) error (a failure to reject the H_0 of genetic homogeneity when it is false). Fisher's exact test was preferred to test genetic differentiation between subsamples because it provides a more stable estimator of α error and power than the alternative chi-square test, particularly when assessing multi-locus genotypes with skewed allele frequencies (Ryman et al., 2006).

As well as using information on the number and sizes of samples, the number of loci and allele frequencies, POWSIM requires an estimate of N_e for the base population in order to simulate genetic drift. The estimate of N_e given has a negligible effect on the estimate of statistical power obtained at any expected F_{ST}, but does control the generations of drift required to attain that level of

differentiation. Because our per-sample estimates were unlikely to be representative of true contemporary N_e due to violation of methodological assumptions (Wang, 2005; Waples & England, 2011; Neel et al., 2013), two values of N_e were tested in POWSIM computations. The lower N_e tested was 2000, close to that estimated for the large Strömstad sample which should be low for the species as a result of historic overfishing (Vucetich et al., 1997; Kalinowski & Waples, 2002; Huserbråten et al., 2013). An upper N_e of 10000 was tested for comparison, which was based on a typical N_e / N_{CENSUS} of 0.005 for highly fecund marine species (Frankham, 1995; Turner et al., 2002; Ovenden et al., 2007; Palstra & Fraser, 2012) and calculated via the estimated stock size for the Cornwall region (CEFAS, 2015).

Separate POWSIM simulations were carried out for broad- and fine-scale base populations, and for the detection of differentiation overall by many samples (5000 subsample replicates per simulation of drift), and pairwise (1000 subsample replicates). The sampling effort (i.e. the number and sizes of subsamples after genetic drift) used to assess overall differentiation were those we applied, as well as a scenario with only 8 individuals per sample (the mean size outside of Sweden and Cornwall), which was assessed to evaluate potential limitations of the small sample sizes in the broad-scale dataset. At a pairwise level, fine-scale sample sizes were set as sampled (n = 24), with broad-scale samples set as mean reduced sizes (n = 8), and a pairwise comparison between the two sample sizes to address the power of detection between Cornwall samples and European outgroups. In addition to analysis by POWSIM, the total number of alleles (across all loci) per sample size was used to assess the relative power of the variable sample sizes to provide accurate representation of population allelic diversity and frequencies. The mean total number of alleles detected by the two largest samples, those from Lysekil and Strömstad in Sweden (each n = 96), was calculated when reducing the sample sizes by intervals of eight individuals via the randomised removal of individuals.

Results

Loci screening and viability

The maximum genotyping error rate was estimated to be 1.8%, with 22 of 1194 allele scores differing between the original screening and independent repeats. The locus HGA8 was found to be significantly deficient in heterozygotes, both globally ($p < 0.001$) and within the majority of geographic samples. This was the result of the failure of one or more alleles to amplify; null alleles at HGA8 were estimated to have a global frequency of 0.08, a maximum frequency of 0.2 among geographic samples, and were confirmed via a separate parentage analysis (Ellis et al., 2015c). As a result, data for this locus was removed from the dataset ahead of analysis. A much rarer null allele at a second locus, HGC129, was detected via parentage (Ellis et al., 2015c) but had an estimated global frequency of only 0.03. Genotype data at this locus was retained since non-amplifying alleles of such low frequency generally have a negligible influence on population genetic analysis (Selkoe & Toonen, 2006; Falush et al., 2007).

After the removal of HGA8, no further locus significantly deviated from HWE (S1 Table). One pair of markers (HGD111 and HGD129) showed evidence of linkage, but these were retained since LD was only detected ($p < 0.05$) in 2 of the 28 regional samples. Three markers were also designated as being potentially influenced by directional or balancing selection; HGC103 exhibited a higher F_{ST} than was expected via H_E (directional) and HGC131b and HGC120 both exhibited a lower F_{ST} than was expected via H_E (balancing). Nevertheless, these results were all marginal under evolutionary models assuming either infinite alleles (Figure S1) or stepwise mutation, so the potential candidate loci were retained.

Genetic diversity

Among geographic samples, only the sample from Tintagel, UK, was found to deviate from HWE ($p = 0.03$; Table 1), although this stemmed from only 3 / 14 loci falling significantly outside HWE ($p < 0.05$). Overall, there was no disequilibrium from HW expectations across all loci and all populations ($p = 0.998$). Among samples, H_O ranged from 0.598 to 0.723, H_E from 0.637 to 0.710, and the global H_E only exceeded H_O by 0.004. Average A_R was lowest for the sample from La Rochelle, France (3.20) and highest for Lysekil, Sweden (3.88), with a weighted global mean of 3.67 (± 0.14). The estimated effective

population size, N_e, from which samples were derived, ranged from 22.0 (Northumberland) to infinity (14 samples). Many of the lower values for N_e were among fine-scale samples in Cornwall; when pooling these samples together N_e in Cornwall was infinite, and 13 of the 16 estimates of sample N_e were >2000.

Table 1. **By-sample genetic variability.** Genetic variability data of geographic lobster samples, with Figure 1 key and approximate location, the number of individuals (n), observed (H_O) and expected (H_E) heterozygosity, inbreeding coefficient (F_{IS}), allelic richness (A_R), p values of exact probability tests of Hardy-Weinberg disequilibrium (HW p), and effective size (N_e).

Map	Geographical	*n*	H_O	H_E	F_{IS}	A_R	HW *p*	N_e
BS	Boscastle, UK	24	0.723	0.686	−0.056	3.738	0.699	60.2*
TT	Tintagel, UK	24	0.598	0.657	0.091	3.521	0.030	146.4*
PW	Padstow, UK	24	0.696	0.677	−0.029	3.690	0.435	203.4*
NQ	Newquay, UK	24	0.705	0.700	−0.008	3.809	0.577	∞
PT	Portreath, UK	24	0.696	0.687	−0.014	3.752	0.213	125.1*
HY	Hayle, UK	24	0.690	0.672	−0.028	3.657	0.729	529.5*
SN	Sennen, UK	24	0.655	0.678	0.034	3.730	0.128	762.3*
MZ	Marazion, UK	24	0.625	0.645	0.031	3.465	0.122	232.4*
LD	Lizard, UK	24	0.661	0.665	0.006	3.588	0.958	∞
FH	Falmouth, UK	24	0.658	0.669	0.016	3.669	0.479	579.9*
SA	St Austell, UK	24	0.616	0.637	0.034	3.561	0.916	168.0*
LO	Looe, UK	24	0.658	0.669	0.017	3.633	0.078	∞
SC	Scilly Isles, UK	24	0.673	0.684	0.017	3.764	0.670	620.2*
BR	Bergen, Norway	8	0.721	0.710	−0.019	3.668	0.904	∞
SV	Stavanger, Norway	8	0.609	0.650	0.070	3.486	0.996	∞
SD	Strömstad, Sweden	96	0.669	0.677	0.012	3.627	0.556	2406.9
LK	Lysekil, Sweden	96	0.715	0.705	−0.014	3.882	0.171	∞
HL	Helgoland,	5	0.714	0.671	−0.072	3.580	1.000	30.9
OI	Orkney, UK	10	0.687	0.643	−0.073	3.566	0.986	∞
NH	Northumberland,	11	0.669	0.658	−0.017	3.474	0.640	22.0
NF	Norfolk, UK	8	0.680	0.707	0.041	3.769	0.514	∞
SX	Sussex, UK	9	0.619	0.651	0.052	3.596	0.731	∞
LY	Llyn, UK	10	0.611	0.647	0.060	3.498	0.315	∞
PM	Pembrokeshire, UK	10	0.629	0.656	0.043	3.646	0.967	248.2
GW	Galway, Ireland	7	0.663	0.662	−0.001	3.540	0.998	∞
LR	La Rochelle, France	7	0.609	0.638	0.054	3.199	0.995	∞
VG	Vigo, Spain	8	0.625	0.652	0.045	3.641	0.945	∞
LZ	Lazio, Italy	7	0.692	0.692	0.000	3.788	0.913	∞
Total / *weighted mean*		**612**	***0.672***	***0.676***	***0.007***	***3.674***	**0.998**	

* = N_e is infinite when Cornwall samples treated as a single population

Genetic Differentiation and Structure

At a broad European scale, overall F_{ST} was 0.007 (P_G = 0.001: 95% C.I. = 0.002 - 0.012; P_{Fish} = 0.000) and *D* was 0.011 (P_G = 0.013: 95% C.I. = 0.000 - 0.023) (S1 Table). Pairwise F_{ST} across all samples ranged from −0.016 to 0.048 (Table 2), with 50 of 378 pairwise comparisons being $p < 0.05$ when permuted by *G*, and 105 of 378 via Fisher's exact test. Control of the false discovery rate (FDR) adjusted the level of statistical significance with 95% confidence to $p = 0.0077$, which was attained by 11 sample comparisons by P_G, and 50 via P_{Fish}. The samples most frequently featuring in significant comparisons were Strömstad (6 by P_G, 20 by P_{Fish}), Lysekil (0 by P_G, 19 by P_{Fish}) and Norfolk (4 by P_G, 6 by P_{Fish}), often when paired with samples from Cornwall (Table 2).

At a fine scale within Cornwall and the Isles of Scilly, overall F_{ST} was 0.0005 (P_G = 0.13: 95% C.I. = −0.002 - 0.003; P_{Fish} = 0.019) and overall *D* was 0.0006 (P_G = 0.43: 95% C.I. = −0.005 - 0.007), and the maximum pairwise F_{ST} was 0.012, with no comparisons significant after FDR control, and only five <0.05 by P_{Fish} (Table 2). Among loci, the total number of alleles ranged from 8 to 20, and the locus HGB6 provided the greatest degree of heterogeneity across all samples via both measures of differentiation (F_{ST} = 0.034, *D* = 0.064; S1 Table). Along with HGB6, four other loci (HGB4, HGC103, HGC6 and HGD129) had confidence intervals for global *D* that did not overlap zero (S1 Table). Isolation-by-Distance (IBD) was detected at a European level, with geographic and genetic distances being significantly correlated ($r = 0.129$, $p = 0.0003$; Figure 2), and was only marginally non-significant at a fine-scale level within Cornwall ($r = 0.063$, $p = 0.06$).

Table 2. Matrix of pairwise F_{ST}. Matrix of pairwise F_{ST} (below diagonal) with statistical significance (above diagonal) between geographical samples. P-values < 0.05 are denoted by † for permuted *G* tests and * for Fisher's exact test, and by ††† (*G* – with corresponding F_{ST} in bold text) and *** (Fisher's) where tests were significance after threshold adjustment ($p < 0.0077$) to account for the false-discovery rate. Fine scale samples from Cornwall, UK, are designated above and left of the dashed line.

x	SC	BS	TT	PW	NQ	LD	FH	SA	LO	PT	HY	SN	MZ	LK	SD	OI	NF	NH	SX	LR	HL	GW	LZ	LY	PM	BR	SV	VG
SC	x													***	†††***										† *			
BS	0.001	x						*					*	***	† ***		†††***								†††***			
TT	0.002	0.002	x			*		*						***	†††***		*	*					*		† ***			
PW	0.002	0.005	-0.005	x										***	***		*						*					
NQ	0.003	-0.001	0.007	0.000	x									***	† ***		†											
LD	0.003	0.004	0.006	-0.004	0.000	x								***	† ***		*	*						† *				
FH	-0.001	0.002	-0.003	-0.005	0.006	0.001	x							***	† ***			*								† *		
SA	0.009	0.012	0.011	-0.002	0.007	-0.002	0.006	x					*	***	***		*	*						† ***			*	*	
LO	-0.003	0.000	-0.002	-0.003	-0.004	0.001	-0.004	0.000	x					***	†††***		† *	*						*		†	*	*	
PT	-0.003	-0.003	0.000	0.000	0.000	0.000	0.004	0.007	-0.001	x				***	† ***		*						†	† *		††† *			
HY	0.004	0.002	0.000	-0.005	-0.004	-0.003	0.003	-0.001	-0.001	-0.002	x			***	† ***									† *					
SN	0.002	-0.005	0.004	-0.001	-0.002	-0.004	-0.003	0.005	-0.002	-0.005	-0.002	x		***	***		†††***							† ***			† *		
MZ	0.004	0.004	0.003	-0.003	0.002	-0.007	-0.002	0.001	0.001	0.003	-0.002	-0.001	x	† ***	†††***		†††***	*						† ***		*	† *		
LK	0.008	0.012	0.011	0.003	0.005	0.008	0.011	0.005	0.003	0.004	0.007	0.003	0.012	x		***	***	***	***	*		*		***					***
SD	**0.016**	0.018	**0.017**	0.010	0.008	0.012	0.017	0.010	**0.012**	0.010	0.008	0.011	**0.017**	-0.001	x	† ***	†††***	† ***	*	† *		††† ***			***	*		***	***
OI	0.011	0.003	0.005	0.002	0.001	-0.002	0.002	0.004	-0.004	0.008	0.001	-0.011	-0.002	0.014	0.026	x	† *									† *		
NF	0.005	**0.028**	0.011	0.007	0.015	0.009	0.009	0.015	0.007	0.018	0.014	**0.026**	**0.013**	0.020	**0.030**	0.026	x	***							*		*	
NH	0.016	0.015	0.014	0.022	0.023	0.016	0.023	0.025	0.021	0.006	0.022	0.020	0.019	0.029	0.034	0.026	0.030	x					† ***		† ***	† *	*	
SX	-0.007	0.009	-0.011	-0.015	-0.005	-0.014	-0.009	-0.015	-0.011	-0.006	-0.007	-0.004	-0.008	0.002	0.006	-0.004	-0.012	0.015	x				† *					
LR	0.013	0.020	-0.001	0.014	0.020	0.008	0.016	0.018	0.014	-0.002	-0.001	0.003	0.015	0.019	0.020	0.015	0.031	0.008	0.008	x			† *		*	*		
HL	0.011	0.003	0.004	0.000	0.007	0.004	0.013	0.015	0.010	0.001	-0.004	-0.004	0.007	-0.013	-0.007	0.029	0.017	0.027	0.007	0.007	x				† *			
GW	-0.001	0.005	-0.006	-0.003	0.008	-0.009	-0.016	0.002	-0.002	0.005	0.004	-0.003	-0.006	0.013	**0.019**	0.010	0.014	0.006	-0.014	0.004	0.002	x	†			† *	*	
LZ	0.013	0.012	0.005	0.014	0.013	0.021	0.012	0.031	0.012	0.016	0.019	0.015	0.018	0.010	0.007	0.022	0.012	0.048	0.025	0.021	0.008	0.029	x	† *	† *			
LY	-0.010	0.007	-0.003	-0.002	0.005	0.002	0.003	0.004	0.003	-0.007	-0.004	-0.001	-0.004	0.009	0.009	0.012	0.008	0.007	-0.007	-0.014	0.007	-0.011	0.020	X		†		
PM	0.026	**0.040**	0.019	0.003	0.011	0.008	0.014	0.004	0.011	**0.025**	0.012	0.016	0.013	0.009	0.007	0.018	0.016	0.043	-0.014	0.032	0.036	0.014	0.034	0.014	x	† *		
BR	0.011	0.021	0.011	0.012	0.000	0.017	0.031	0.025	0.016	0.011	0.013	0.018	0.020	-0.003	-0.007	0.037	0.013	0.028	0.009	0.033	0.000	0.022	-0.005	0.012	0.018	x		*
SV	0.010	0.018	0.001	0.002	0.010	0.012	0.014	0.009	0.014	0.003	0.010	0.009	0.007	0.000	-0.005	0.013	0.024	0.019	-0.010	0.021	0.002	0.027	0.005	0.001	0.006	0.006	x	
VG	-0.006	-0.006	-0.007	-0.006	-0.003	-0.009	-0.011	0.001	-0.011	-0.002	-0.004	-0.007	-0.011	0.013	0.019	-0.014	0.002	0.009	-0.008	0.003	0.021	-0.001	-0.003	-0.010	0.019	0.029	0.005	x

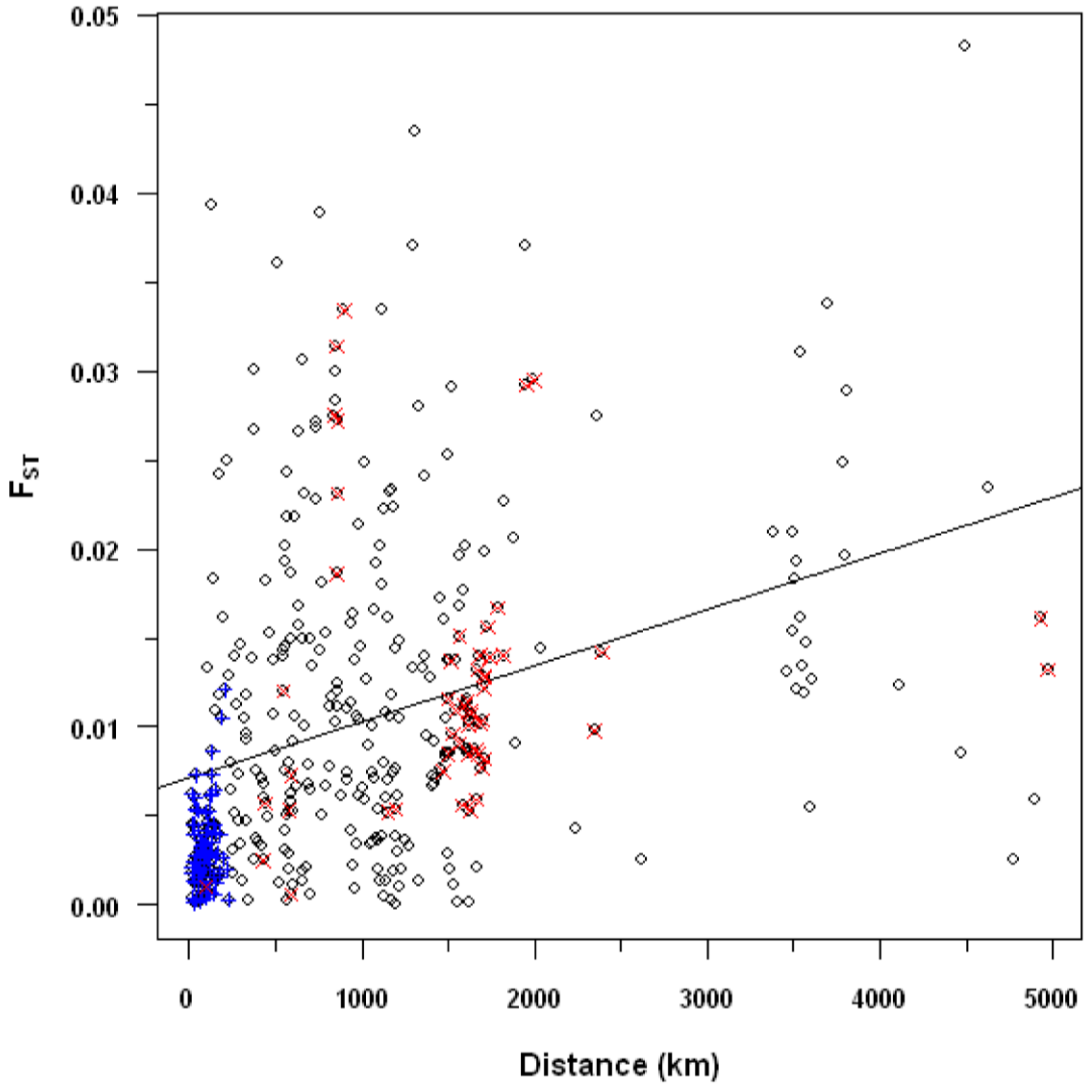

Figure 2. Isolation by Distance plot. Plot of regression between pairwise geographic (km) and genetic (F_{ST}) distance, for which a significant correlation from a 10,000-permutation Mantel test suggests samples demonstrate isolation-by-distance (r^2 = 0.129, p = 0.0003). Pairwise fine-scale samples from Cornwall, UK, are highlighted by blue plus points, and all samples paired with the two samples from Sweden by red crosses. Negative values of F_{ST} are converted to positives, although retaining negative values did not alter significance and had negligible effect on explanatory power (r^2 = 0.136).

At a European level, consistent population structure among samples was detected by STRUCTURE outputs (Figure 3), although only when coalescent algorithms featured *a priori* information of sample composition, another indication that the inferred population structure is weak (either as a result of genuinely subtle divergence or insufficient markers or individuals – Hubisz et al., 2009). Differentiation was absent when spatial priors were omitted, when Swedish samples included only individuals re-genotyped during this study, and when fine-scale samples from Cornwall were run in isolation (Figure S2). All divergent clustering was a result of differentiation at two of the 14 loci; HGB6

and HGC111 (Figure S3). Evanno's delta-K method (Evanno et al., 2005) suggested that K = 2 was the optimum number of clusters among the full dataset, although the likelihood and standard deviation of coalescence only changed sharply beyond K = 4 (Figure 3a, 3b). However, increasing the number of clusters beyond K = 2 was not informative in revealing any additional population clusters (Figure S4), and K = 2 provided the greatest convergence proportion (0.92 - 0.95) for any value of K (Figure 3d, S3).

Heterogeneity in cluster stratification showed that there was a clear trend for the two samples from western Sweden to be differentiated from those of a main Atlantic cluster featuring all samples from Western Europe, including the UK. Samples from the eastern North Sea from Norway and Germany appeared to be a mixture of these two main clusters, as did, unexpectedly, the single Mediterranean sample from Italy. Mean cluster proportions across iterations showed that all UK and Atlantic samples were >68% assigned to cluster 1 (orange; Figure 3c, 3d), while samples from the eastern North Sea (except Stavanger) and Italy were >60% assigned to cluster 2 (blue; Figure 3c, 3d). The sample from Stavanger was the most evenly assigned, with marginally greater (55%) assignment to cluster 1. The mean allele frequency divergence between clusters was 0.013, and the mean H_E was 0.66 for cluster 1 and 0.69 for cluster 2. The global AMOVA showed that ~1% of the total genetic variation arises among the two inferred clusters, five times more variation than occurs among the samples within each cluster (Table 3). The results were not meaningfully altered when samples with marginal assignments (SV and LZ) were grouped oppositely (i.e. by geographic positioning – data not presented).

Table 3 – **Results of global AMOVA**, as a weighted average of locus-by-locus tests. Atlantic (NF, SX, NH, OI, GW, LY, PM, BS, TT, PW, NQ, PT, HY, SN, SC, MZ, LD, FH, SA, LO, LR, VG & SV) and Eastern North Sea (LK, SD, HL, BR & LZ) sample groups were defined by majority assignment in cluster analysis.

Source of variation	Mean d.f.	Sum of squares	Variance components	Percentage variation	Fixation index
Among groups	1	30.00	0.05	1.02	0.008
Among populations within groups	26	132.79	0.01	0.20	0.002
Among individuals within populations	444	2,540.56	0.04	0.76	0.010
Within individuals	567	2,640.00	4.67	98.01	0.020
TOTAL	-	5,343.35	4.76	100.00	-

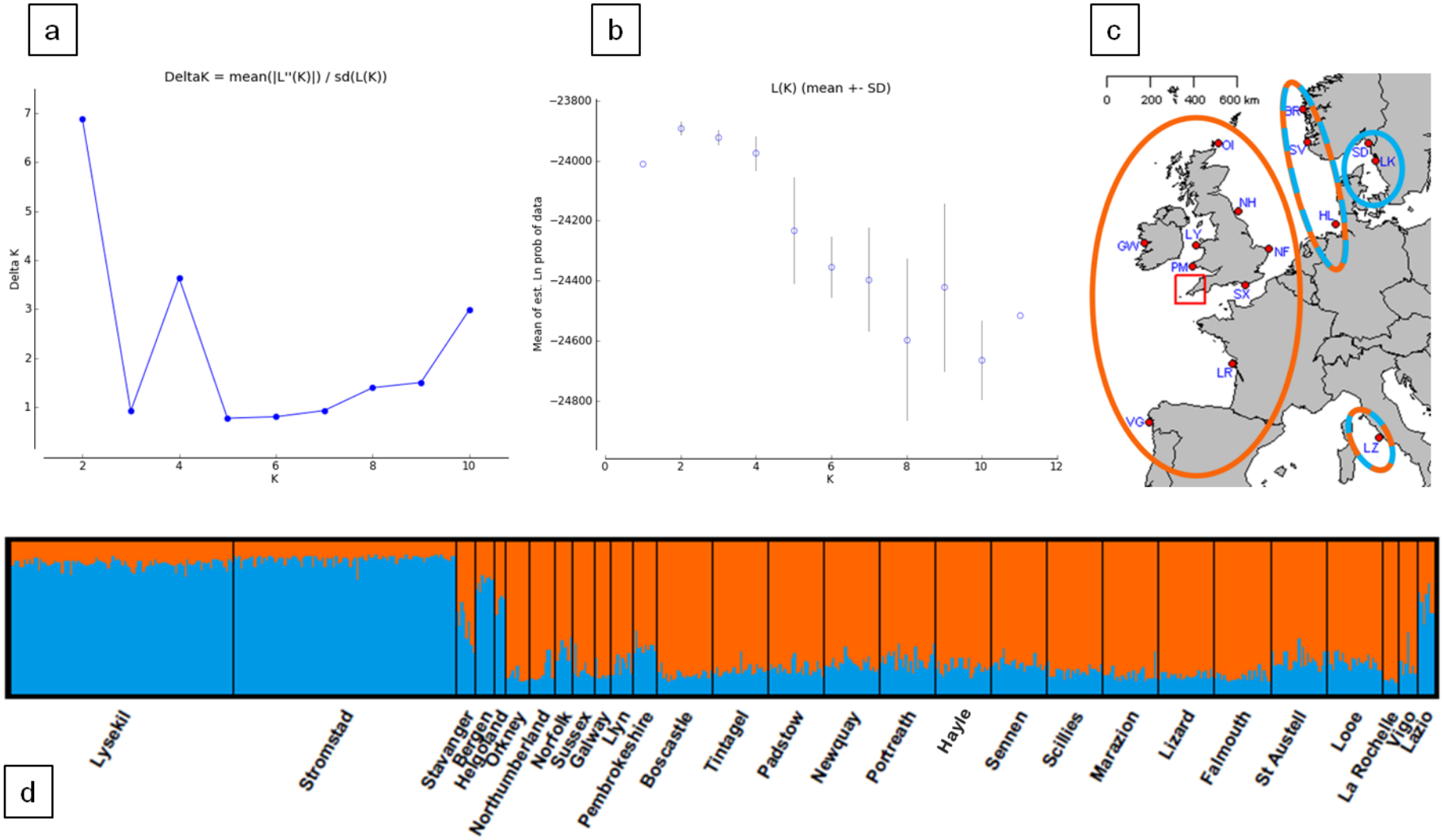

Figure 3. Likelihood, assignment and map of population clusters. Clockwise from top left: plots of cluster likelihood via **[a]** Evanno's delta-K and **[b]** the mean log likelihood; **[c]** map showing sample groupings as indicated by the colour composition of individual samples (vertical bands) in **[d]** a Distruct plot of convergence from 5 iterations of K = 2 using *a priori* location data. Sample names are given in full in **[d]**, whereas in **[c]** they are abbreviated as per Table 1.

Power to detect genetic structure

POWSIM estimated that there was high statistical power to detect overall genetic differentiation, even at low F_{ST} (Figure 4). With the sampling effort we applied, there was significant power (β p <0.05) to reject fine scale homogeneity when overall F_{ST} was >0.0035, and to reject broad scale homogeneity when overall F_{ST} was >0.0025 (Figure 4a). The probability of falsely rejecting overall genetic homogeneity was low at both sampling scales (α p < 0.08). Although POWSIM estimated α error rates of slightly greater than 0.05, this is normal for multi-allelic, skewed frequency markers values, and rates closer to zero are associated with datasets providing very low resolution (Ryman et al., 2006). Restricting the size of all samples to that of reduced European outgroups (n = 8) still provided 95% confidence in the rejection of genetic homogeneity whenever overall differentiation reached F_{ST} = 0.0075.

Within Cornwall, POWSIM estimated that there was significant power (>95%) to detect pairwise differentiation whenever F_{ST} >0.014. However, even when expected pairwise F_{ST} = 0.025 between Cornwall samples and European outgroups of reduced size, and among the latter, the power to detect significant differentiation was only 88% and 52%, respectively (Figure 4b). POWSIM also estimated that genetic drift was expected to generate differentiation of F_{ST} = 0.00025 per generation at N_e = 2000, and of F_{ST} = 0.00005 per generation at N_e = 10000 (Figure 4a). When reducing the sample sizes of the two Swedish samples to match others used in this study there was a clear loss of coverage of the total alleles present in a population. While sample sizes from Cornwall (n = 24) still provided the resolution to detect an estimated 79% of the allelic diversity detected by samples of 96 individuals, the mean size of broad-scale samples outside Sweden provided less than 64% of the detection rate from that of 96 individuals (Figure 5).

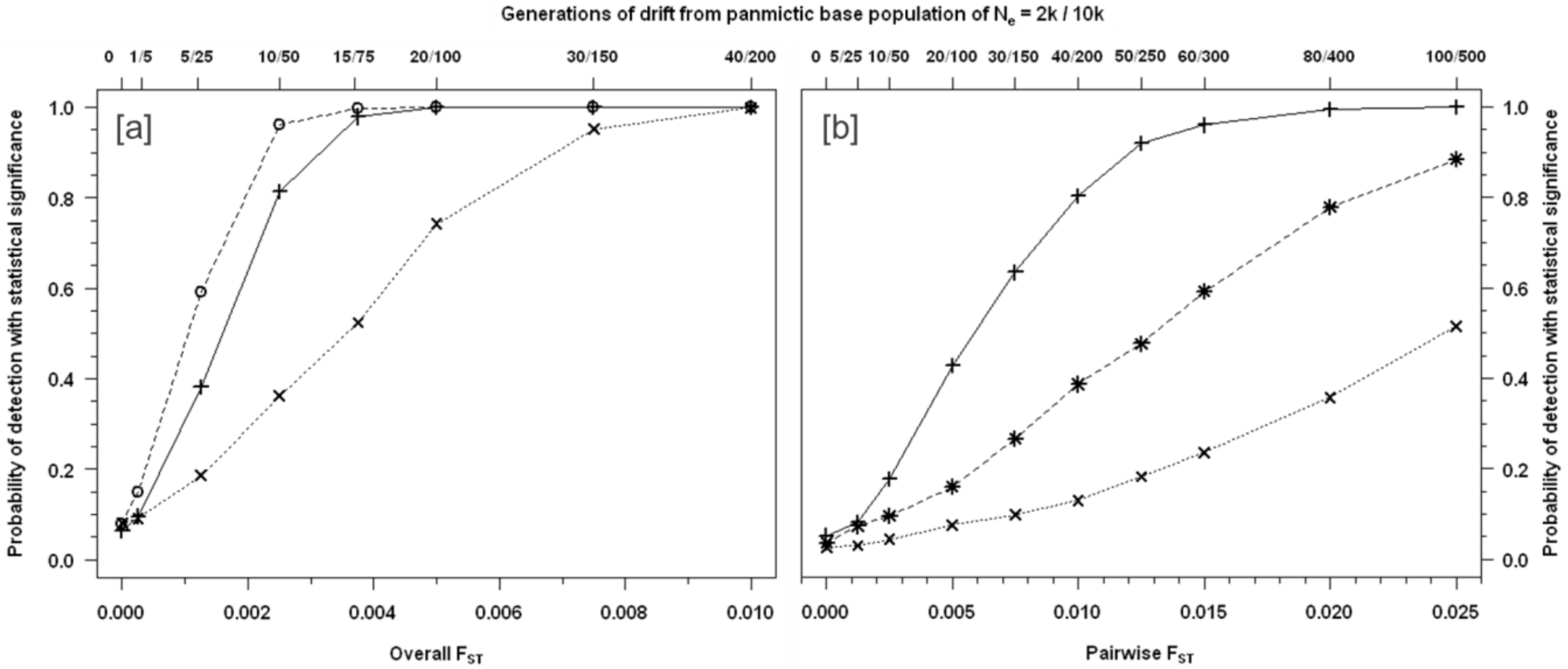

Figure 4. **Estimated probability of detecting significant genetic differentiation**. Results of POWSIM tests of the power of the microsatellite panel to detect significant genetic differentiation, overall **[a]** and between pairwise samples **[b]**, after a simulated base population undergoes genetic drift. Probabilities of detection express p-values of Fisher's exact tests (*df* = 28), calculated as the proportion of 5000[a] or 1000[b] simulations that provided significant power ($p > 0.05$) to reject the H_0 of genetic homogeneity. Where generations of drift is zero (F_{ST} = 0.0000), p-values equate to the probability of falsely rejecting genetic homogeneity. In [a], data points denote fine-scale as sampled (+), broad-scale as sampled (o), and broad-scale with mean minimum sample sizes (x). In [b], data points denote pairwise comparisons as sampled at a fine-scale (+), between fine-scale as sampled and broad-scale with mean minimum sample sizes (✳), and at a broad-scale with mean minimum sample sizes (x).

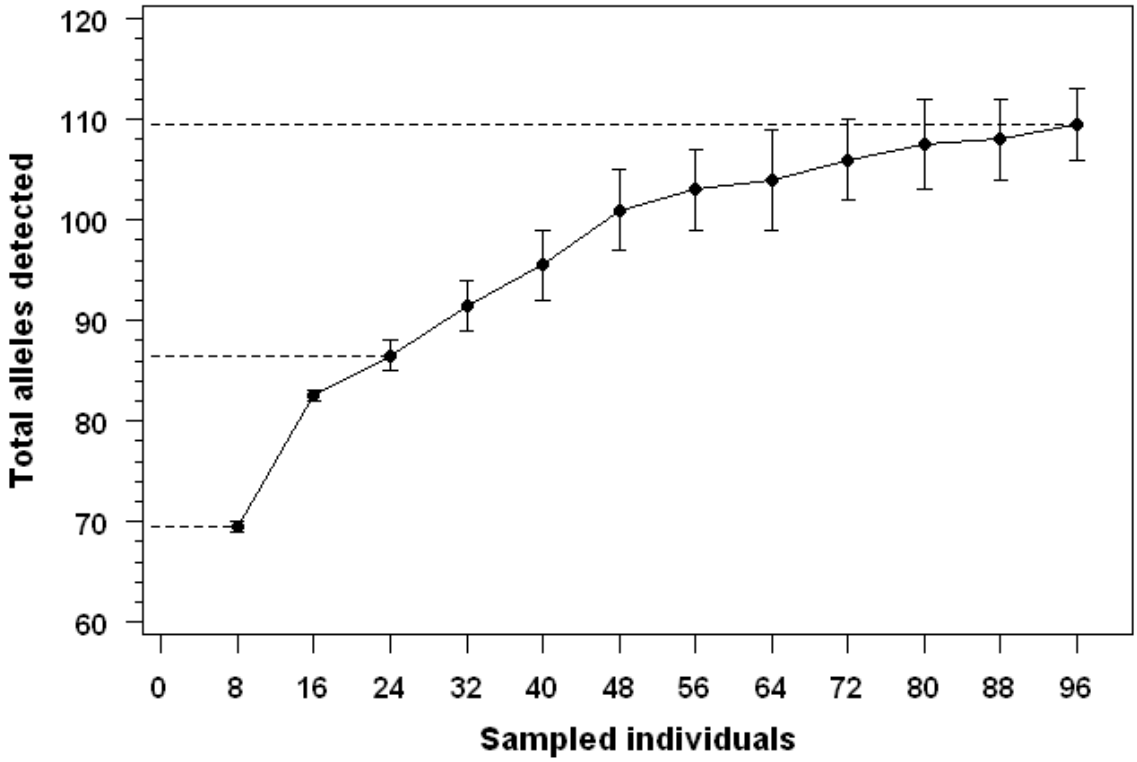

Figure 5. Allelic discovery with number of sampled individuals. The mean total number of alleles detected across all loci in the two samples from Sweden (Lysekil and Strömstad), when reducing the sample sizes via the removal of individuals. Dotted lines show the level of detection for 96 individuals (the sizes of both Swedish samples), 24 individuals (the sizes of all fine-scale samples from Cornwall) and 8 individuals (the mean size of broad-scale samples discounting those from Sweden).

Discussion

Our results, obtained from the most geographically extensive appraisal of contemporary population structure conducted in this species to date, provide an indication that European lobsters do not exhibit extensive spatial genetic structure throughout large areas of the species' range, but that genetic heterogeneity is apparent within parts of their distribution. This heterogeneity is evidenced in particular by relatively weak but consistent differentiation between samples from the Swedish Skagerrak and those from open Atlantic areas to the west. A small but significant portion of the total genetic variation was attributed to differences between samples (~1% via F_{ST} and D), for which confidence intervals did not overlap zero (via F_{ST}, but reach zero via D). There was also a strong association between genetic and geographic distances, suggesting that larval dispersal, the presumed mechanism of primary connectivity (Huserbråten et al., 2013), may become increasingly limited beyond adjacent regions.

However, we found no evidence of substantial genetic differences between geographically distant samples (e.g. 2000 km between NE Scotland and NW Spain) throughout the open Atlantic portion of the range. This result aligns with the previous findings of Triantafyllidis *et al* (2005), who detected negligible differentiation in mitochondrial DNA haplotypes among all samples from the UK, and the French and Iberian Atlantic, but may be a consequence of insufficient analytical power for the detection of weak differentiation, caused by limitations of our broad-scale sampling.

As a second recent study to utilise microsatellite markers for the assessment of fine-scale *H. gammarus* population structure, our results conform to those obtained by Huserbråten *et al* (2013) which detected no substantial differentiation throughout a comparable expanse of the Skagerrak. Our results indicate that hatchery releases in Cornwall, southwestern UK, do not exceed the spatial extent of population connectivity by natural dispersal, and are important in the wider context of lobster stock conservation and fisheries management, for reasons we further outline below.

Broad-scale population differentiation

The strongest indication of restricted gene flow within *H. gammarus* was found among samples from Western Sweden, where broad scale sampling was concentrated. The phylogeographic study of *H. gammarus* by Triantafyllidis *et al* (2005) found no differentiation between a sample from Western Sweden and those from the UK/Atlantic, although this was based on mitochondrial DNA, which often mutates at a slower rate than microsatellites (Whittaker et al., 2003; Wan et al., 2004) and thus may underestimate contemporary divergence (e.g. Monsen & Blouin, 2003). A fundamental difference between samples we analysed from Sweden and those we analysed from elsewhere is that the majority of Swedish lobsters were genotyped by other researchers rather than during this study. Additionally, one of the two loci found to drive divergence in cluster assignment, HGC111, had been previously been allocated several dinucleotide alleles by Huserbråten *et al* (2013). However, we only found tetranucleotide repeats at this locus, either when re-genotyping sub-samples of the Swedish samples or in samples from elsewhere, and instead attributed all

Swedish dinucleotide scores to the nearest larger tetranucleotide allele. Although it cannot be totally discounted that the observed genetic divergence may have arisen from differences in allelic scoring, the re-analysis of 1/12 individuals should have robustly calibrated the two datasets. Additionally, significant clustering variation was based on a second marker which showed no discrepancy in allele sizing between datasets. As such, we believe that the observed divergence of Swedish lobsters is genuine.

Especially where evidence suggests population structure is weak, biophysical models can prove informative in interpreting the spatial scales at which population units exhibit demographic independence (Selkoe et al., 2008; Crandall et al., 2012; Teacher et al., 2013a). Although the extensive geographic scope of this study made it unfeasible to account for detailed oceanographic connectivity, regional ocean currents and population genetic structure in some other species support the apparent lack of gene flow between Skagerrak populations and those inhabiting more open waters to the west. Baltic Sea discharge means there is a mean annual net outflow of 450-500 km^3 from the Skagerrak into the North Sea (Omstedt et al., 2004), so Skagerrak lobster populations at the boundary of the species' range may experience a net deficit of larval migration due to asymmetric dispersal (e.g. Pringle et al., 2011). Perhaps more importantly, prevailing currents circulate the Skagerrak (Lekve et al., 2006; Stenseth et al., 2006), hydrological conditions which could promote the extensive larval dispersal and genetic homogeneity reported within region (Huserbråten et al., 2013), while also creating weak differentiation from Atlantic populations through regional larval retention. By example, Atlantic cod (*Gadus morhua*) are generally self-recruiting in the Skagerrak, and only occasional annual influxes of larvae from North Sea populations inhibit more comprehensive genetic stock divergence (Knutsen et al., 2004; Stenseth et al., 2006). Combining the findings of Huserbråten *et al* (2013) and those of the population clustering from our analysis, microsatellite DNA evidences a discrete lobster population unit in the Skagerrak/Kattegat region, with connectivity to the UK/Atlantic maintained largely via indirect gene flow through intermediary sub-populations of the eastern North Sea.

Not all our results neatly fit this narrative, however. Cluster analysis showed that the sample from Stavanger (collected from the nearby Kvitsøy archipelago), aligned towards the Swedish group less well than other samples of the eastern North Sea from Bergen and Helgoland. It is possible that this might be an artefact of hatchery-induced gene flow, since a small fraction of broodstock initially used to restock the heavily depleted Kvitsøy area was imported from Scotland (Agnalt et al., 1999; Jørstad & Farestveit, 1999).

Another unexpected grouping involved the sample from Lazio, western Italy, which clustered with the Scandinavian samples almost 5000 km away. Although several evolutionary mechanisms could account for the observed pattern of differentiation among lobsters from the eastern North Sea and the Mediterranean, none can reasonably explain the apportioning of those samples to a single population cluster by our coalescent analysis, since these areas could not be linked by natural dispersal without high connectivity via open Atlantic populations. The area from which the Italian samples were collected has undergone hatchery stocking in recent times (Ellis et al., 2015a), but broodstock were strictly sourced from the local stock, and no juveniles were released prior to the collection of tissues (Roberta Cimmaruta, CISMAR, pers. comm.). The deficiency of the sample size means that confidence in the reliability of this result is not high, but cluster definition was consistent between all individuals. An alternative, anthropogenic explanation may stem from the routine export of lobsters captured in northern Europe to markets further south. Prior to marketing, imported lobsters are usually stored alive in land-based tanks, often adjacent to marine environments, into which adults may escape and hatched larvae may be released via effluent. Such mechanisms of introduction are presumed to have led to recent wild captures of the American lobster, *Homarus americanus*, throughout many coastal European waters (van der Meeren et al., 2000, 2010; Jørstad et al., 2007; Stebbing et al., 2012), and cannot be dismissed as an explanation for either the presence of putatively Scandinavian-type genetic profiles in the Mediterranean, or the apparent connectivity between distant UK and continental populations.

Our results add to existing evidence that genetic differentiation occurs within *H. gammarus* populations that are located towards the limits of the species'

distribution, with discrete biological units being previously identified in Northern Norway and the Aegean Sea (Jørstad & Farestveit, 1999; Jørstad et al., 2004a; Triantafyllidis et al., 2005). Increased genetic drift and fragmentation of population structure towards range margins is often a consequence of relatively infrequent immigration and emigration (Slatkin, 1993; Vucetich & Waite, 2003; Gaston, 2009; Hellberg, 2009). Interestingly, the geographic samples we found to be differentiated from Atlantic populations also share a recent history of heavy depletion by recruitment overfishing (Dow, 1980; Schmalenbach et al., 2011), which would be expected to accelerate genetic drift (Waples & England, 2011). However, where genetic divergence is caused by genetic drift under conditions of limited gene flow, this is usually characterised by a relative reduction in genetic diversity (Alleaume-Benharira et al., 2006; Eckert et al., 2008) which was not detected in the Swedish lobster samples. Divergence of these fringe populations may instead result from adaptation to environmental conditions that are at the limits of the species tolerance (e.g. Ledoux et al., 2015). Population genetic differentiation has been frequently found to correlate with temperature or salinity gradients where abiotic data is coupled with that from genetic markers associated with selection (e.g. Limborg et al., 2012; Teacher et al., 2013a; Orsini et al., 2013; Berg et al., 2015; Jorde et al., 2015). In our study, the locus HGC103 suggested divergence via *D* and was marginally identified as a candidate for directional selection. However, the markers we used largely evidenced selective neutrality, even when divergent populations were analysed separately (Figure S5).

Fine-scale population connectivity

We found no evidence of significant genetic differentiation amongst lobsters collected across Cornwall and the Isles of Scilly, the area throughout which *H. gammarus* stock enhancement is undertaken as a routine objective of the National Lobster Hatchery (National Lobster Hatchery, 2015). Despite high power (>80%) to detect relatively low levels of pairwise differentiation (F_{ST} = 0.01) with significance, only five of 78 pairwise comparisons were significant via Fisher's exact test even before FDR correction, only one more than would be expected from the α error rate. Coalescent analysis revealed no divergence overall and very little genetic variability (<0.1%) was attributed to differences

between samples. Although the overall differentiation (F_{ST} = 0.0005; D = 0.0006) was significant via Fisher's exact test, POWSIM estimated that the confidence in this test was very low when heterogeneity was so limited, and confidence intervals overlapped zero via both differentiation measures. Cluster patterns for the locus HGC111 did hint at some localised differentiation (Figure S3), and regional IBD was only marginally rejected, so the existence of some weak spatial heterogeneity cannot be completely discounted. Nevertheless, fine-scale samples comprised enough individuals to represent population allele frequencies relatively accurately (Hale et al., 2012) and were expected to detect a high proportion of total allelic diversity, so the power to detect overall differentiation was significant, even when only a tiny fraction (0.33%) of the total genetic variability arose between samples. As such, the existence of robust, hierarchical, fine-scale population structure can be rejected with high confidence.

Given the paucity of cultured lobsters released to date compared to the size of the local stock, and the lack of differentiation between Cornish samples and nearby areas where stocking has not occurred (Figure S2), it is considered highly unlikely that the observed homogeneity in allelic diversity is a result of any recent erosion of spatial structure by hatchery stocking. Instead, it appears likely that the low levels of genetic drift detected (F_{ST} = 0.00005 per generation when N_e = 10,000) are counteracted by gene flow. The rate at which genetic drift acts is slow (unless N_e is drastically reduced by bottlenecking – Waples & England, 2011), so a relatively low effective number of migrants (Nm) can generate enough gene flow to prevent populations becoming differentiated (Wright, 1969). Even in isolated subpopulations, only one to ten migrant individuals per generation is usually sufficient to inhibit strong divergence (Mills & Allendorf, 1996). Although the failure of most natural populations to adhere to model assumptions mean that measures of gene flow calculated from those of differentiation should be treated only as a tentative approximation (Waples, 1998; Whitlock & McCauley, 1999; Meirmans, 2012), the overall level of F_{ST} we detected in Cornwall correspond to 500 migrants entering the effective

population per generation, according to the Wright (1969) island model, $Nm = \frac{\left(\frac{1}{F_{ST}}\right)-1}{4}$.

Isolation by distance

Low values of F_{ST} and high genetic connectivity in marine species does not necessarily correspond to an absence of population structure (Waples, 1998). Triantafyllidis *et al* (2005) had previously detected significant broad-scale Isolation by Distance (IBD) among *H. gammarus* populations, and our results provide further robust evidence that dispersal may be restricted throughout the species' range. High site-fidelity has been observed among adult lobsters (Smith et al., 2001; Moland et al., 2011; Øresland & Ulmestrand, 2013; Huserbråten et al., 2013; Skerrit et al., 2015) and the pelagic larval duration is a relatively modest two to four weeks under natural thermal niches (Schmalenbach & Franke, 2010). It is therefore highly unlikely that distant populations, such as those from Orkney, in northern Scotland, and Vigo, in western Spain, are directly connected despite showing no evidence of differentiation. The areas are over 2000 km apart, so high levels of direct migration or dispersal between them is improbable. The lack of consistent heterogeneity between distant samples may simply result from a lack of power, since moderate differentiation (F_{ST} = 0.01) was estimated to be significantly detectable at an overall level, but only detectable in a small minority of pairwise cases (13%).

If the genetic homogeneity between geographically disparate samples that our results evidence is a true reflection of population connectivity, then gene flow most adequately fits a stepping-stone model (Kimura & Weiss, 1964; Slatkin, 1985). Stepping-stone structure is often exhibited where marine populations are fragmented by restriction to patchy habitats (e.g. Lejeusne & Chevaldonné, 2006), and this appears to fit the preference for shelter-providing rocky substrata of adult *Homarus* (Wahle et al., 2013). It may be that all differentiation we observe is a consequence of IBD. Untangling whether genetic heterogeneity arises from IBD or actual barriers to gene flow is problematic, although testing for IBD within each identified population cluster allows for the effect of

hierarchical structure, unlike global testing (Meirmans, 2012). When analysing clusters individually, IBD is significant within the Atlantic cluster (r = 0.10, p = 0.002) but not the Eastern North Sea cluster (r = 0.41, p = 0.17; neither data presented), although this may simply result from the lack of power attributed to the latter from reduced pairwise comparisons (10, vs 253 for the Atlantic cluster).

Methodological power and resolution

More genetic markers, different markers, more samples, and particularly a greater number of individuals within samples may reveal more extensive differentiation in *H. gammarus* populations than we have identified. While simulations show that considerable confidence can be assigned to our findings of fine-scale homogeneity in Cornwall and the heterogeneity of Swedish lobsters at a broad scale, improved analytical resolution would be required to attach a robust degree of confidence to our finding of extensive homogeneity throughout the UK and Atlantic Europe. Although the sizes of Swedish and Cornish samples were sufficient to distinguish clear signals of differentiation between them, all 13 of the samples we analysed from elsewhere comprised fewer than 12 individuals, which may have prevented the detection of existing genetic structure more widely. The inferiority of this resolution was identified by power simulations and allelic detection rates, and was confirmed by a run of STRUCTURE which found no divergence among Swedish lobsters when sample sizes were reduced to the mean of other European outgroups.

The ratio of statistical signal to noise is often prohibitively low among marine species when genetic samples comprise few individuals (Waples, 1998), with samples obviously prone to yielding calculations of allele frequencies and total heterozygosity unreflective of the wider population, which often biases and inflates estimates of pairwise differentiation (Hale et al., 2012). This latter bias may explain why high pairwise differentiation (F_{ST} ≥0.025) was observed relatively frequently (18/78 comparisons) among the broad-scale samples comprising fewer than 12 individuals, of which only three comparison attained adjusted significance via Fisher's test, and none by G tests. In contrast, larger samples from Cornwall and Sweden produced only 13 instances with equivalent

differentiation when paired among themselves, with each other, and with the small broad-scale samples, but led to 47 instances of adjusted significance via Fisher's test, and all 11 by *G* tests. Overall, there was clear evidence that European samples genotyped during this study were too small to facilitate any conclusive interpretation of broad-scale population genetic structure other than the absence of extreme differentiation. The extent of *H. gammarus* population genetic structure would clearly be more robustly represented by a greater number of individuals among all geographic samples outside of Cornwall and Sweden, and also by more geographic samples across the southern part of the range.

Although simulations showed that the panel of microsatellites we utilised provided high power to discriminate differentiated populations, methods of genotyping-by-sequencing have become increasingly favoured in the study of population structure, and have proven especially useful in detecting extremely small signals of differentiation among weakly structured marine species (e.g. Corander et al., 2013; Reitzel et al., 2013; Wit & Palumbi, 2013; Willette et al., 2014). One recent example is the determination of population structure in the nearest relative of *H. gammarus*, the American lobster *H. americanus*. Kenchington *et al* (2009) used a panel of 13 microsatellites in an attempt to delineate the population structure of *H. americanus*, and identified the existence of two population clusters, divided into the northern and southern portions of the species' range. Since then, Benestan *et al* (2015) have confirmed this broad-scale divergence using over 8,000 neutral single nucleotide polymorphisms (SNPs), but also discovered robust evidence for IBD and considerable fine-scale differentiation within each region, a level of hierarchical structure that was not detected by Kenchington *et al* (2009). Although SNP-derived estimates of pairwise differentiation were typically an order of magnitude lower than those from microsatellites, confidence intervals were extremely small and did not overlap zero, and 11 genetically distinct populations were inferred among 17 geographic samples. The study by Kenchington *et al* (2009) had mean sizes per geographic sample that were more than double those of Benestan *et al* (2015) – 75 and 34 individuals, respectively – so the additional resolution generated by the more recent study clearly comes from genotyping-by-sequencing. Especially

given the species shared life-histories (Wahle et al., 2013), it seems very likely that applying the same methods to *H. gammarus* would reveal a greater degree of population sub-division than that which we were able to detect.

Implications for fisheries management and hatchery stocking

A lack of data on lobster population structure and connectivity has prevented the informed definition of stock units and therefore the implementation of effective management at spatial scales which reflect stock boundaries (Wahle et al., 2013). Genetic evidence has previously revealed distinct, isolated, self-recruiting *H. gammarus* sub-populations (e.g. in northern Norway; Jørstad et al., 1999, 2004a), but our results indicate that such strong differentiation is likely to be rare. Range-wide restrictions on fishing lobsters are minimal, with the current European Union fisheries legislation limited to a minimum landing size (of 87 mm CL), and more rigorous international management would help prevent overexploitation and further regional collapses. However, many national or regional authorities have introduced additional management measures in an attempt to conserve lobster fishing in their jurisdictions (e.g. closed seasons, closed areas, gear restrictions, landing bans on v-notched or ovigerous lobsters, maximum landing sizes and extended minimum landing sizes – Ellis et al., 2015a). This type of localised management is likely to be of considerable benefit to the sustainability and conservation of lobster fisheries within individual jurisdictions, since our evidence for IBD suggests that recruitment is driven, at least partially, by local spawning stock biomass. Proactive management is especially important in preventing localised overexploitation, since where lobster fisheries have been severely depleted, they have generally failed to recover (Kleiven et al., 2012). That IBD was evidenced alongside high levels of genetic connectivity among proximal populations also suggests that, where local spawning stock biomass can be successfully increased, larval dispersal is likely to extend recruitment benefits to adjacent regions.

The implications of our findings to fisheries management apply similarly to hatchery stocking given that cultured lobsters produce viable offspring (Agnalt et al., 2007; Agnalt, 2008). Increased abundance of spawning stock from hatchery interventions may extend far beyond the immediate areas of releases

over multiple generations, although unintended impacts expected to weaken long-term stock fitness, such as the introduction of maladapted traits (e.g. Kitada et al., 2009; Christie et al., 2012a) may also be widely dispersed. However, negative genetic impacts of hatchery releases are most common and most damaging where natural stock is depleted, extensively structured, and demonstrate considerable adaptation to localised environments (Lorenzen et al., 2012), all of which can be discounted with reasonable confidence for lobsters throughout Cornwall and the Isles of Scilly. Overall, we detected no evidence that the current hatchery protocol of admixing releases in this area has any detectable effect on local genetic structure. Gene flow throughout the area appears high, and it is unlikely that releases exceed the spatial extents of natural dispersal. However, it cannot be ruled out that further investigation using more markers, or adaptive markers (located in or close to genes under selection in the environment) might reveal more than one biological unit within the region.

From a practical perspective, extensive hatchery production requires the communal rearing of larvae, and for broodstock to be sourced from fishery stakeholders who are often unable to provide fine-scale details of capture origin. As a result, where broodstock are sought over a wide region, the precise return of juveniles to the location of maternal capture remains practicably impossible for many hatcheries. It may not be desirable either: individual clutches in Cornwall exhibit single paternal fertilisation (Ellis et al., 2015c), so releasing batches of full-siblings together could increase the likelihood of inbreeding. Overall, the deleterious erosion of genetic structure by hatchery stocking appears most likely where stocking occurs over significant distances, towards range margins, or across strong environmental gradients, but it is not obvious how to alleviate negative genetic impacts. Indeed, culture and release strategies to mitigate the disruption of population structure directly contrast those to avoid inbreeding depression. Nevertheless, given our discovery of broad-scale differentiation and isolation-by-distance, and the likelihood of further population structure being revealed by increased methodological resolution, we recommend that operators of lobster stocking projects use locally-sourced broodstock to rear juveniles for release wherever possible, and

should not import juveniles originating from distant areas without empirical evidence of extensive natural genetic connectivity.

While this study has undoubtedly contributed considerable additional information on lobster population genetics, a larger assessment which tests more markers on more lobsters (i.e. comparable to that of Benestan et al., 2015) would be a vital tool with which to improve fisheries management and hatchery release strategies. Until such a study provides further resolution to the spatial structure of European lobster populations, hatchery stocking should not be extended beyond existing operations unless the targeted natural stock is known to exhibit genetic homogeneity, or is too depleted for natural strategies of fisheries conservation to viably restore stock abundance.

Supplementary material

Table S1. Global descriptive statistics of microsatellite loci. The total number of alleles (N_A), *p*-value of test for Hardy-Weinberg equilibrium (HW *p*), total heterozygosity (H_T), and two measures of differentiation; the fixation index (F_{ST}) and Jost's differentiation (*D*), with associated confidence intervals (95% C.I.) or standard error [s.e.], and *p*-values derived from Fisher's exact test (F_{ST} *p*) or 1000 bootstrap replicates (*D p*).

Locus	GenBank accession	N_A	HW *p*	H_T	F_{ST} [s.e.]	F_{ST} *p*	*D* (95% C.I.)	*D p*
HGD106	GU233670	12	0.833	0.715	−0.001 [0.004]	0.08	0.011 (−0.018-0.048)	0.01
HGC118	GU233666	9	0.819	0.583	0.008 [0.005]	0.00	0.017 (−0.002-0.041)	0.00
HGB4	GU233661	12	0.548	0.612	0.006 [0.006]	0.31	0.028 (0.008-0.055)	0.01
HGD117	KT240104	12	0.519	0.569	0.003 [0.006]	0.03	0.005 (−0.027-0.048)	0.43
HGC103	GU233664	9	0.707	0.693	0.019 [0.009]	0.00	0.057 (0.022-0.095)	0.07
HGB6	GU233662	11	0.951	0.737	0.034 [0.010]	0.00	0.064 (0.028-0.107)	0.76
HGD129	KT240105	11	0.935	0.556	0.004 [0.007]	0.40	0.024 (0.007-0.046)	0.79
HGC6	GU233663	8	0.989	0.408	0.013 [0.009]	0.01	0.015 (0.007-0.026)	0.91
HGC129	GU233668	14	0.207	0.754	0.002 [0.006]	0.05	−0.006 (−0.068-0.062)	0.01
HGC111	GU233665	11	0.216	0.732	0.018 [0.007]	0.00	0.002 (−0.032-0.044)	0.21
HGD111	GU233671	15	0.826	0.573	−0.005 [0.005]	0.51	0.007 (−0.013-0.031)	0.47
HGD110	KT240103	13	0.961	0.802	−0.001 [0.004]	0.18	−0.007 (−0.075-0.072)	0.27
HGC131b	GU233669	13	0.369	0.821	−0.003 [−0.002]	0.95	−0.036 (−0.085-0.016)	0.31
HGC120	GU233667	20	0.986	0.866	−0.003 [−0.003]	0.36	−0.026 (−0.083-0.033)	0.03
Overall (95% C.I.)	**-**	**170**	**0.998**	**0.673**	**0.007 [0.003] (0.002 - 0.012)**	**0.00**	**0.011 (0.000 - 0.023)**	**0.01**

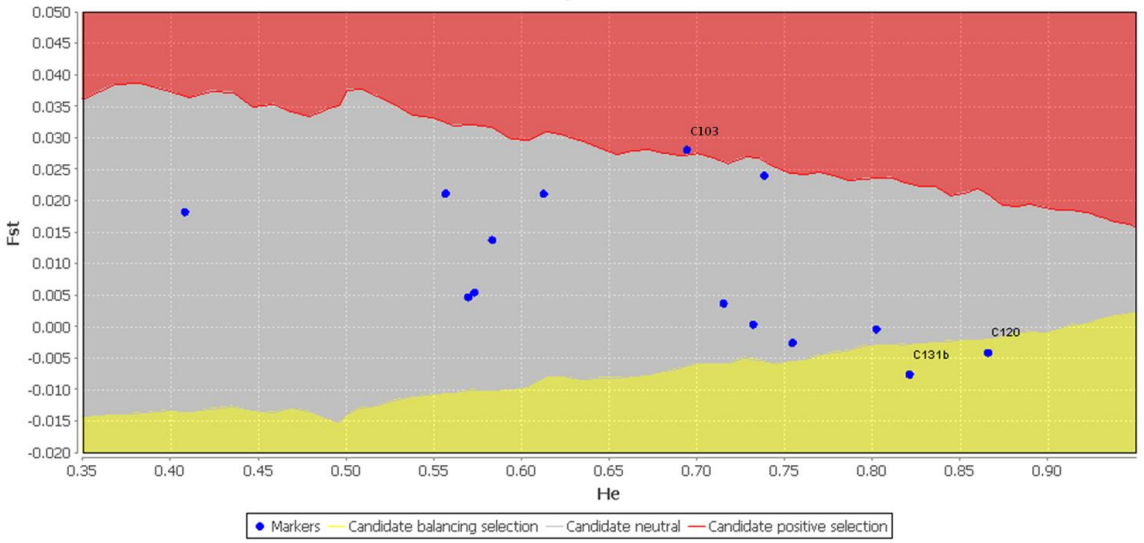

Figure S1. Plot of markers under selection across all samples. LOSITAN plot of H_E vs F_{ST} for all samples and all markers. The grey zone denotes selective neutrality; markers (blue dots) falling into estimated regions of directional (red) and balancing (yellow) selection are labelled.

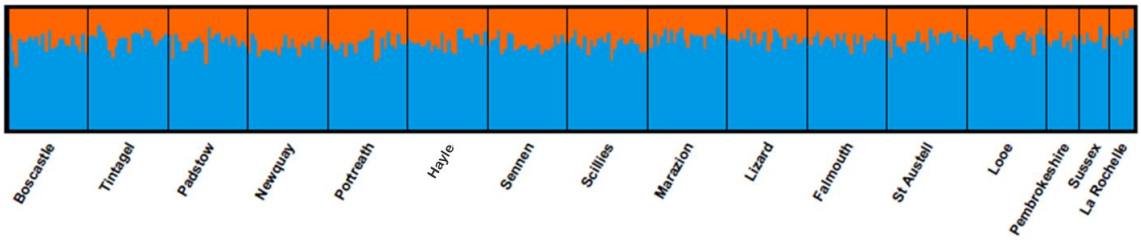

Figure S2. Plot of fine-scale cluster assignment. Distruct plot of convergence of K = 2 for five iterations of the fine-scale dataset of samples from Cornwall, U.K., and nearby outgroups, from STRUCTURE models with *a priori* location data.

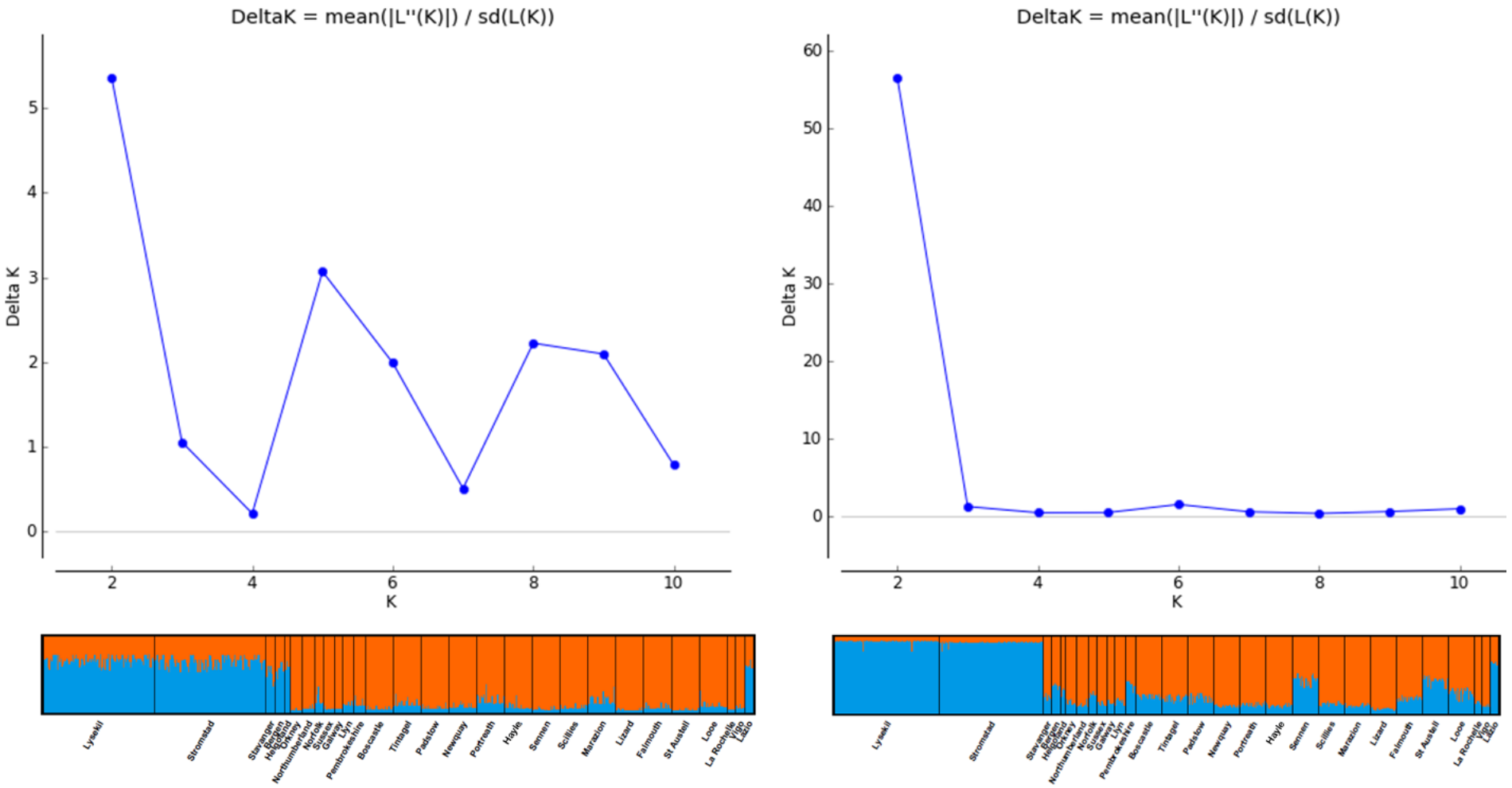

Figure S3. Single-locus plots of cluster likelihood and population assignment. Plots of Evanno's delta-K (top) and Distruct plots of convergence (bottom; min. 5 iterations) from single-locus Structure analyses of the European-scale dataset (with *a priori* locations) at HGB6 (at left) and HGC111 (at right).

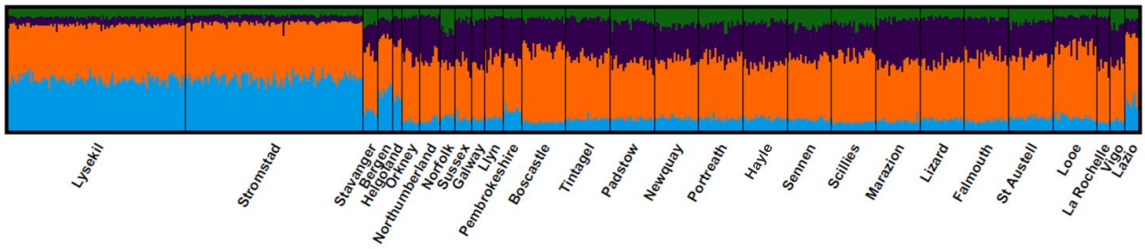

Figure S4. Plot of assignments with four clusters. Distruct plot of convergence of K = 4 for five iterations of the full dataset from STRUCTURE models with *a priori* location data.

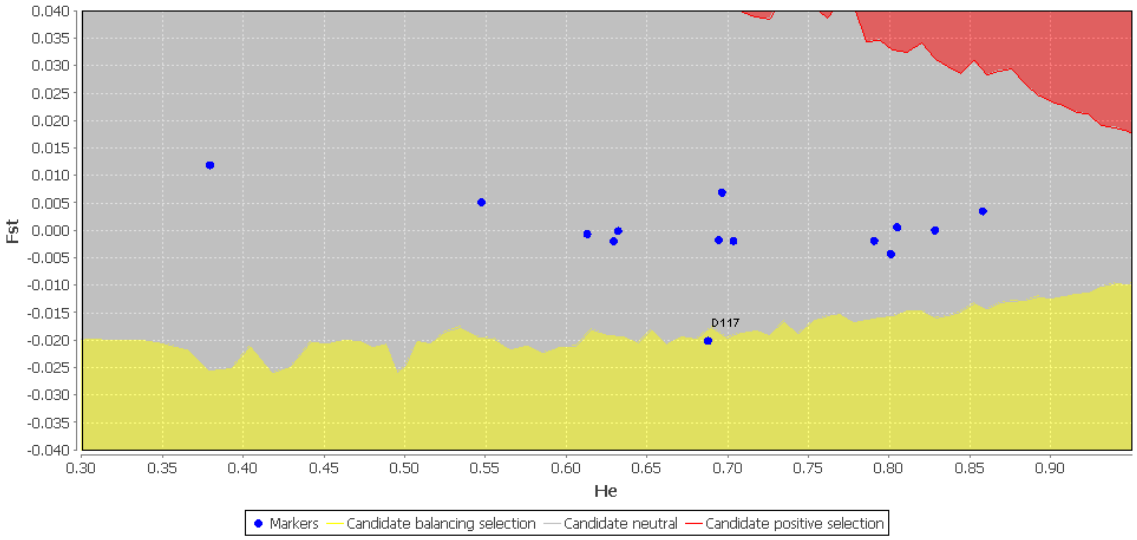

Figure S5. Plot of markers under selection at Swedish samples only. LOSITAN plot of H_E vs F_{ST} for Swedish samples only across all markers. The grey zone denotes selective neutrality; markers (blue dots) which fall into estimated regions of directional (red) and balancing (yellow) selection are labelled.

Chapter 6: Evaluating parentage-based tagging for the identification of released hatchery lobsters

Abstract

Recaptures of hatchery-reared European lobsters (*Homarus gammarus*) in the wild have provided a proof-of-concept that the release of cultured individuals can enhance the species' valuable capture fisheries. However, several recent hatchery stocking initiatives are yet to monitor the wild stock for recaptures, in part due to unfavourable methods with which to tag released animals in order to distinguish them from natural conspecifics. To evaluate the suitability of parentage-based tagging to identify hatchery lobsters among admixed populations in the wild, we quantified the power and error of assignment to hatchery parents for stock samples simulated from known microsatellite genotypes. Assignment accuracy was improved where stock samples contained a greater proportion of hatchery individuals. Assignment solely via maternal candidate led to frequent false positives (>9.8% of allocations; >2.1% of natural stock) which increased in proportion to the number of candidates and always resulted in an overestimation of hatchery recaptures. In contrast, parent-pair assignment never overestimated the released component of the sample, greatly reducing false positives (to ≤2.0% of allocations; <0.3% of natural stock) and more accurately estimating hatchery stock size at all ratios of admixture. Parent-pair assignment yielded minor underestimates of the number of hatchery recaptures, but provided ≥86.0% power to distinguish hatchery and natural stock accuracy, and ≥96.8% power whenever hatchery recaptures comprised at least a fifth of sampled stock. Our results show that, where false positives can be controlled, genetic parentage assignment presents a powerful method for monitoring the contribution of released lobsters to admixed wild stocks, and should be used to inform the optimisation and appraisal of hatchery stocking programs.

Introduction

Alongside control of fishing effort and the protection or restoration of habitats, the release of captive-reared stock (alias 'hatchery stocking'), is an important strategy by which capture fisheries can be conserved and enhanced in the wake of increasing global seafood demand (Lorenzen et al., 2010). Ensuring that released animals can be identified upon recapture is a fundamental objective of hatcheries attempting to augment capture fisheries, as it enables the empirical assessment of stocking impacts on target populations and the ecological and economic optimisation of culture and release strategies (Blankenship & Leber, 1995; Leber et al., 2005; Bell et al., 2006; Lorenzen et al., 2010).

Attempts to sustain and augment natural stocks of the European lobster (*Homarus gammarus*, L.) date back 150 years, over which time the species has always been a prized seafood catch of commercial and recreational pot fishers (Nicosia & Lavalli, 1999). During the 1980's-90's, the development of physical implant tags – coded microwires (CWT) and visible implant elastomers (VIE) – first enabled cultured lobsters to be distinguished from natural stock (Wickins et al., 1986; Addison & Bannister, 1994; Bannister & Addison, 1998). Since then, monitored stocking trials in the UK, France, Norway and Germany have recaptured tagged lobsters, providing data on the survival, growth and fecundity of cultured lobsters in the wild (Henocque, 1983; Latrouite & Lorec, 1991; Burton, 1992; Bannister et al., 1994; Cook, 1995; Agnalt et al., 1999, 2004; Agnalt, 2008; Schmalenbach et al., 2011). Yet, although many of these results were encouraging and established a proof of concept for hatchery stocking of *H. gammarus* (Bannister & Addison, 1998; Ellis et al., 2015a), assessments of the economic viability of lobster stocking found that recapture rates did not adequately offset production costs (Whitmarsh, 1994; Moksness et al., 1998). Despite this, the release of cultured juvenile lobsters has been far from abandoned as a fisheries conservation tactic, with several new hatchery stocking ventures launched in recent years. Although these hatcheries have provided useful biotechnical innovations to advance production efficiency, none currently monitors wild stocks for hatchery recaptures (Ellis et al., 2015a).

Cultured juvenile lobsters are fully benthic by the attainment of the fifth instar, 1-2 months after hatching, and have overcome the assumed recruitment bottleneck associated with predation in the wild during previous pelagic life-stages (Richards & Wickins, 1979; Addison & Bannister, 1994). However, tag retention and lobster survival are both compromised when tagging these early post-larval life-stages with CWT or VIE (Uglem et al., 1996; Linnane & Mercer, 1998; Agnalt et al., 2004; Neenan et al., 2015). As a result, all empirical evaluations of lobster stocking to date are based on recaptures of individuals on-grown in captivity for about a year (Latrouite & Lorec, 1991; Burton, 1992; Cook, 1995; Bannister & Addison, 1998; Agnalt et al., 1999; Schmalenbach et al., 2011), at which point increased body sizes improve tag retention and post-tag survival (Linnane & Mercer, 1998). Although post-release survival is likely to be improved by increased body size (Daniels et al., 2015), whether the considerable additional investment required to produce large juveniles is offset by presumed improvements in recruitment success is unknown (Leber et al., 2005; Ellis et al., 2015a), and the absence of a tag suitable for all instars has prohibited the assessment of which life-stages may be ecologically and/or economically optimised for release (Ellis et al., 2015a). The lack of economic viability in releasing late-stage juveniles, let alone their tagging and the subsequent monitoring of catches for recaptures, has led most active *H. gammarus* hatcheries to release early-stage juveniles as standard (Ellis et al., 2015a). Such a strategy enables hatcheries to maximize numerical release outputs and avoid the expense of on-growing (Ellis et al., 2015), although relies on key assumptions – that early-stage post-larval lobsters have high settlement success and may eventually recruit to the fishery – which have never been empirically tested (i.e. via mark-recapture monitoring).

In the absence of a more satisfactory tag, genetic markers offer an alternative identification resource. Early applications of genetic markers in admixed stock designation in finfish relied on extensive genetic heterogeneity between natural and released stock (e.g. Murphy et al., 1983; Hansen et al., 1995). However, the release of highly divergent hatchery stock is at odds with the responsible management of stocked populations (Lorenzen et al., 2010), and is unlikely where stocking operations utilising wild broodstock (Tringali, 2006), as is typical

of *H. gammarus* enhancement (Ellis et al., 2015a). Multi-locus genotypes can identify hatchery recaptures at an individual basis where each animal's tissue can be archived prior to release (Letcher & King, 1999), but this type of tagging – DNA fingerprinting – is financially and practically inefficient due to the requirement of a very large marker panel (Rew et al., 2011), and could necessitate the attainment of a particular body size before release, a flaw of available implant tags (Wang et al., 2014). Instead, genetic methods of parentage assignment (the allocation of an offspring to a particular parent or parent-pair – Queller et al., 1993; Blouin et al., 1996; Jones & Arden 2003; Jones et al., 2010), are more likely to provide a viable technique to discriminate released lobsters from natural stock (Neenan et al., 2015; Ellis et al., 2015a).

Early uses of molecular markers to assign parentage in hatchery stocking included assessments of culture dynamics, such as the variance in reproductive success among broodstock or offspring family sizes (Perez-Enriquez et al., 1999; Sekino et al., 2003; Jørstad et al., 2005a; Vandeputte & Haffray, 2014). Simulations of parentage-based tagging also showed that accurate post-release allocation of recaptured individuals to hatchery broodstock would be possible using as few as 7-15 highly polymorphic microsatellite loci (Letcher & King, 1999, 2001; Bernatchez & Duchesne, 2000; Hayes et al., 2005; Vandeputte & Haffray, 2014) or 60-100 single-nucleotide polymorphisms (SNPs – Hayes et al., 2005; Anderson & Garza, 2006), even when testing against many hundreds of candidate parents. Such methods have since been employed to detect released individuals among wild, admixed stocks in several aquatic species, including walleye (Eldridge et al., 2002), Japanese flounder (Sekino et al., 2005), red drum (Renshaw et al., 2006; Saillant et al., 2009; Denson et al., 2012), black sea bream (Jeong et al., 2007; Gonzalez et al., 2008) steelhead trout (Araki et al., 2007a; Christie et al., 2012a, 2012b; Steele et al., 2013; Miller et al., 2014), red sea bream (Shishidou et al., 2008) and Chinese shrimp (Wang et al., 2014), all of which have facilitated rigorous monitoring of hatchery stocking.

In addition to replacing physical tags in providing mark-recapture style data to assess the survival, growth and dispersal of hatchery-reared stock, parentage-based tagging offers significant analytical opportunities which traditional tagging

methods cannot (Blouin, 2003; Jones & Arden, 2003; Araki & Schmid, 2010; Ellis et al., 2015a). Genetic allocations of parentage can be used to construct pedigrees to identify second-generation hatchery stock (the wild-born offspring of released individuals – Letcher & King, 2001; Blouin, 2003; Araki et al., 2007b), to identify integration and fitness differences between released and natural stock (Hansen et al., 2001; Araki et al. 2007a, 2007b; Christie et al., 2012a; Miller et al., 2014), to infer the extent of trait heritability (Abadía-Cardoso et al., 2013), and to assess the wild fitness of different released families or genotypes (Sekino, 2005; Tringali, 2006). The validation of parentage-based tagging to identify hatchery lobsters in the wild would therefore facilitate more comprehensive assessments of the impacts of stocking, enabling its optimisation and an informed appraisal of its usefulness as a fisheries conservation strategy in *H. gammarus* (Ellis et al., 2015a). We aimed to assess the suitability of parentage-based tagging for the discrimination of natural and hatchery-derived lobsters among an admixed stock, using population genetic data from lobsters in Cornwall, southwestern UK, to create a case study. We achieved this by using existing microsatellite genotype data to simulate a wild lobster population containing cohorts of cultured individuals, and then assessing the accuracy and consistency of parentage-based tagging by estimating the power and error rates associated with genetic parental allocation.

Materials and Methods

Data Collection and Simulation

We used existing genetic data to simulate the natural and hatchery-reared portions of an admixed lobster population, which were combined to form theoretical stock samples in order to test the accuracy of parentage-based tagging. To simulate natural stock, we collated existing multi-locus microsatellite genotypes for 375 wild lobsters from Cornwall, southwestern UK; 309 wild-caught individuals from a study of local genetic diversity (Chapter 5, this thesis), 34 wild-caught ovigerous females from a study of paternity structure in broodstock of the local hatchery (Ellis et al., 2015b), and 34 unsampled wild males reconstructed with 99.9% power from fertilised egg clutches by the same

paternity study (which showed clutches were singly sired). Using allele frequencies from these 375 individuals, the software SHAZA (Macbeth et al., 2011) was used to simulate the genotypes of 1000 individuals – the natural stock used in admixed stock samples. The number and allelic diversity of loci (the most important factors in obtaining accurate parentage assignment – Bernatchez & Duchesne, 2000; Webster & Reichart, 2005; Harrison et al., 2013a, 2013b) was sufficient for all simulated genotypes to be unique, as established by a test for repeatedly-sampled individuals in SHAZA.

To represent released hatchery stock among admixed stock samples, the genotypes of 340 progeny of hatchery broodstock – 10 fertilised eggs from each of the 34 ovigerous females used by Ellis *et al* (2015b) – were used. We used genotypes as recorded after initial allele scoring, since Ellis *et al* (2015b) repeated genotyping procedures to correct allele scores which were initially mismatched to those of the known mother or reconstructed father: no prior knowledge of parentage or relatedness would be held for individuals sampled from a real admixed population in the wild, so we retained original uncorrected genotypes. For all samples, the data were from 13 microsatellite loci (André & Knutsen, 2010; Ellis et al., 2015b) selected for reliable amplification as per the quality controls outlined by Selkoe & Toonen (2006). There was no missing data for any sample, so multi-locus genotypes provided an estimated power of 99.14% to exclude two unrelated individuals from first-order relatedness via Mendelian incompatibility (Dodds et al., 1996; Ellis et al., 2015b).

Parental Allocation Software

An experimental simulation of mark-recapture-style analysis used the program COLONY 2.0 (Wang, 2004; Jones & Wang, 2010) to estimate the number of hatchery recaptures among an admixed stock via parentage-based tagging. This was achieved by running stock samples of 1000 individuals, featuring a mix of both our simulated natural and hatchery stocks, for parental assignment against the known mothers and reconstructed fathers of the hatchery stock. COLONY establishes full- and half-sibling family groups among the screened individuals, and then categorically allocates these families to specified maternal and paternal candidates (Jones & Wang, 2010). COLONY was preferred to

alternative parentage assignment software because it can accommodate genotyping error / mutation, is able to assign statistical confidence to parental allocations, provides easily interpretable results, and because it offers multiple analysis modes (Jones et al., 2010; Harrison et al., 2013a, 2013b). In particular, COLONY was used because it can apply a full-likelihood method (Wang, 2004; Wang & Santure, 2009), which assigns parentage more accurately than pairwise-likelihood (Marshall et al., 1998; Gerber et al., 2003) or Bayesian (Christie, 2010) approaches, as confirmed by extensive simulations (Wang, 2004; Wang & Santure, 2009; Wang, 2012; Harrison et al., 2013a, 2013b) and empirical datasets (Hauser et al., 2011).

Simulation Settings and Assumptions

In all tests, a total of 1000 individuals (natural and released) were included as the admixed stock sample being assessed for parentage, and population allele frequencies were calculated by COLONY from this sample. All known mothers and reconstructed fathers (both n = 34) of the hatchery stock were supplied as the candidate parents. The mating system was assumed to be without inbreeding but with polygyny and polyandry (as is required by COLONY where half-siblings may be present among the tested offspring; i.e. breeders are not life-time pair-bonded – Wang, 2004). COLONY also requires an *a priori* probability of each offspring's father or mother being included among the specified candidates; given that released stock is necessarily derived from many fewer parents than natural stock, this was estimated as 0.05 (i.e. the 34 candidates of each sex represented only 5% coverage of the total number of parents from which the whole stock sample were descended – an estimated 680 mothers and 680 fathers, in this case). Although the true coverage of total parents would vary according to sibling family sizes and the fraction of the sampled stock which was released, in any practical application using wild lobsters this value would never be known, and COLONY is robust to uncertainty in the estimation of parent population size (Wang & Santure, 2009; Harrison et al., 2013a). COLONY was not given any known sibship, and no sampled individuals were *a priori* excluded from any paternity, maternity or sibship assignment, but a weak prior sibship size (1.1; the harmonic mean number of

offspring per parent) was given for both parental sexes in order to minimise erroneously extensive pedigrees (Jones & Wang, 2010).

For all assignment runs, the anticipated frequency of genotyping errors was set to 1.8% for all loci, the maximum mean mistyping rate for this data as estimated by tests of allele scoring consistency (C. Ellis, Chapter 5: this thesis). To ensure the benefit of factoring genotyping errors was not outweighed by the costs to overall assignment accuracy (Morrissey & Wilson, 2005), genotyping errors were set to zero and results compared to those allowing errors (Supplementary Material, Table S1). The COLONY algorithm converged to the best configuration of assignment at the default settings of medium likelihood precision and computation run-length (Wang, 2004), as evidenced by largely consistent outputs from multiple replicates of the same stock sample run using different random number seeds (Supplementary Material, Table S1). Across all stock samples tested for hatchery parentage, any parental assignment with a probability of <0.5 (i.e. a secondary allocation) was discarded from the results.

Assignment criteria and appraisal measures

A power analysis was carried out to determine the empirical conditions under which COLONY most accurately allocates and excludes hatchery parentage to samples of admixed stock. From all stock samples tested for parentage assignment, the stock-specific frequency (% of natural or hatchery individuals) of false positives (Type I error; allocation of parentage to natural stock) and false negatives (Type II error; exclusion of parentage in hatchery stock) were calculated. Because these false allocations and false exclusions generally represent a trade-off, there is a need to calculate both error types in order to appraise the true accuracy of parentage assignment (Morrisey & Wilson, 2005; Harrison et al., 2013b). False allocations and false exclusions were extracted from the assignment results to calculate positive and negative error rates. The fraction of natural stock allocated to individual candidates across all assignment runs was extracted to quantify the cumulative rate and mean frequency of false positives per parental candidate(s). Overall error was calculated by comparing the true number of hatchery recaptures, N_{HR}, in the stock sample to that as estimated via parental assignment (% ± true N_{HR}). Power, effectively a measure

of the overall accuracy of assignment, was calculated by subtracting this overall error from the maximum power (100% ± Error). This approach enabled us to identify assignment conditions achieving high accuracy, as well as to identify if overall accuracy might have been achieved despite a large error rate, in which false positives and negatives were relatively frequent but were close to equilibrium.

Power and error rates were calculated separately for two criteria of parental allocation; sole maternal assignments (to any candidate mother) and parent-pair assignments (to both a candidate mother and a candidate father). These criteria were compared since they present discrete strategies in assigning sampled stock, and in the assembly of candidate parent genotypes; a maternal genotype can be tested directly from broodstock tissue, whereas the paternal genotype requires reconstruction via the genotyping of 5-10 fertilised eggs (96.9-99.9% reconstruction power, assuming single paternity). Candidates of each sex are specified separately to COLONY, so that sampled individuals may be allocated to any male-female candidate combination. As such, parent-pair assignments could be made to non-mated pairs as well as known-mated pairs, with only the latter therefore eligible to be allocated as hatchery stock.

Assessment of assignment accuracy

The ratio of released to natural stock in admixed lobster populations varies greatly across regions, depending on the number and survival of releases, and especially the abundance of natural stock (Ellis et al., 2015a). The proportion of hatchery individuals among stock samples was varied across COLONY allocation runs to explore the origins of assignment error and to assess the power of parentage-based tagging at different admixtures of released and natural stock. The assessed stock samples comprised 1, 5, 10, 15, 20, 25, 30, and 34% hatchery stock (34% being the upper limit as imposed by the number of hatchery individuals relative to the size of the stock sample). Although the proportions of stock types were fixed, natural and hatchery individuals were included at random, creating variance in the sibling family sizes of hatchery stock, as is known to occur in real lobster release cohorts (Jørstad et al., 2005a).

Results

COLONY provided considerable power to assign parentage in all analyses, with >99% of assignments producing an assignation likelihood of >0.99, regardless of whether or not assessed individuals were allocated to, or excluded from, candidate parentage. Although some hatchery individuals were falsely excluded, all 'recaptures' correctly identified as hatchery stock were allocated to the correct parental candidate(s) via both assignment criteria. There was an overall trend for maternal assignment to always overestimate true N_{HR}, whereas parent-pair assignment always underestimated true N_{HR}. However, estimates of hatchery stock size were consistently more accurate via parent-pair assignment (Table1; Figure 1; Figure 2). Based on the accuracy of assignment-estimated N_{HR}, parent-pair assignment provided ≥94.0% power to discern natural and hatchery stock wherever at least 10% of the sampled stock was released, and ≥96.8% power where at least 20% of the sampled stock was released, whereas maternal assignment failed to attain >91.0% power at any stock admixture (Table 1). There was a general tendency for increased hatchery representation to improve estimates of hatchery stock size via both assignment criteria, although this effect appeared to plateau once released individuals comprised at least 20% of the admixed stock (Figure 2).

Table 1. Results of assignment-estimated hatchery stock size. The number of hatchery recaptures, N_{HR}, as included in samples and as estimated via maternal and parent-pair assignment, and the overall error of assignment-estimated N_{HR}.

True N_{HR} (per 1000 stock sampled)	Maternal assignment			Parent-pair assignment		
	N_{HR} (of which false positives)	Error (% ± true N_{HR})	Power (100% ± Error)	N_{HR} (of which false positives)	Error (% ± true N_{HR})	Power (100% ± Error)
10	30 (21)	200.00	−100.0%	9 (0)	−10.00	90.0%
50	95 (48)	90.00	10.0%	43 (0)	−14.00	86.0%
100	150 (51)	50.00	50.0%	99 (2)	−1.00	99.0%
150	184 (39)	22.67	67.3%	141 (1)	−6.00	94.0%
200	233 (38)	16.50	83.5%	195 (0)	−2.50	97.5%
250	283 (37)	13.20	86.4%	242 (1)	−3.20	96.8%
300	327 (32)	9.00	91.0%	293 (0)	−2.33	97.7%
340	375 (38)	10.30	89.7%	336 (1)	−1.18	98.8%

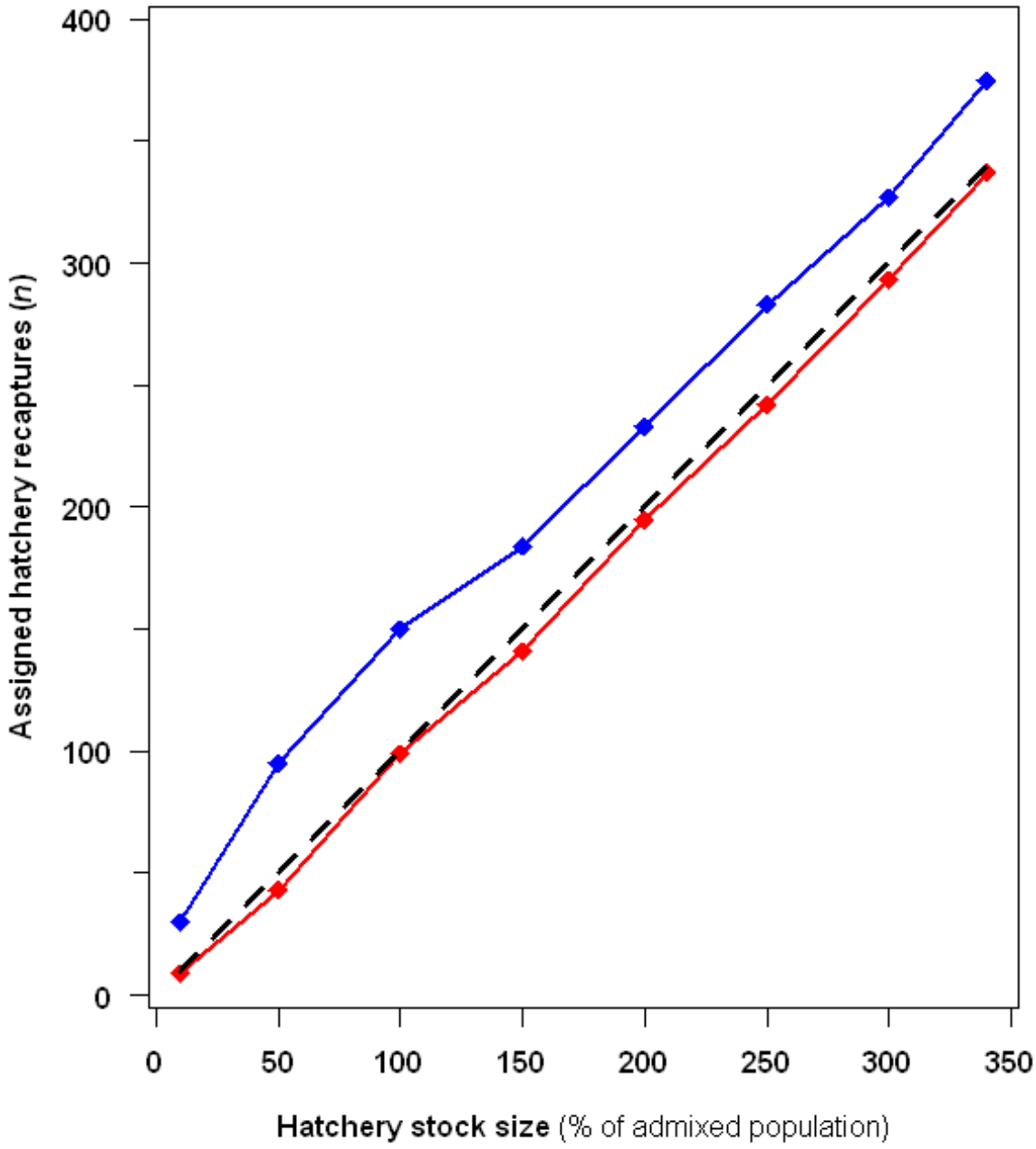

Figure 1. Accuracy of assignment-estimated hatchery stock size. The number of hatchery recaptures estimated via maternal (blue) and parent-pair (red) assignment (points joined with solid lines), against the true number of recaptures (black; dashed line), with varied hatchery contribution to the stock sample.

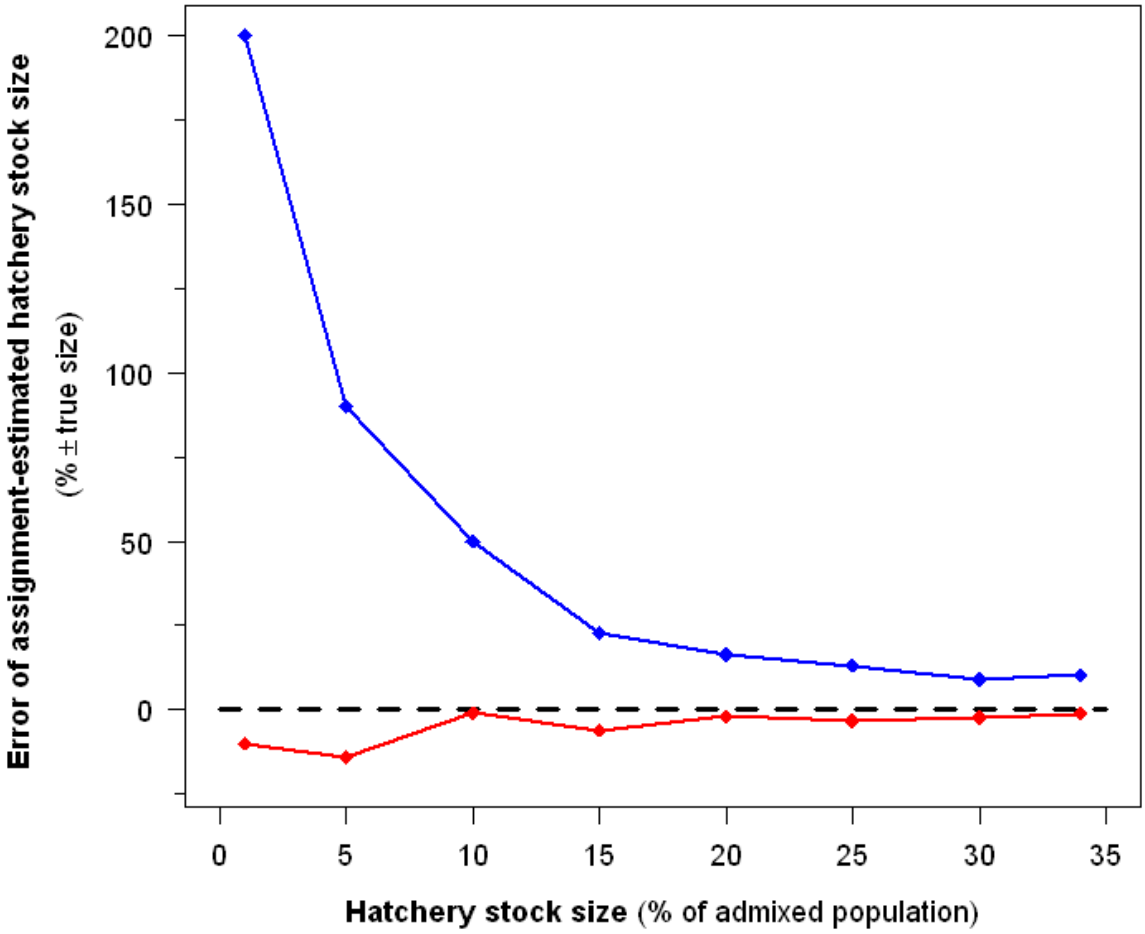

Figure 2. Error of assignment-estimated hatchery stock size. The overall error of hatchery stock size estimates, as per maternal (blue) and parent-pair (red) assignment, with varied hatchery contribution to the stock sample. The dashed black line shows zero assignment error (i.e. the true hatchery stock size).

There were clear and anticipated differences in the types of error suffered by the different assignment criteria. Almost all of the error in parent-pair assignment resulted from false exclusions, whereas maternal assignment suffered many more false positives than false negatives (Figure 3; Supplementary Material, Table S2). Although parent-pair false negatives yielded the highest rate of any stock-specific error and generally exceeded the negative error from maternal assignments across all stock samples, the false negative rates were closely correlated between each assignment criteria and generally decreased for both as hatchery representation increased. In contrast, maternal false positives showed no corresponding decrease, with between 4.5% and 5.8% of natural stock being wrongly identified as descendent from

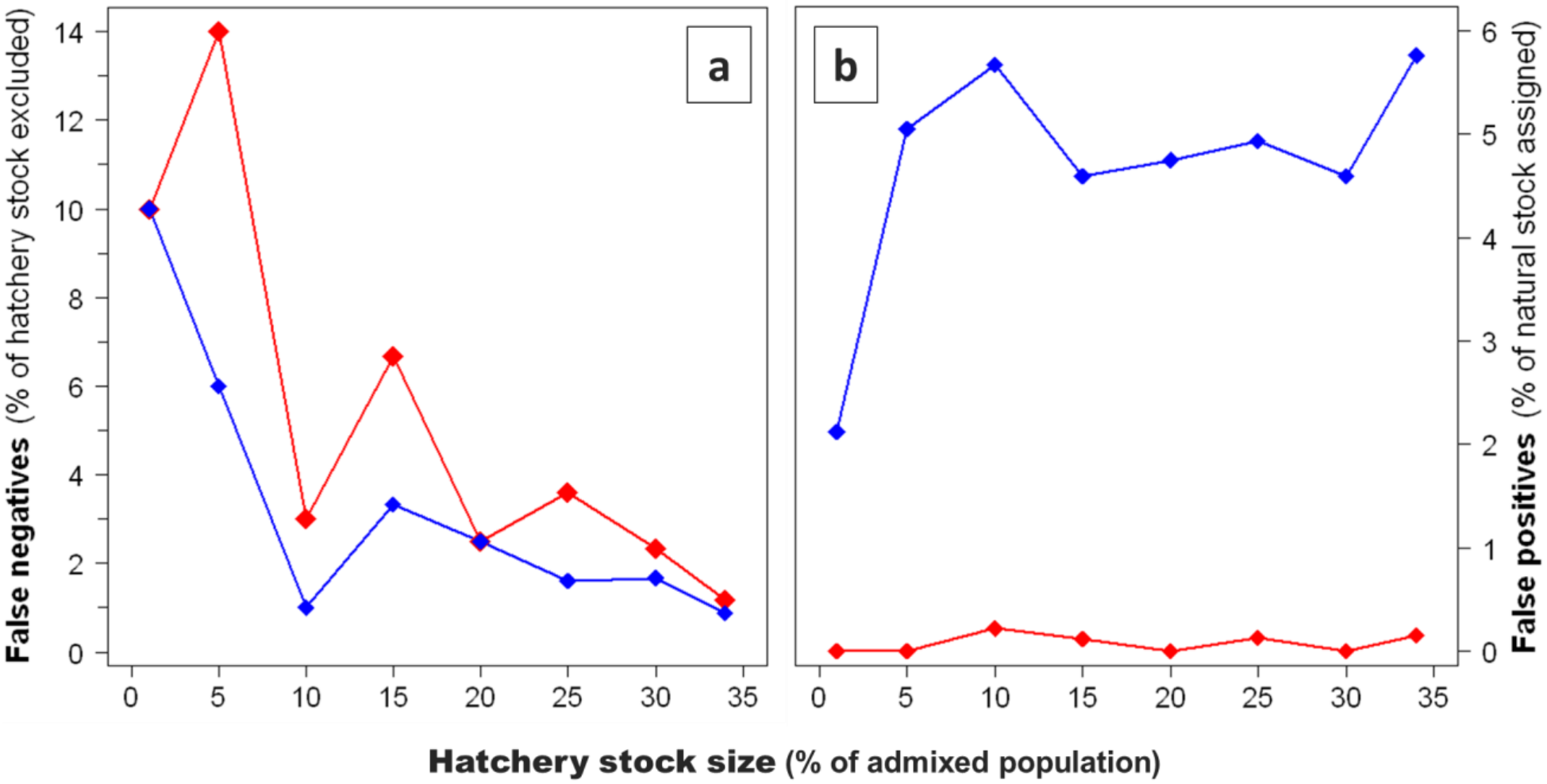

Figure 3. Rates of false exclusion and allocation. Stock-specific rates of false negatives [**a**] and positives [**b**] from maternal (blue) and parent-pair (red) assignments, with varied hatchery contribution to the stock sample.

hatchery broodstock whenever hatchery representation in the stock sample exceeded 1%. Even if allowing parent-pair assignment to occur to non-mated pair combinations, less than 0.3% of natural stock was ever falsely positive via parent-pair assignments. As a result, the weighted mean rate of false negatives across all stock samples was less than 1.4% higher for parent-pair than maternal assignments, but the equivalent rate for false positives was higher via maternal assignments by a margin of over 4.5% (Supplementary Material, Table S2).

Across all stock samples there were only five instances (attributed to three individuals) of a 'natural stock' individual being allocated to both a maternal and paternal candidate. However, all but one of these could be discounted from being genuine hatchery stock because parent-pair assignment was made to non-mated parent combinations (Supplementary Material, Table S3). In contrast, cumulative false allocations correlated positively (linear r^2 >0.99) with the number of candidate mothers via maternal assignment (Figure 4). Of the 34 maternal candidates, 30 generated false positives, with 304 instances of natural individuals being falsely allocated parentage across all stock samples (from a total of 6600 individuals assigned; max. per female = 26 instances). The frequency of maternal false positives increased by an average of 0.14% per female candidate, such that every 7 candidate mothers led to an additional 1% of natural stock being allocated hatchery parentage, with 4.8% of natural stock being falsely allocated to the 34 hatchery broodstock tested. This is a higher rate of false positives than would be expected by the total exclusion probability of the markers (Dodds et al., 1996; Ellis et al., 2015b), from which 2.9% of natural stock would be predicted to show artefactual compatibility to one of the 34 maternal candidates. This discrepancy may arise from our method of simulating natural stock genotypes, although likelihood-based methods typically have higher assignment rates than those based on exclusion, especially where genotyping errors are factored (Wang, 2004; Wang & Santure, 2009; Jones & Wang, 2010; Vandeputte & Haffray, 2014).

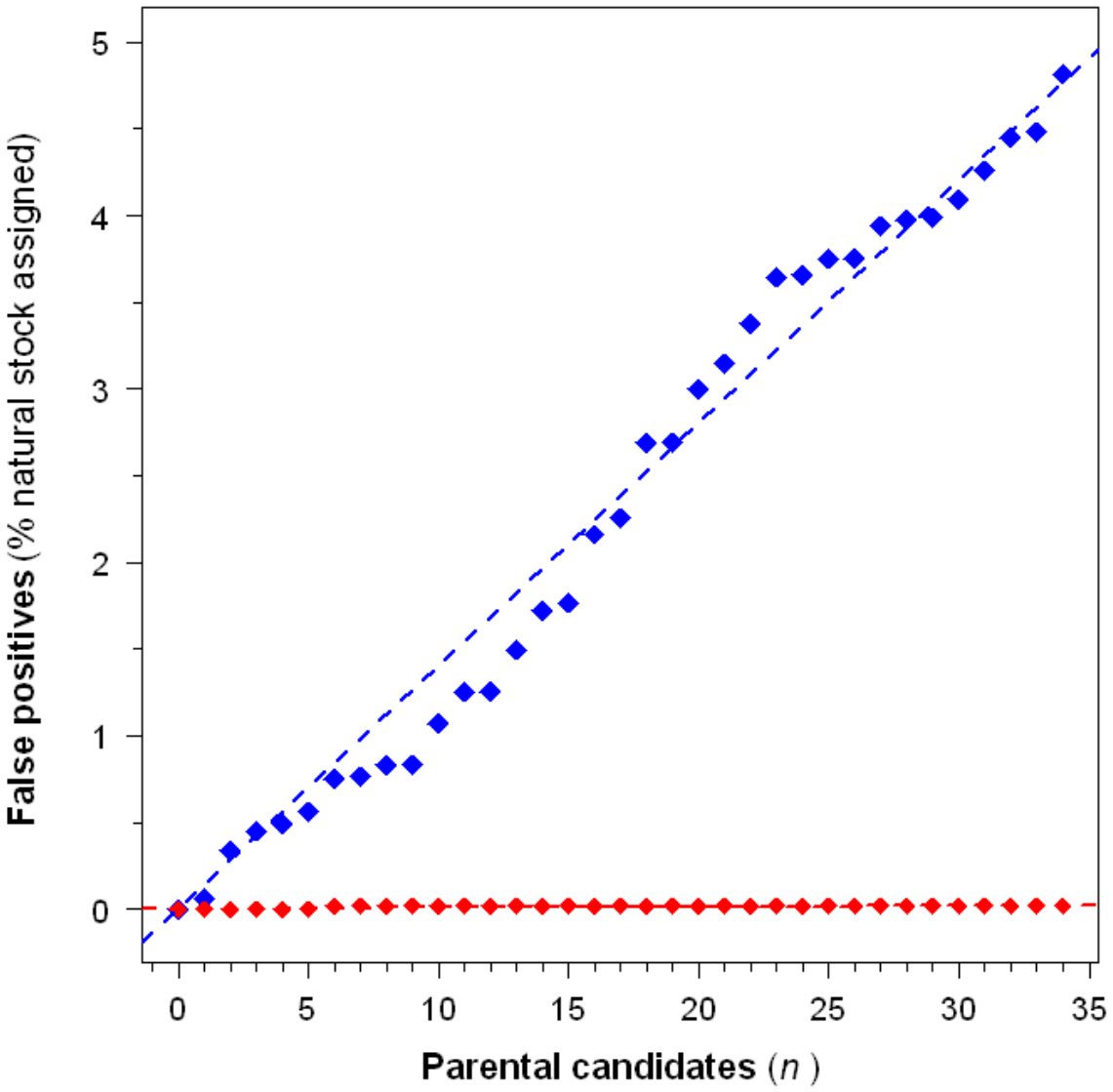

Figure 4. Accumulative false allocations with candidate numbers. The cumulative mean rate of false positives from maternal (blue) and parent-pair* (red) assignment, against the number of candidate parents. Linear regression fits are shown as dashed lines. * = Parent-pair false positives are shown for known parent-pair combinations (0.02% of natural stock across all assignments) as oppose to any paternal/maternal combination (0.08% of natural stock across all assignments).

Among the 340 individuals forming the hatchery stock were eight offspring (from seven sibling families) whose original allele scores included Mendelian mismatches to the genotypes of the known mother and/or reconstructed father (Supplementary Material, Table S4). Of these eight individuals, three had allelic mismatch(es) to the maternal genotype, one to the paternal genotype, two to both parental genotypes, and two where the mismatch(es) could not be resolved to an individual parent (i.e. where an offspring was heterozygote, with one allele shared by both parents and another mismatching both). COLONY

was more robust in the correct allocation of these individuals when only one parental genotype was mismatched, with 12 instances of parent-pair assignment and two of maternal assignment from the 15 instances of a sampled individual having uniparental genotypic mismatch. In contrast, COLONY generally failed to assign released stock to the correct hatchery families when mismatches were to either or both parent(s), with only three instances of parent-pair assignment and one of maternal assignment across 18 instances of a sampled individual having biparental or unresolvable genotypic mismatch (Supplementary Material, Table S4).

Discussion

At most admixtures of natural and hatchery stock, we found parentage-based tagging to be an accurate and consistent method of quantifying the number of hatchery recaptures, with parent-pair assignment clearly outperforming assignment made solely via maternal lineage. Even where hatchery individuals comprised very minor fractions of the stock admixture (e.g. 1%), parent-pair assignment provided high power (≥86%) to estimate the hatchery stock size because the stringency of this allocation criteria all but eliminated false positives. Both assignment criteria were shown to control false negatives much more effectively than the estimated 10% of coded wire tags (CWT) lost per year after physical implantation among hatchery-reared *H. gammarus* released in Norway (Agnalt et al., 2004) Parent-pair assignment correctly identified >96% of hatchery recaptures whenever they comprised at least a fifth of the stock sample. This rate of power is comparable to the performance of 17 microsatellites or 188 SNPs used and approved by Steele *et al* (2013) for parentage-based tagging in stocked populations of trout (*Oncorhynchus mykiss*) in the wild.

Validity of simulation conditions

Our estimates of negative error are likely to be reflective of those which might be expected in a field application of parentage-based tagging because we included hatchery stock known to have genotypic mismatches to parental candidates, and because there was little variation in assignment success

among the 34 release families tested, despite variation in sibling family sizes. Our estimates of positive error are higher than those expected via multi-locus exclusion probability and are likely to be maximum estimates, because the genotypes of natural stock were simulated from a wild survey which included a considerable proportion (18.1%) of individuals specified as parental candidates in assignment runs. More natural individuals may therefore have been allocated to hatchery parents than might be expected when screening a real admixed population. Because maternal assignment is far more affected by false allocations than the parent-pair assignment, the disparity in methodological power between the criteria in this case study may not be as great in real applications of parentage-based tagging. Further validation of assignment accuracy would be advisable at the onset of any application of parentage-based tagging in wild lobsters, and if the true rate of false allocations were shown to be much lower than we estimated among natural stock, the performance of maternal assignment may be closer in accuracy to that obtained by parent-pairs.

The inferior performance of maternal assignment compared to that by parent-pair was also a consequence of the acceptance by COLONY of anticipated genotyping errors from mistyping or mutation. The acceptance of genotyping errors in parentage analysis is expected to reduce the number of false negatives (Hoffman & Amos, 2005) at a cost of increased false positives (Morrissey & Wilson, 2005), which decreases the error rate of the parent-pair criterion and increases that of maternal assignment. Indeed, where no genotyping error was allowed in assignments, the maternal criterion attained power of >94% and was more accurate than parent-pairs for estimating the hatchery stock size (Supplementary Material, Table S1). However, maternal assignment still overestimated hatchery stock size and suffered from imprecision; the total combined error (positive plus negative error rate) was only slightly reduced by the absence of genotyping errors, and still exceeded that of comparable parent-pair assignments incorporating a realistic rate of allelic error (Supplementary Material, Table S2). Where the number and variability of loci provide sufficient assignment power, the benefits of allowing non-zero error rates typically outweigh the costs to overall assignment accuracy (Morrissey &

Wilson, 2005), and that appears to be the case for parentage assignment using the microsatellites available for *H. gammarus*.

Potential effects of stock dynamics and compatibility with genetic management

Although we only assessed the performance of parentage-based tagging when limiting hatchery stock size to 1-34% of the population, this covers the range of hatchery representation reported in enhanced lobster stocks (e.g. an annual peak of 10% in NE England - Addison & Bannister, 1994). Only following the restocking of heavily depleted populations has the abundance of hatchery stock been found to match or exceed that of natural stock (Agnalt et al., 2004), and our results suggest that the accuracy of parentage-based tagging is generally improvedwhere hatchery releases comprise a greater proportion of sampled stocks. The requirements of parentage-based tagging do pose a challenge to applications in hatchery stocking, however, because they present a disparate goal to that of the genetic conservation of stocked populations in terms of the targeted number of releases per broodstock parent ($n_{\mathbf{Rel}}$:$n_{\mathbf{Par}}$). In order to minimise both genotyping expenses and the possibility of false positives from the wild stock, the identification of hatchery recaptures via parentage assignment requires $n_{\mathbf{Rel}}$:$n_{\mathbf{Par}}$ to be as high as possible. In contrast, hatcheries need to limit $n_{\mathbf{Rel}}$:$n_{\mathbf{Par}}$ to maintain high genetic diversity among releases (Blankenship & Leber, 1995; Bell et al., 2006; Gaffney, 2006; Bert et al., 2007; Lorenzen et al., 2010), so as to reduce the risk of inbreeding depression and preserve the genetic effective size of admixed populations (Ryman & Laikre, 1991; Hamasaki et al., 2010; Laikre et al., 2010; Christie et al., 2012b; Satake & Araki, 2012). The use of wild-mated broodstock which are only stored in captivity temporarily means that typical lobster enhancement initiatives are likely to buffer admixed stocks against negative impacts on genetic diversity (Ellis et al., 2015a), but parentage-based tagging would still ideally be applied in focussed, one-off experiments where any effects from releasing stock with high relatedness should be better contained.

Cost and development of parentage-based tagging

Parentage-based tagging has strengths and weaknesses, in practice. In contrast to the advantage of not having to physically tag released individuals, a distinct disadvantage of parentage-based tagging compared to subcutaneous implants is the lack of any capability to distinguish hatchery and natural stock in situ (i.e. at point of capture or landing). To enable parentage-based tagging, all hatchery broodstock contributing to release batches must be genotyped to resolve parental candidates (along with a portion of each egg-clutch when assigning to parent-pairs), and all sampled stock must be genotyped to assign parentage. Assuming that releases and subsequent sampling for recaptures could be integrated into the regular activities of fishers, the cost of monitoring by parentage-based tagging is therefore dependent on: (i) the number of hatchery broodstock and eggs requiring genotyping to resolve parental candidates; (ii) the number of sampled individuals genotyped for comparison to parental candidates, and; (iii) the proportion of hatchery stock among sampled individuals. Because $n_{\mathbf{Rel}}$:$n_{\mathbf{Par}}$ is necessarily high in hatchery culture, (ii) contributes much more to the total cost of parentage-based monitoring than (i), and the cost per recapture is dependent on (iii), with increased hatchery representation improving efficiency.

Using costs associated with the genetic analyses carried out for this study, we calculate the outlay per genotyped sample (mother/egg/sampled individual) to be approximately GB£7 (including all laboratory reagents and consumables, but not including any costs for labour or use of thermocycler/sequencer equipment). Applying this cost to a monitoring study using parentage-based tagging at the same scale of our simulations – a sample of 1000 individuals tested against 34 parental candidates – would cost ~£240 (maternal, M) or ~£1900 (parent-pair, P-P) to resolve parental candidates, plus a further ~£7000 to assign the sampled stock. In reality, bulk-purchasing of reagents for such a large-scale genotyping assay would decrease the expense to ~£7000 (M) or ~£8000 (P-P) and reduce the additional outlay required to implement parent-pair over maternal assignment.

Both our estimates of accuracy of parentage-based tagging and these projected costings are based on genotype data from 13 microsatellite loci we tested across four multiplexes, with data from two further loci removed because of bias by null alleles (Ellis et al., 2015b). The development of more markers would provide more assignment power, although this would increase genotyping costs unless new loci could be incorporated into existing multiplexes, or were used to replace less informative current loci. Ellis *et al* (2015b) found that only three *H. gammarus* loci, amplified and analysed together in a single multiplex, gave power to detect multiple paternity among progeny clutches that was only reduced by <4% compared that of the full panel of 13 markers (requiring screening at three further multiplexes per sample). The addition of similarly informative new markers to this or another multiplex may well increase the power of parentage-based tagging while simultaneously decreasing the cost.

Optimisation of parentage-based tagging to monitor hatchery stocking

Our results recommend parentage-based monitoring of hatchery stocking of *H. gammarus* in the wild, and also indicate best-practice protocols. Our findings clearly indicate that allocation to known-mated parent-pairs is the most powerful and reliable criteria with which to distinguish released and natural stock using parentage assignment. Although the utility of parentage-based tagging need not be limited by geographic or numeric scale in any mark-recapture-style application, the general fidelity of cultured lobsters to release sites (Bannister et al., 1994) means that the proportion of hatchery recaptures in the catches made close to release sites can be at least an order of magnitude higher than it is across the total catch of inshore fishers throughout the their range of effort (Addison & Bannister, 1994). As such, both accuracy and efficiency would clearly be improved where focussed on a small number of carefully selected and rigorously monitored sites, rather than being spread across a broad region in which low-level stocking is widespread.

To implement such an assessment of hatchery stocking, suitable release sites should first be sought, where habitat-types and site use are likely to be compatible to high survival of released lobsters, where the density of natural stock is well below the expected carrying capacity, and where little or no

previous stocking has occurred. To ensure released animals are genetically compatible to existing stock, wild broodstock should be sourced relatively locally, although not from immediately within or adjacent to release sites to minimise the potential occurrence of true false positives (natural stock locally recruited from the previous progeny clutches of hatchery broodstock). Since clutch-size is generally increased by increasing maternal size (Tully et al., 2001; Agnalt et al., 2007; Agnalt, 2008; Ellis et al., 2015c), $n_{\mathbf{Rel}}$:$n_{\mathbf{Par}}$ would be maximised by using the largest broodstock available. However, given that the offspring of larger females may experience heightened survival (Moland et al., 2010), a broodstock size distribution typical of that used in regular culture operations would better ensure recapture results were representative of standard releases. To ensure $n_{\mathbf{Rel}}$:$n_{\mathbf{Par}}$ is compatible with accurate parentage-based tagging, the production of hatchery juveniles should be maximised from a small number of high quality sibling clutches (e.g. <100), and maternal and paternal genotypes resolved. These juveniles should be released, save for a small number from each family retained as controls to enable the verification of parentage assignment (as well as for ecological studies, such as comparing the rates of growth and survival between released and on-grown hatchery cohorts). Annual monitoring of the release area for recaptures should commence a year after releases, at first using fine-meshed traps designed for prawns or modified to retain emerging phase lobsters (Wahle et al., 2013), and the multi-annual recapture profile of hatchery stock determined via parent-pair assignment over ensuing years.

Assuming complete resolution of parental genotypes, the maximum number of possible progeny genotype possibilities from each parent pair (pg_{max}) can be calculated as $pg_{max} = 4^n \times 3^n \times 2^n \times 1^n$, where 1-4 is the possible progeny genotypes at a locus (dependent on the zygosity and allelic sharing of parents), and n is the frequency at which that number of genotype possibilities is recorded across all loci. Across the 13 loci we amplified, the maximum possible number of genotype combinations derived from the 34 parent-pairs we tested ranged from 8.19×10^3 to 8.39×10^7 with a mean of 6.70×10^5, so it is likely that repeat recaptures could be identified with high probability. Nevertheless, designating sampled individuals with some visible mark (e.g. a ‘v-notch’ – Tully,

2001) upon re-release would help prevent repeated samplings of individuals during the same season and limit wasted genotyping effort.

Comparison to physical tagging

Using parent-pair assignment, we measured power to distinguish hatchery and natural stock that is well in excess of that provided by physical implant tags, given recorded rates of retention of visible elastomer (VIE: 88% per ≤3 moults – Neenan et al., 2015) and coded wire tags (CWT: 90% yr^{-1} – Agnalt et al., 2004), even in on-grown *H. gammarus* juveniles. The cost for a tagging a release batch of 1000 individuals with VIE is approximately £1100 (60ml elastomer kit with one month rental of air-injection tagging system; Northwest Marine Technologies, 2015a), but the equivalent cost for CWTs is ~£8000 (CWTs with handheld injector and detector; Northwest Marine Technologies, 2015b), without even factoring the potential excision of tags after preliminary detection by magnetometer (e.g. Bannister et al., 1994). Although VIE may superficially represent the cheapest option of monitoring released lobsters in the wild, these costs do not include expenses associated with the extensive on-growing required to tag successfully (Neenan et al., 2015), which may be avoided using parentage-based tagging. Compared to implant tags, parentage-based tagging also delivers the considerable benefit of providing data from which reproductive ecology can be studied (e.g. Gosselin et al., 2005; Ellis et al., 2015b) and genetic impacts on the target stock can be monitored, providing important information such as the trait heritability (Christie et al., 2012a; Abadía-Cardoso et al., 2013) and breeding fitness (Araki et al. 2007a; Miller et al., 2014) of hatchery stock in the wild. Crucially, unlike tagging by CWT or VIE, parentage-based tagging could facilitate the monitoring of hatchery lobsters released at any life-stage.

Potential to monitor other stock conservation measures

The same techniques we have applied here to distinguish released lobsters among wild stocks could be equally applicable in quantifying the effect of other fisheries management measures designed to preserve the recruitment contributions of individual breeders or clutches. Where ovigerous females are released as part of landing bans or v-notching schemes, maternal and egg

tissue sampling would facilitate the parentage-based identification of those progeny which recruit from these clutches to be recaptured. However, compared to the high site-fidelity of hatchery individuals released directly onto habitable seabed (Bannister et al., 1994), wild-hatched individuals undergo larval life-stages in nature so may be dispersed over a much greater area by oceanic currents. A relatively vast stock survey may therefore be required to yield recaptures, reducing the efficiency and greatly increasing the cost of parentage-based monitoring (Hauser & Carvalho, 2008) compared to applications within confined sites of hatchery stocking. Nevertheless, it may prove possible to quantify the contribution of a pool of candidate parents to local recruitment (e.g. Jones et al., 1999, 2005), and any comparison between different strategies (i.e. hatchery stocking vs. ovigerous female landing ban) would enable fisheries managers to identify the most effective stock conservation measures.

Conclusions

We have demonstrated the clear potential of parentage-based tagging to be a powerful method with which to identify hatchery-reared *H. gammarus* in the wild, and that 13 existing microsatellite markers are sufficient to practically eliminate false positives among natural stock when assignment is made to known-mated parent-pairs. Our assessment of parentage-based tagging provides a foundation for the methods' implementation in empirical assessments of hatchery stock size among admixed lobster populations, and of post-release performance in juveniles released immediately following the onset of benthic settlement behaviours. Parentage-based tagging can offer a more effective and informative tool for discerning natural and hatchery stock than traditional implant tags, and could support experimental designs that compare culture, conditioning, acclimation and release protocols in order to facilitate both the optimisation of lobster stocking, and the informed appraisal of the conservation benefit of hatchery release programmes.

Supplementary material

Table S1. Results of repeat computation runs and a run factoring no genotyping error. The number of hatchery recaptures, N_{HR}, as estimated via maternal and parent-pair assignment, and the overall error of assignment-estimated N_{HR}, from multiple runs of the same stock sample (true N_{HR} = 340) and in the absence of genotyping error (i.e. zero rate of mistyping/mutation for all loci).

COLONY run (true N_{HR} = 340 per 1000 stock sampled)	Maternal assignment			Parent-pair assignment		
	N_{HR} (of which false positives)	Error (% ± true N_{HR})	Power (100% ± Error)	N_{HR} (of which false positives)	Error (% ± true N_{HR})	Power (100% ± Error)
Run 1	375 (38)	10.30	89.7%	336 (1)	−1.18	98.8%
Run 2	376 (38)	10.59	89.4%	334 (0)	−1.76	98.2%
Run 3	371 (36)	9.12	90.9%	333 (0)	−2.06	97.9%
Run without genotyping error	360 (26)	5.88	94.1%	307 (0)	−9.71	90.3%

Table S2. Rates of false positives, false negatives and overall error. The percentage of natural and hatchery stock being falsely assigned and total resultant error rate via maternal and parent-pair criteria with varied hatchery contribution to the stock sample. Parent-pair false positive rates are uncorrected for the occurrence of natural individuals assigning to non-mated parent-pair combinations. Results from assignments with 0.0% anticipated rates of mistyping and mutation are denoted by *, and are not included in the calculation of means weighted for the contribution of the stock-type to each admixed sample.

Hatchery stock size (% of all sampled stock)	Maternal assignment			Parent-pair assignment		
	False positives (% of natural stock)	False negatives (% of hatchery stock)	Total error rate (positive + negative error %)	False positives (% of natural stock)	False negatives (% of hatchery stock)	Total error rate (positive + negative error %)
1	2.12	10.00	12.12	0.00	10.00	10.00
5	5.05	6.00	11.05	0.00	14.00	14.00
10	5.67	1.00	6.67	0.22	3.00	3.22
15	4.59	3.33	7.92	0.12	6.67	6.79
20	4.75	2.50	7.25	0.00	2.50	2.50
25	4.93	1.60	6.53	0.13	3.60	3.73
30	4.57	1.67	6.24	0.00	2.33	2.33
34	5.76	0.88	6.64	0.15	1.18	1.33
34*	3.94*	1.76*	5.70*	0.00*	9.71*	9.71*
Weighted mean	***4.61***	***1.93***	***6.53***	***0.08***	***3.29***	***3.36***

Table S3. The assignment results of natural individuals allocating to male and female candidates in the same computation run. The parental allocation results of the three natural stock individuals which were falsely allocated via parent-pair assignment in at least one stock sample. Where assignment occurred to a maternal ('M') or paternal ('P') candidate, this is designated as such with the numeric family code of the hatchery clutch (i.e. assignment to the mother of clutch 16 = 'M16'). 'None' denotes no assignment to any parental candidate, and 'X' denotes where any individual was not included in a particular stock sample. Parent-pair assignments are denoted in bold italics, and additionally underlined where the parent-pair were a known family combination of a hatchery clutch.

Individual	**Hatchery representation (% of sampled stock)**							
	1	**5**	**10**	**15**	**20**	**25**	**30**	**34**
Natural#651	None	M16	M16	None	M16	***P31, M16***	None	M16
Natural#786	None	P17	***P17, M18***	***P17, M18***	None	P17	P17	P17
Natural#967	M6	M16	***P12, M6***	X	M6	X	X	***P6, M6***

Table S4. The assignment results of hatchery individuals with genotypic mismatches to known parents. The assignment types of the eight hatchery stock individuals whose genotypes contained Mendelian mismatches to one or both parents. 'None' denotes no assignment to any parental candidate, and 'X' denotes where any individual was not included in a particular stock sample. Three individuals (Hatchery31.10, 32.10 and 34.10) were not detected as hatchery stock via either maternal or parent-pair assignment in any stock sample.

Individual (# Family.# ID)	**Mismatch**	**Hatchery representation (% of sampled stock)**							
		1	**5**	**10**	**15**	**20**	**25**	**30**	**34**
Hatchery28.9	Maternal	None	X	X	X	X	Parent-pair	Parent-pair	Parent-pair
Hatchery28.10	Either/both parent(s)	X	Maternal only	X	X	Parent-pair	X	Parent-pair	Parent-pair
Hatchery29.10	Maternal	X	X	X	X	X	Parent-pair	Parent-pair	Parent-pair
Hatchery30.10	Maternal	X	X	X	X	Parent-pair	Parent-pair	Parent-pair	Parent-pair
Hatchery31.10	Both parents	X	None	X	None	None	X	None	None
Hatchery32.10	Both parents	X	X	X	X	Paternal only	None	None	None
Hatchery33.10	Paternal	X	X	X	Maternal only	X	Maternal only	Parent-pair	Parent-pair
Hatchery34.10	Either/both parent(s)	X	Paternal only	X	X	Paternal only	Paternal only	Paternal only	Paternal only

Note – the mismatching offspring all come from highest numbered families and are the highest numbered individuals, although this is an artefact of the numbering of these families chronologically in the order in which their parentage was resolved by Ellis *et al* (2015c), and not any indication of bias in these samples from tissue collection or processing.

Chapter 7: Discussion

Whether for purposes of sustainable resource use or wildlife conservation, knowledge of the biology and ecology of pressured species is vital to inform management strategies which aim to ensure their persistence. This thesis has examined aspects of the reproductive and molecular ecology of European lobsters (*Homarus gammarus*) in order to improve our understanding of biological characteristics which, applied through strategies of fisheries management and hatchery stocking, are vital for the conservation of lobster populations. I have evaluated the performance of lobster stocking and identified important knowledge deficits which require resolution in order to sustainably manage lobster fisheries and appraise the value of hatchery interventions (Ellis, Hodgson, Daniels, Boothroyd, Bannister & Griffiths, 2015a [Chapter 2]). I have then addressed some of these fundamental information deficits, demonstrating that there is spatial variation in reproductive potential (Ellis, Knott, Daniels, Witt & Hodgson, 2015b [Chapter 3]) and genetic diversity (Ellis, Hodgson, & Griffiths, submitted [Chapter 5]) between lobster populations, an important indication that flexible, localised fisheries management is required to safeguard stocks. I have characterised the mode of fertilisation of individual broods within an important regional lobster stock, showing that clutches are sired by single rather than multiple males (Ellis, Hodgson, André, Sørdalen, Knutsen & Griffiths, 2015c [Chapter 4]). Finally, I have evidenced the suitability of genetic parentage assignment for the identification of released hatchery lobsters in the wild (Chapter 6), providing a pathway for the impacts of stock enhancement and restocking to be monitored more rigorously than is feasible using implant tagging techniques, so that the overall usefulness of stocking can be properly appraised. Because each chapter includes its own Discussion section in which potential implications of the results to hatchery interventions and catch regulation are presented, this chapter focuses on the brief synthesis of my findings and considers the future direction of conservation management and ecological investigation in the European lobster.

Reproductive ecology of lobsters

I have produced novel investigations showing that singly-sired clutches are the standard paternal fertilisation mode in an important regional fishery (Chapter 4), and have demonstrated that variations in female fecundity correlate with environmental gradients (Chapter 3). Both are potentially important findings for the management of lobster fisheries, though further investigation should be a priority of ecologists and fisheries biologists. I postulate that fecundity indices are driven by temperature range and that spatial variation in clutch size may indicate local adaptation among stocks (Ellis et al., 2015b). If adaptation were present it would suggest that population genetic structure may be more defined than the depiction produced by our own study (Chapter 5). While we related fecundity to a natural driver, it was also considered that this association might be a proxy-correlate evidencing an adaptive response to overfishing (Ellis et al., 2015b). This requires further investigative attention given the capacity for fisheries-induced evolution to extinguish adaptive genetic variation and reduce capture harvests (Kuparinen & Merilä, 2007). It is also imperative to test paternity in other areas of the species distribution to check whether populations elsewhere are similarly typified by singly-sired clutches. Paternity information would be a derivative of applications of parentage-based tagging, but the presence of multiple paternity would complicate genetic reconstructions of lineage (Ellis et al., 2015c). Further study of paternity would be hugely valuable even in isolation, to establish whether the link postulated between overexploitation and multiply-sired clutches in American lobsters (*H. americanus*) may also apply in *H. gammarus*, and whether paternal fertilisation contribution might therefore be a useful reference point with which to characterise the conservation status of lobster stocks.

Molecular ecology of lobsters

I have determined that fine-scale population structure appears to be absent throughout an Atlantic peninsula, but that natural connectivity is generally diminished with increasing spatial separation (Chapter 5). This finding is of vital importance in evidencing that current releases of hatchery-reared juveniles are

not incompatible with the localised distribution of genetic variation (Lorenzen et al., 2010). Nevertheless, given some limitations with this investigation that may have concealed more defined population structure existent at a broad geographic scale, further and more rigorous evaluation of population genetic structure in *H. gammarus* is essential in order to ensure that the spatial zonation of management units reflect those of biological populations, and that the true extent of natural dispersal can be ascertained (Kenchington et al., 2003; Waples et al., 2008; Reiss, 2009; Knutsen et al., 2015).

Rapid recent technological developments offer molecular ecologists greater power than ever before to detect patterns of population structuring (DePristo et al., 2011; Elshire et al., 2011). Such an application of high resolution genotyping has already been used to detect weak but important population structure in *H. americanus*, information crucial to the preservation of fishery yields (Benestan et al., 2015) that was not previously revealed by markers of the type that we have applied to assess *H. gammarus* population genetics (Kenchington et al., 2009). A similar genotype-by-sequencing approach is urgently required to define spatial genetic structure and identify isolated populations and barriers to gene flow across the range of *H. gammarus*. Such a study would benefit from international collaboration given the complexity of sourcing samples from the diverse range of nations, fisheries and stock statuses encompassed by the species' distribution. High resolution genotyping could also validate the lack of fine-scale spatial structure across the region of southwestern UK currently undergoing stock enhancement, as well as potentially improving the accuracy of applications of parentage-based tagging to discern cultured and natural individuals in the wild. Testing whether prolonged hatchery stocking can erode existing structure (e.g. Ayllon et al., 2006) or create genetic patchiness (e.g. Blanco-Gonzalez et al., 2015) is also important to confirm in lobsters. Although findings based in one region may not be applicable elsewhere, if improved methods are to be employed for this purpose, overall evaluation of hatchery stocking would be most impactful if the study encompassed stocks in Scotland and included sampling of the Orkney Islands, the site of by far the largest and most prolonged *H. gammarus* release program to date (Ellis et al., 2015a).

Creating sustainable lobster fisheries

Despite some problems with bycatch and ghost fishing (where lost pots continue to capture and cause mortality of animals, until their eventual degradation), static gear lobster fisheries have a relatively low ecosystem-level impact compared to many other marine fisheries which utilise destructive and indiscriminate gear types (Jennings & Kaiser, 1998). As such, the primary sustainability issue for European lobster capture fisheries is in ensuring that harvests of the target species are conducive to the long-term conservation of its wild populations.

Reported landings of *H. gammarus* have increased in recent years (Fisheries and Aquaculture Department, 2016a), and many fisheries stakeholders and even some fisheries scientists have argued that this demonstrates that productive stocks are healthy and sustainably fished. However, the observed rise in landings is less a reflection of widespread stock abundance, and more of a considerable increase in capture effort throughout Britain and Ireland, as well as the result of improved data collection (CEFAS, 2014; Fisheries and Aquaculture Department, 2016a). When assessed via temporal indices such as days-at-sea, fishing effort fluctuates between years and regions and shows no clear directional trend (CEFAS, 2014), and even appears to be decreasing when judged via vessel numbers (e.g. des Clers et al., 2014). However, when the number of pots fished or number of pot-lifts per year is taken into account, which better represent overall effort, fishing effort has increased substantially throughout England and Northwest France in recent years (CEFAS, 2014; des Clers et al., 2014), and probably in other fisheries without effort regulation, too. In areas without limitation on trap numbers, even some inshore fishers routinely deploy >1,000 pots during the peak capture season, almost an order of magnitude higher than was typical only a few decades ago. As a result, and in stark contrast to indices of fishery sustainability, even relatively abundant stocks are currently fished far beyond maximum sustainable yield (MSY) targets, and often in excess of biological reference points at which populations are considered highly vulnerable to collapse (CEFAS, 2014).

Given that the potential of pre-recruit lobsters (<87 mm CL: the current EU MLS) to sustain the abundance and fitness of *H. gammarus* populations is unknown, the widespread adoption of more stringent management guidelines, capable of supporting the long-term health of stocks without significantly endangering the fishing industry in the short-term, may well be required in order to safeguard lobster populations and industry livelihoods. Consultation and involvement of fishery stakeholders is crucial in developing measures to ensure fisheries sustainability (Beddington et al., 2007; Gardner et al., 2013; Henry & Johnson, 2015). European lobster capture effort typically occurs inshore but is often distributed across vast stretches of coast, and is exacted by a diverse array of stakeholders, from recreational part-timers, to commercial fishers whose livelihoods are principally supported by the species. Since management organisations often have scant resources with which to enforce restrictive legislation (i.e. size/effort/area limitations) that a significant fraction of fishers refuse to adopt, some conservation measures only perform as intended if they are widely accepted by the fishing industry. In place of authoritative control-based regulation, rights-based management offers a powerful alternative (Ostrom, 1999; Ostrom et al., 1999; Dietz et al., 2003) to preserve future harvests of other low-yield, high-value lobster species, by fostering ownership among fishers and rewarding conservative management of stocks (Gardner et al., 2013). An example such as the spatial usage rights co-operatively operated for a self-regulated regional spiny lobster (*Panulirus argus*) fishery in Caribbean Mexico (Seijo, 1993) may be transferrable with similar success to *H. gammarus*, although there are fundamental differences between the species and fisheries.

Overall, in order to gain the support of fishery stakeholders for conservation strategies and ensure that the most suitable regulations are in place, a number of management measures require rigorous assessment to enable empirical comparisons of their effectiveness. Theoretical investigations using mechanistic model frameworks to simulate fisheries management scenarios (e.g. Pelletier et al., 2009) offer a valuable tool to ascertain which regulation options most effectively balance the prosperity of capture industries with the protection of the stock (Lehuta et al., 2010; Simons et al., 2014), but such simulations are plagued by substantial uncertainties without robust empirical inputs specific to

the species targeted and fishing practices used (Gasche et al., 2013). A lack of information on lobster demography continues to be problematic, especially among early life-stages not encountered by fishers, but we have shown that identifying lobsters in the wild via genetic parentage assignment offers the chance to track the dispersal and survivability of natural individuals as well as hatchery releases (Chapter 6). Such studies should be prioritised to improve our understanding of the reproductive success and spatial reach of individual breeders, and provide data on the effect of specific regulatory strategies.

The role of lobster hatcheries

It is yet to be evidenced that hatchery stocking interventions can reliably make an economically worthwhile contribution to the productivity and sustainability of capture fisheries (Araki & Schmidt, 2010; Lorenzen et al., 2012). Nevertheless, a proof-of-concept has been established in the case of *H. gammarus*, for which cultivation methods are likely to avoid many serious drawbacks which have afflicted similar interventions in other species, and conclusions are largely positive that stocking may present a viable management approach (Bannister & Addison, 1998; Ellis et al., 2015a). Continued interest in hatchery stocking is understandable and founded in logic, if not in the available data from monitored release programs (e.g. Burton, 1993; Bannister et al., 1994; Cook, 1995; Bannister & Addison, 1998; Agnalt et al., 1999, 2004; Agnalt, 2008; Schmalenbach et al., 2011). However, more comprehensive investigation of the economic and ecological impacts of the large-scale release of cultured individuals into wild populations is urgently needed using the improved analytical methods now available, both in the general field of conservation (Laikre et al., 2010) and in the specific case of lobsters (Ellis et al., 2015a). Presently, the wisdom or success of hatchery stocking of *H. gammarus* simply cannot be properly evaluated given the limitations in the monitoring of past hatchery interventions and the lack of any data at all on some existing stocking initiatives. It is hoped that the findings of this thesis will make new and existing stocking operators aware of measures to mitigate potential negative impacts (e.g. Chapter 2), and will contribute to a renewed drive to rigorously monitor hatchery releases to appraise their benefit (e.g. Chapter 2, Chapter 6).

Despite the obvious potential for hatchery stocking to induce negative 'side-effects' by bypassing aspects of natural selection, current protocols for rearing *H. gammarus* reduce the scope for deleterious consequences because they do not involve many of the practices shown to be problematic in other stocked species (Ellis et al., 2015a). Female broodstock are obtained readily 'berried' with an egg clutch after capture from the wild, so sexual selection is unaffected and hatchlings from a group of hatchery clutches would have approximately the same genetic diversity as those from a same-sized group of wild clutches (since 'hatchery clutches' are essentially wild clutches). Nevertheless, even where hatchlings entering into the rearing process are comparable to wild conspecifics, juveniles comprising release batches may not be. The hypothetical development of a selectively-bred, domesticated broodstock strain may boost juvenile production but would be incompatible with the goals of stock enhancement and restocking due to the consequent bottlenecking of genetic diversity (Sekino et al., 2002, 2003; Araki & Schmidt, 2010) and the disparity in adaptive selection generated between wild and hatchery environments (Araki et al., 2007b, 2008; Araki & Schmidt, 2010; Christie et al., 2012a). Ecological conditioning of hatchery releases has barely been considered to date and offers a clear opportunity to increase the positive impacts of stocking (i.e. greater abundance of stock and spawning biomass) at existing levels of production (Ellis et al., 2015a).

The rapid rise in human population size and shift in trends of consumption, from local outlets to international markets, is creating global demand for seafood (Fisheries and Aquaculture Department, 2016b), including the European lobster. While the species has long been transported as a live export commodity, chiefly from the UK and Ireland to France and the Iberian peninsula, emerging markets, particularly those in East Asia (Uglow, 2010), threaten to create additional demand for the species which far exceeds current capture yields. Given the problematic histories of sustainably managing capture fisheries via traditional regulation (Pauly et al., 2002; Beddington et al., 2007) and of environmental and ethical efficiency in full-grow-out aquaculture (Goldburg & Naylor, 2005; Olesen et al., 2011), it seems reasonable that lobster hatcheries can have an important role to play in meeting projected expansions

in demand. As a seafood commodity, the quality of a hatchery-reared, wild-released lobster should match that of a natural individual, whereas aquaria-farmed or even sea-ranched lobsters might be comparatively inferior products. Nevertheless, if the potential for hatchery stocking to negatively impact wild stocks (Araki & Schmidt, 2010) is realised in *H. gammarus*, the rearing of cultured individuals would really only be compatible with contributing to lobster fishery sustainability where integrated into a realisation of a commercial aquaculture industry capable of absorbing increased market demand. The assumed effectiveness of natural regulatory strategies to prevent the overexploitation of wild fisheries should mean that lobsters can be managed sustainably without the need for hatchery stocking. However, until we are better able to understand and explain the performance of hatchery stocking and the fitness of cultured lobsters, hatcheries should not be written off as a viable tool to help conserve lobster populations, either via direct contribution to wild stocks or the creation of an alternative seafood resource.

Conservation management of exploited marine species

Because of the high cost of capital assets required to enter the industry, and the value of accumulated knowledge passed between generations, lobster fishing as a commercial endeavour often runs in families (Henry & Johnson, 2015). Though all generations naturally wish to pass on a healthy resource to the next (Seijo, 1993), where management and regulation fail to adequately protect a common resource, the interdependence of decision-making by competitive users (Schelling, 2006) mean that its overexploitation is the most likely outcome (Hardin, 1968, 1998). Ensuring that the exploitation of marine species is mitigated by effective conservation strategies is therefore critically important and, given the recognised responsibility of humanity to maintain global biodiversity (Wilson, 1989; Ehrlich & Wilson, 1991), the issues raised in this thesis have far greater resonance than the biology and management of the focal species. When considered in this context, extensive further research can be easily justified; *H. gammarus* can become a model organism for investigating the fisheries management and conservation biology of habitat-restricted, long-lived and iteroparous benthic species with pelagic larval dispersal.

Modern population ecology can contribute to ensuring that the exploitation of marine biota is sustainable in several ways. Molecular studies can elucidate the scale of connectivity and dispersal in order to dictate the approach to governance (Gaines et al., 2010), establishing the boundaries of management units based on demographic association. Direct studies of demography can be used to identify factors determining apparent stochasticity in recruitment, such as the effects of bottlenecks, density-dependence, and carrying capacity (e.g. Wahle & Steneck, 1991, 1992; Wahle & Incze, 1997; Steneck & Wahle, 2013; Davies et al., 2015). Finally, theoretical ecology can be applied to determine probabilistic outcomes of conservation management strategies via simulation modelling (e.g. Gardner et al., 2015). When combined, these disciplines can provide a powerful approach, indicating, by example, whether a proposed network of marine protected areas or restocking venture is likely to provide enough additional recruitment to support simple harvest regulation in adjacent areas, or whether only the ubiquitous application of a diverse and extensive capture regulation strategy will safeguard future yields.

Generally, the findings of studies in population biology should be more fully integrated into fisheries management (e.g. Hauser & Carvalho, 2008; Waples et al., 2009; Reiss et al., 2009). There is vast room for improvement in the way that advancements in scientific understanding are implemented into governance, but it is not only the responsibility of the policy-maker or the fisheries regulator to keep up to date with academic consensus (Dietz et al., 2003). For ecological research to contribute most effectively to conservation, a paradigm shift is required in the way that the scientific community measure success, with greater emphasis required on how investigative understanding impacts the world beyond academia (Bornmann, 2013). Traditional publication-based metrics are an important if flawed indicator of success within the scientific community (Seglen, 1997; Vanclay, 2011; Laurance et al., 2013; Bradshaw & Brook, 2016), and science which is impactful via citations may often also be impactful via real-world improvements to environmental policies. However, that the latter should be the ultimate motivation of conservation biologists is not always reflected in efforts to interpret and disseminate their research, key factors in its uptake by society and policymakers (Landry et al., 2001, 2003;

Priem et al., 2012; Priem, 2013). Encouraging progress in this matter is emerging through the increasingly multifaceted involvement of fisheries scientists (Dankel et al., 2016), through novel tools for researchers (e.g. Impactstory, 2016) and through the push towards openly accessible research findings (Antelman, 2004; Harnad & Brody, 2004) and data (Piwowar et al., 2007). Still, researchers should further prioritise the engagement of conservation managers, industry, governance and society as a whole with their scientific findings to maximise their contribution to real conservation.

It was my profound aspiration upon conducting the research which compiles this thesis that its findings be strongly linked to knowledge deficits inhibiting current efforts to conserve populations of European lobsters, and I hope that it can be applied over the coming years to further the creation of sustainable marine fisheries.

Figure 1. The author deploys a fishing pot carrying hatchery-reared juvenile lobsters to release them onto the seabed at Helford, Cornwall.

Appendix: The suitability of VIE tags to assess stock enhancement success in juvenile European lobsters

The following appendix is a supplementary chapter, a previous version of which has been submitted as an MSc thesis by S.T.V. Neenan to the University of Exeter (2012). I co-supervised Sarah and was senior author on the publication arising from this work. This study was the first carried out as part of this collection of research, and is included for perspective in this thesis as it was a catalyst to our considering the potential of a genetic method to distinguish hatchery- from natural-derived stock.

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Abstract

Assessments of stock enhancement programmes for European lobsters (*Homarus gammarus*) require mark-recapture analysis of stocked individuals. However, established tag technology is deemed unsuitable for extensive use by many current lobster hatcheries, particularly upon the early juvenile stages. We tested the suitability of fluorescent Visible Implant Elastomer (VIE) tags for use in five-month-old juvenile lobsters. Three treatment groups comprising 348 cultured lobsters in total were used to examine survival, growth and tag retention, and to assess mobility, shelter-use and moulting behaviours. Tagging had no significant effect on lobster survival, growth, mobility, shelter-use or moult frequency. Survival over seven weeks was 75% among lobsters tagged with two elastomers, 76% in those with one elastomer, and 74% among untagged controls. Mortality during moulting did not increase in tagged (6%) compared to untagged lobsters (9%). We found no evidence that VIE tags cause any negative effects that would be expected to inhibit survival upon wild release, but tag loss had reached 12% in both tagged treatments after seven

weeks and showed no sign of abating. Our study suggests that VIEs effectiveness in discerning cultured lobsters long after wild release may be limited when used in smaller juveniles.

Introduction

In response to reduced supply caused partially by collapse in other established fisheries throughout Europe, the European lobster, *Homarus gammarus* (Linnaeus, 1758), has become increasingly prized by UK fishers as a seafood export commodity. As a result, attempts have been made to locally enhance lobster stocks by rearing lobster larvae through their planktonic life-stages to produce and release large numbers of benthic dwelling juveniles (Bannister et al., 1994). The collapse of commercially-fished populations became a major driver for not only re-stocking efforts in those areas (e.g. Norway – Agnalt et al., 1999, 2004; Germany – Schmalenbach et al., 2011), but of stock enhancement of comparatively abundant fisheries around the UK (Bannister et al., 1994). The rationale for current cultivation of *H. gammarus* is typical for stock enhancement schemes, caused by some severe wild population depletions and well-functioning rearing technology but with a comparative lack of biological and ecological data (Svåsand et al., 2004). However, stock enhancement activity should be partnered with quantitative analyses of its impacts on wild populations (Lorenzen, 2005), and efforts to appraise stocking as a method to enhance lobster fisheries have been carried out in the UK (Bannister et al., 1994), Norway (Agnalt et al., 1999) and Germany (Schmalenbach et al., 2011). The ability to tag juvenile lobsters allows the generation of data vital to the creation of sustainable management models, such as growth, migration and survival rates, and enables stock enhancement programmes to quantify their impact on the wild population (Blankenship & Leber, 1995). Current, full-time lobster stock enhancement programmes in the UK comprise of a single operation based in northern Scotland (Orkney Lobster Hatchery) and another in southwest England (The National Lobster Hatchery). Neither has yet undertaken quantitative impact assessments of their stocking work, principally due to the prohibitive costs and sub-optimal development of available tagging and monitoring technology, and because previous studies have established a

proof-of-concept (Bannister et al., 1994).

The most commonly-used method of identifying individuals within populations are artificial external tags, which are attached to the exterior of the organism from where they can be visually logged by samplers (CATAG, 2000). Because such tags would be lost during moulting in marine crustaceans, externally-visible tags to mark adult crabs and lobsters have been developed that are able to persist across exoskeleton moults via attachment to the underlying musculature, often using barbed anchors (dart tags), or by passing through the abdomen (streamer tags). However, some of these tags are too physically invasive for use on small juveniles, causing mortality by tagging injury or prolonging the duration of moulting (Linnane & Mercer, 1998). Internal tags have been developed that can be inserted into the body cavity or musculature, where they should not be lost during moulting or inhibit moult success. Any physical trauma of tagging is minimised by tags being small and injected into transparent adipose tissue or just under the skin. Subcutaneous tags reduce abrasion and/or tag loss, are less conspicuous to predators, and do not hinder foraging or predator evasion (Malone et al., 1999). This aids long-term fitness and survival, and therefore is appropriate for use in stock enhancement programmes. It also enables operators to infer findings of mark-recapture analyses to untagged stocked individuals or the wider natural population.

The internal coded wire tag (CWT) - a tiny magnetised stainless steel wire embossed with a numeric code - has been successfully implanted into the pereiopods (walking legs) and abdomen of juvenile clawed lobsters (Linnane & Mercer, 1998; McMahan et al., 2012). This has facilitated the identification of hatchery-reared lobsters in the wild up to fourteen years after implantation as three-month-old juveniles, and has been used in some of the most encouraging assessments of marine stocking to date, with cultured lobsters forming the majority of the landings following intensive re-stocking of a highly depleted population around Kvitsøy in southwestern Norway (Agnalt et al., 2004). However, CWTs have to be removed from the organism in order to retrieve the identification number of the tag, potentially destroying the animal and inhibiting the collection of longitudinal, multiple-recapture datasets. The detection of CWTs also requires the use of specialised scanning equipment, so the use of

CWTs does not enable fishery stakeholders to provide cheap and widespread recapture reports, and estimates suggest that tag loss routinely exceeds 25% and can reach 47% (Agnalt et al., 2004). Some hatcheries, like that on Kvitsøy, utilised juveniles for release at approximately 12 months old (approx. Stage XII, 20-25mm carapace length [CL]) because survival rates both in the wild, and from tagging methods, are increased in juveniles of this size (Agnalt et al., 1999). However, on-growing in this way creates additional economic challenges to stocking programs and can hinder efforts to make lobster stocking a financially-efficient fisheries management tool.

Subcutaneous coloured markers have been retained successfully in aquatic species to enable mark-recapture trials, including acrylic paint (trout – Kelly, 1967); liquid latex (flatfish – Riley, 1966); small pieces of plastic imprinted with alpha-numeric codes (rockfish & lingcod – Buckley et al., 1994); and the Visible Implant Elastomer (VIE) tag (shrimp – Godin et al., 1996). The VIE tag (Northwest Marine Technology Inc., Shaw Island, Washington, United States) was developed specifically for tagging large batches of small or juvenile fish (Willis & Babcock, 1998). The VIE tag is a two-part liquid chemical compound; a fluorescent coloured elastomer and a translucent catalyst which, when mixed, cure into a pliable biocompatible solid (Jerry et al., 2001). Injected into transparent or translucent tissue as a dot or line, it is visible in ambient sunlight and enhanced when exposed to UV light (Jerry et al., 2001; Reeves & Buckmeier 2009). By tagging various body locations and using multiple combinations of colours, it is possible to compose unique markers to identify batches or individuals (Uglem et al., 1996). Where animal size restricts the insertion of multiple VIEs, the tags cannot readily provide individualised data from large samples, only more generalised release-cohort data. However, continuous data collection is possible as tag detection causes no harm to the lobster upon recapture, and further VIE tags can be added as lobsters grow between recaptures (Schmalenbach et al., 2011).

Previous studies have already shown VIE tags to be an effective tool for marking juvenile crustaceans of very small body size (e.g. 2 g giant freshwater prawns – Dinh et al., 2012), and several studies have assessed VIE tags in cultured European lobster juveniles. Of 25 hatchery-reared juvenile *H.*

gammarus tagged with VIE at approximately one year old by Uglem *et al* (1996), retention after three moults was 100% and total survival was 92%. Used upon large on-grown juveniles, VIE tags have already been successful assessing the wild survival of hatchery-reared lobsters, with 5,400 one-year-old *H. gammarus* tagged and released around the North Sea island of Helgoland from 2000 to 2005 (Schmalenbach et al., 2011). By 2009, 14% of the juveniles released into the semi-enclosed harbour had been recaptured, as well as 3% of those released into open coastal habitats (Schmalenbach et al., 2011). Linnane & Mercer (1998) also tagged hatchery-reared juvenile *H. gammarus* with VIE, but while they deemed them successful in seven-month-old lobsters, they concluded their use was inappropriate for those smaller juveniles of 6-7 weeks old due to high mortality and obvious tag migration. Identifying the optimum lobster age/size for tagging – young enough to alleviate on-growing expenses but large enough to exhibit very high survival from tagging and tag retention – would be of significant benefit to lobster stock enhancement operations.

Our study concentrated on the suitability of VIE for use in a five-month-age *H. gammarus* that better represents the typical age and size at release from current UK hatcheries (11mm CL ±4mm). In controlled experiments, we measured the impact of single- and double-tagging on tag retention, survival, growth, shelter-use, mobility, and the frequency and success of moulting. We hypothesised that tag retention and post tag survival would be high, and that tagging would have no influence upon lobster growth, shelter-use, mobility, or the frequency and success of moulting. Additionally, we hypothesised that double tagging would have no influence on the measured criteria when compared to single tagging.

Materials and methods

Study species

The investigations were carried out during 2012 at the National Lobster Hatchery (NLH), Padstow, England. Of a total sample of 348 five-month old juvenile *H. gammarus,* of approximate equal size (11mm CL, ±4mm), derived from rearing facilities on-site and having hatched from mixed-sourced, wild-

caught broodstock, 116 lobsters were randomly assigned to three treatment groups - double tagged, single tagged and untagged controls. All juveniles were reared in separate 5.5cm x 11cm cells of mesh-bottomed trays in two shallow raceway tanks. Raceway flow-rates were 10 L hr^{-1} (±2 L hr^{-1}) from a recirculation system with modular filtration, which maintained seawater at 17^{o}C (±3^{o}C), pH 7.7-8.0 and salinity at 34gmL^{-1} (±1gmL^{-1}). Twice daily, juveniles were fed a 5mg food pellet formulated specifically for hatchery-reared lobsters. The raceways were cleaned daily using a fine mesh net to remove faeces, food waste, and any mortalities.

Tagging procedure

Lobsters were tagged with fluorescent yellow and fluorescent red Visible Implant Elastomers (Northwest Marine Technology, 2015a); of the 6 fluorescent colours available, yellow and red were the most noticeable colours as identified in natural light and before tag insertion by a panel of 30 marine biologists and fishermen. Yellow elastomer was used for the single-tag treatment, and an additional red tag used for the double-tag treatment. Both single- and double-tagged treatments were employed since double-tagging juvenile lobsters had not been previously assessed, despite individualised or cohort designation requiring multiple VIE locations and/or colours, and double-tagging having shown to more than double both the period (Gonzalez-Vicente & Diaz, 2012) and quantity (Bjornsson et al., 2011) in which recaptures are reported for other marine species. A handheld manual injector (0.5cc hypodermic syringe) was used to ventrally insert the yellow VIE tags into the first abdomen segment (that nearest the thorax) and the red VIE tags into the second segment, avoiding the pleopods (Appendix Figure 1C).

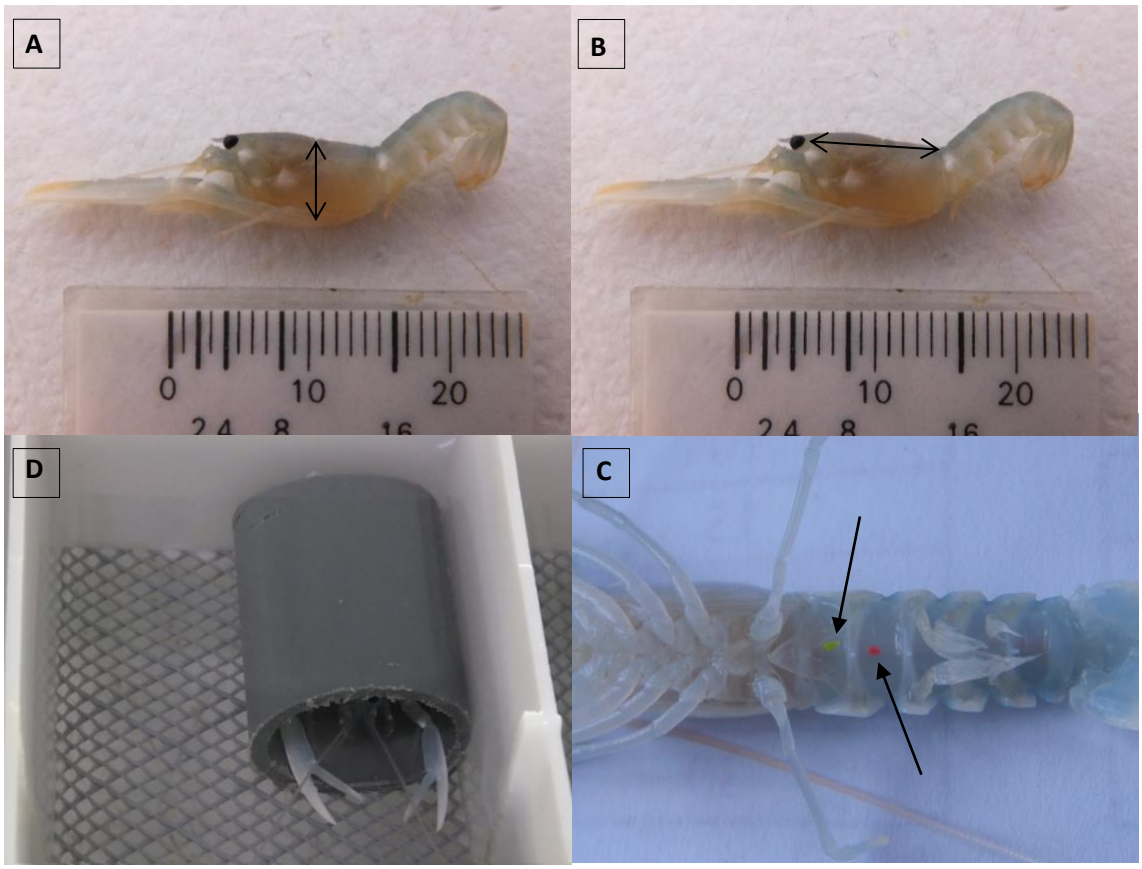
Assessment criteria

The assessment criteria were chosen as the most important indices to evidence the performance of VIE-tagged lobsters in intrinsic (tag retention, survival), physical (growth, moult frequency/success) and behavioural (mobility, shelter-use) measures, so that comparisons to untagged conspecifics may reveal any detrimental effects of tagging in traits linked to short-term survival and long-term recruitment after release. Tag retention and survival from tagging are

fundamentally important aspects of a tag's performance and had been previously assessed as the key indices of previous VIE tagging trials on juvenile lobsters (Uglem et al., 1996; Linnane & Mercer, 1998). Growth rate and the frequency of successful moults are important factors in juvenile recruitment and had been previously assessed in trials of CWTs on lobster juveniles, but not in trials using VIE (McMahan et al., 2012). Effects of tagging on shelter-use and mobility in juvenile lobsters had not been previously assessed, although shelter-seeking behaviour strongly influences the ability of hatchery-reared lobsters to avoid predation upon wild release (van der Meeren, 2000; Ball et al., 2001), and high mobility is advantageous to lobsters in foraging for food, seeking shelters and mates, and for predator evasion (Phillips, 2006).

Survival, tag retention and growth experiment procedure

Mortalities were recorded daily, and tag retention was recorded weekly for seven weeks. In double-tagged lobsters, each tag was considered independently (i.e. if both tags were lost from one individual then two losses were recorded). To assess the influence of juvenile size, tag retention was analysed by allocating lobsters into four groups of similar carapace length (CL) (Appendix Figure 3). CL size groups, from smallest to largest, included 83, 126, 38 and seven lobsters; uneven group compositions reflected the range of sizes in the five-month-old juveniles in this study. The weight, carapace depth (CD), and carapace length of each individual was recorded every two weeks to assess growth. To obtain morphometric measurements, juveniles were dried carefully with a paper towel and photographed against a scale. ImageJ software was used to calculate the carapace depth (Appendix Figure 1A) and length (Appendix Figure 1B). To measure weight, juvenile lobsters were blotted dry using paper towels and weighed to the nearest 0.001g using an electronic balance (Acculab VICON electronic top loading balance, Sartorius AG, Göttingen, Germany).



Appendix Figure 1. Lobster growth measurements, shelter use and VIE tag positioning. A-D, clockwise from top left; A) Carapace depth, CD, was measured from the cervical groove to the ventral extreme of the carapace. B) Carapace length, CL, was measured from the eye-socket (the base of the rostrum) to the posterior dorsal margin of the carapace. C) VIE tag locations and colours in the abdomen of a double-tagged lobster. D) A juvenile lobster utilising a shelter provided during behavioural trials. Photos: A, B, D; Sarah Neenan. Photo C: Charlie Ellis. All courtesy of The National Lobster Hatchery, UK.

Movement experiment

To test whether the VIE tags had an adverse effect on the lobster's ability to move, two mazes were built to assess mobility in 60 lobsters randomly selected from each treatment group. Each lobster was only tested once due to time restraints. This experiment was conducted after the 7 week tag retention, growth, and survival study in weeks 8 and 9. Mazes were created using the same raceway trays used for rearing cells, with plastic walls and mesh bottom, with an area of 27.5cm (w) x 44.0cm (l) x 5.5cm (h). The mazes were submerged to a depth of 4cm in tanks supplied by the same recirculation system as the rearing vessels, with the lobsters allowed to acclimate for three minutes upon entering the maze. Water flow (10 L hr^{-1}, ±2 L hr^{-1}) ran from the top right corner of the maze to the bottom left, where the lobster started the trial.

Focal individuals were filmed navigating the maze for 10 minutes. The underlying grid of 5.5cm x 5.5cm cells allowed calculations of distance and speed. Distance (cm) was measured by the number of squares the lobster moved through, while mean speed (ms^{-1}) was calculated from the total distance moved during 10 minutes. Mazes were chosen instead of large open tanks to assess motility, to maximise wall-space and minimise open spaces, as lobsters prefer to move thigmotactically along the walls of a tank (Mehrtens et al., 2005). No cues were administered to encourage locomotion, so all movement was presumed to be exploratory behaviour.

Shelter experiment

To test shelter use, 30mm (±5mm) sections of 20mm gauge UPVC pipe was placed into rearing cells to provide a makeshift burrow (Appendix Figure 1D). 60 lobsters from each treatment group were tested 9 weeks after tagging, being recorded as either 'in' or 'out' of the shelter every 10 minutes for one hour, over three hours daily. The shelters were introduced to the rearing trays 1 hour before the first trial started to allow juveniles acclimation to a novel object and recovery from disturbance, and for 10 minutes prior to the second and third trials. Flow rate was 10 L hr^{-1} (±2 L hr^{-1}). Water temperature and time of day were controlled for as random effects during statistical analysis. Experiments were conducted in indoor rearing facilities with both artificial and natural lighting, so the three daily trials were run in a morning, afternoon and evening test to account for any diurnal fluctuations in activity.

Moulting experiment

Moulting was monitored throughout the 7 week trial to investigate whether the presence of a tag had an effect on the frequency and success of the exoskeletal moult. Successful moulting was evidenced by the remains of all or part of a shed exoskeleton. Moult induced mortality was recorded where lobsters showed evidence of having undertaken a moult (e.g. lifted carapace) but had died during the process. Unsuccessful moults were recorded among individuals who became entangled during moulting, and were logged as such until they completed the moult or died.

Statistical analyses

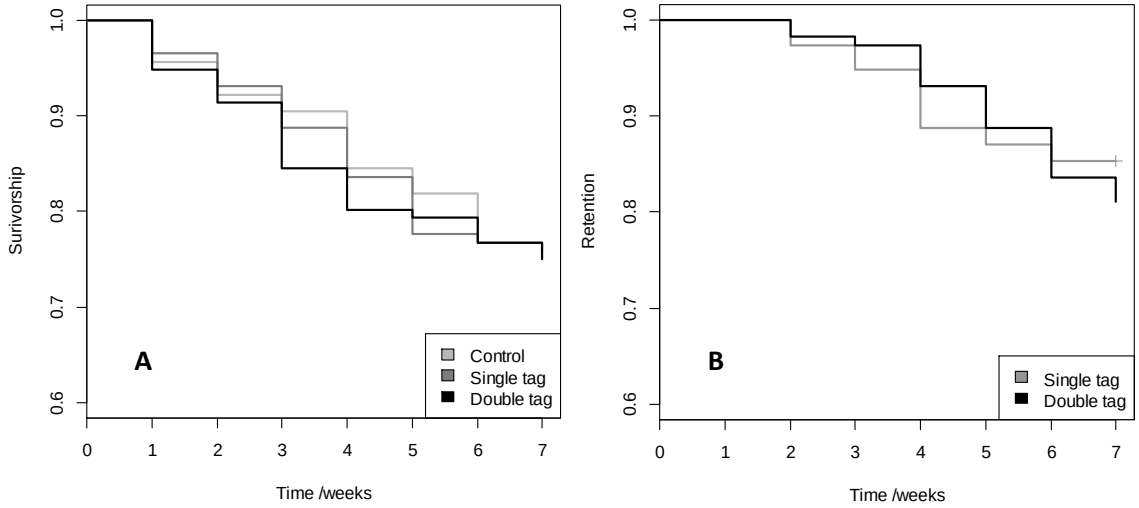
For the shelter-use trial, proportional data was collected and generalised linear modelling was used with binomial error structures. Censored survival analyses ('survreg' and 'survfit') were used to analyse both the lobster survival and tag retention data. Survival analyses censored individuals that survived beyond the end of the trial: censoring allows these individuals to contribute information on rates of survival, but not timing of death. Tag retention analyses censored individuals that died during the course of the trials: these individuals contributed information on tag retention prior to death, but not timing of loss. When looking at the influence of carapace length on tag retention, the lobsters were divided into 4 groups of equal length (2mm). Mobility and speed in the movement experiment were tested with general linear modelling, with water temperature, the maze the lobster was placed in, and the carapace length being controlled for as covariate effects. Growth analyses used mixed effects modelling to compare growth rates among tagging treatments, accounting for repeated measures by absorbing the random slope of lobster size through time for each individual. This model used likelihood ratio tests, which are χ^2 distributed, to test the significance of tagging treatment on weekly rates of increase in weight, carapace length, and carapace depth. Chi-squared contingency tables were used to assess moulting behaviour. All statistical tests were carried out in R 2.14.1 for Windows (R Core Team, 2012).

Results

Survival, tag retention and growth

There was no significant difference in survival among single tagged, double tagged and untagged lobsters (χ^2_2=0.506, *P*=0.983, Appendix Figure 2A), and no significant difference in tag retention between single-tagged and double-tagged treatments (χ^2_1=0.600, *P*=0.453, Appendix Figure 2B). Lobster size (CL) did not show a statistically-significant influence on tag retention (χ^2_3=1.65, *P*=0.65, Appendix Figure 3). Survival was 74-76% in all treatments, and overall tag retention, independent of tag treatment, was 88% to seven weeks among surviving lobsters. No significant difference between treatments was found in

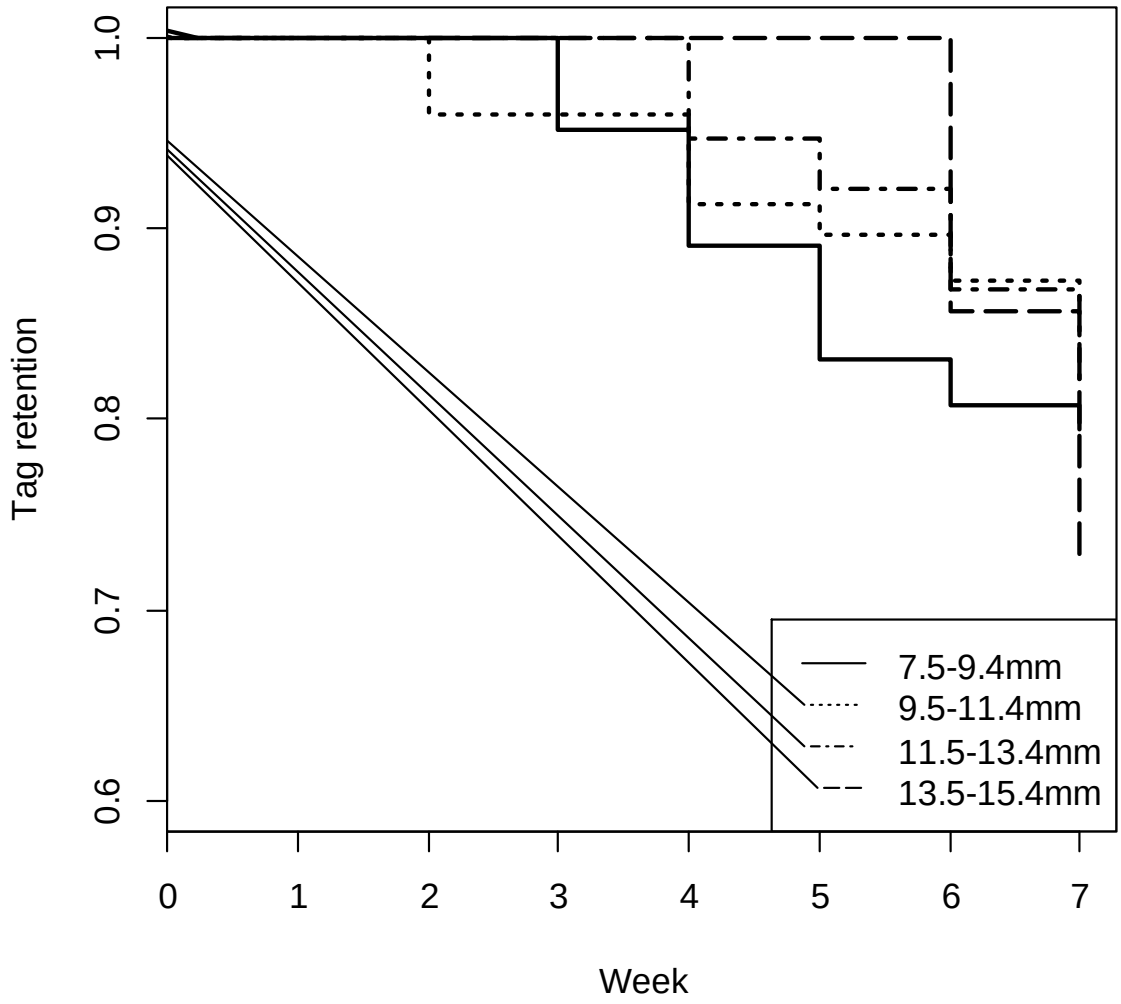
growth rate as evidenced by either carapace length (χ^2_2=0.04, *P*=0.98), carapace depth (χ^2_2=0.41, *P*=0.81), or weight (χ^2_2= 3.89, P=0.14, Appendix Figure 4). Appendix Figure 4a suggests an identifiable reduction in weight increase in the two-tag treatment, but this was not significant.



Appendix Figure 2. Lobster survival and tag retention over the study duration. A) Survivorship of lobsters in each treatment over 7 weeks with censoring (taking into account that lobsters survived the study). Treatment groups had no effect on survival rate (χ^2_2=0.506, *P*=0.983). B) Tag retention over 7 weeks with censoring (taking into account that the tag was still in place when the experiment ended). Tag loss between treatments was non-significant (χ^2_2=0.600, *P*=0.453).

Moulting

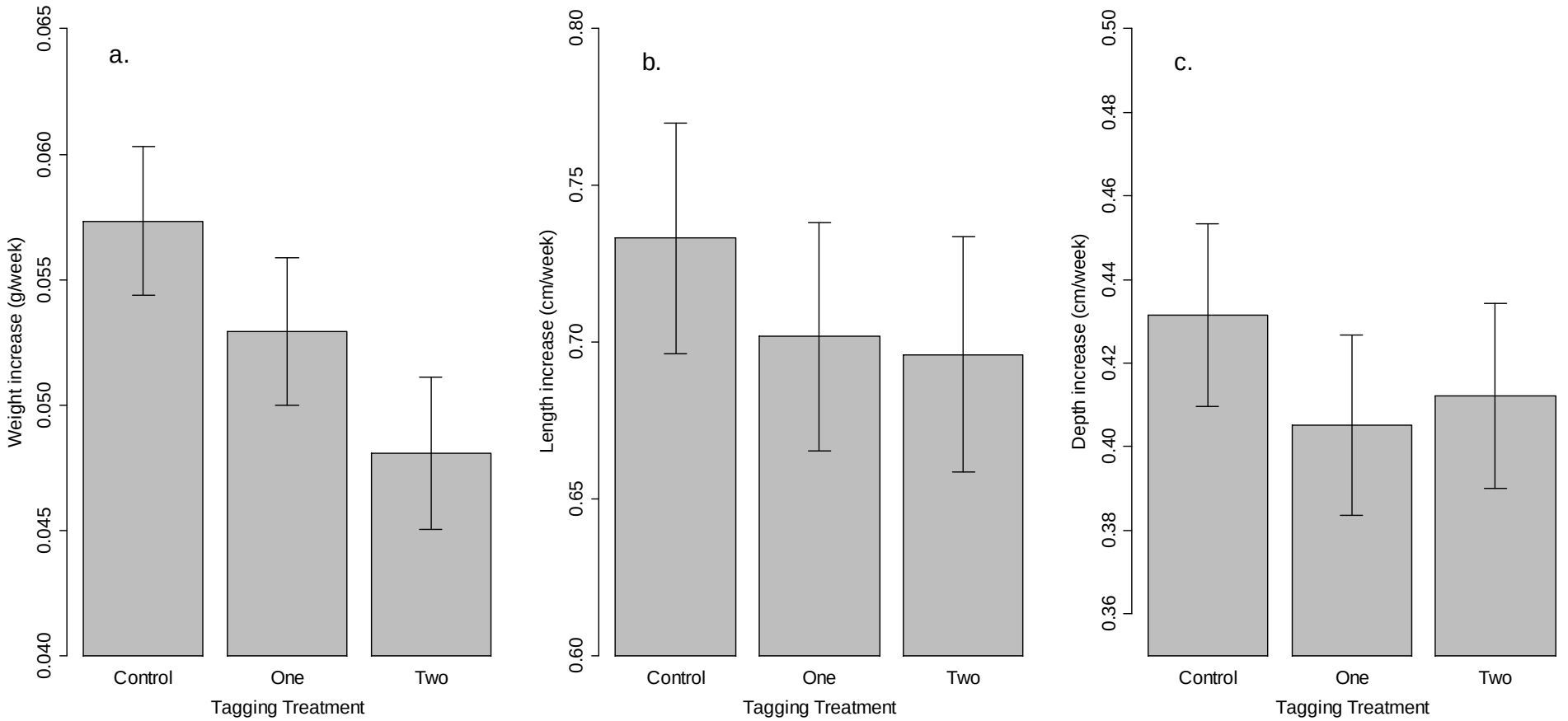
Tags did not influence mortality attributed to becoming stuck during moulting (χ^2_2=0.237, *P*=0.888). Mortality during moulting did not differ significantly between tagged (6%) and untagged lobsters (9%). Successful moulting occurred as soon as 2 days after tagging, and occurred once in 231 lobsters and twice in 57 lobsters during the seven week study period, with only 2 lobsters moulting a third time, and 58 lobsters not moulting throughout the study period. Moult frequency did not show any significant difference between controls and VIE tagged lobsters (*P*=0.199, Fishers exact test). The number of moults per treatment group (consisting of 116 lobsters) per week also showed no significant difference among all three treatments (*P*=0.846, Fishers exact test).



Appendix Figure 3. Tag retention by size cohorts. Tag retention for difference sized lobsters (grouped by carapace length at start of study). Length of lobster did not have a significant influence on the retention of the tag (χ^2_3=1.76, *P*=0.62).

Movement and Sheltering

Neither speed nor distance travelled varied significantly among treatment and control groups (speed, $F_{2,161}$=1.39, *P*=0.253; distance travelled, $F_{2,161}$=1.300, *P*=0.275). Mean speeds and distances were 1.31ms^{-1} across 7.85m over the 10 minute trial for single tag group, 1.26ms^{-1} across 7.56m for double tag group, and 1.16ms^{-1} across 6.97m in control, untagged juveniles. Shelter-use behaviour was not significantly different between the tagged and untagged groups (χ^2_2=0.047, *P*=0.977). The double-tag group utilised the shelters on average 63% of the time, and the single tag and control lobsters on average 62%.



Appendix Figure 4. Mean growth of lobster treatment groups. Mean growth rate per week of lobsters (in (a) weight; (b) carapace length; (c) carapace depth) in each treatment. Error bars show standard errors, having accounting for random variation in slopes for each individual lobster.

Discussion

The analyses of all trials indicated that the VIE tag had no significantly negative effects on the survival, mobility or moulting process of juvenile lobsters. There was no overall significant difference in growth by carapace length or depth, or growth rate between the tagged and untagged treatment groups. Our results endorse the conclusions of previous studies on the use of VIE in aquatic crustaceans which found no extensive tag-associated mortality (e.g. Uglem et al., 1996; Jerry et al., 2001; Woods & James, 2003). Although this trial led to lower overall survival rates than previous trials of VIE in clawed lobster juveniles, this could have been caused by sub-optimal rearing conditions, and neither rearing conditions nor mortality rates differed among treatment groups. After seven weeks, tagged juveniles had experienced very similar levels of mortality (24% in single tagged; 25% in double tagged) as that of untagged controls (26%). For a tag to be suitable for use in stock enhancement monitoring it must have minimal negative effects on representative survival, and this is particularly important in *H. gammarus*, which usually take 3-4 years to reach the size (>50mm CL) at which they are routinely caught by fishers (Addison & Bannister, 1994). In mark-recapture studies, an increase in mortality caused by tagging would result in inaccurate population estimates (Woods & Martin-Smith, 2004). Similarly, in the assessment of enhanced stocks, excessive tag-induced mortality would lead to under-estimations of survival.

High mobility is advantageous to lobsters in foraging for food and shelter, finding mates, and evading predators (Phillips, 2006), so it was a positive sign that VIE tagging did not influence this. Lobster mobility, as assessed by speed and distance travelled, did not differ significantly among either the control lobsters or the tagged treatments. Shelter-seeking behaviour also strongly influences lobster survival in the wild, particularly in early benthic phase (EBP) juveniles unable to defend themselves from predation by demersal fish (van der Meeren, 2000). EBP American lobsters, *H. americanus*, are known to be restricted to shelter-providing habitats upon benthic settlement (Wahle & Steneck, 1991), and the ability of hatchery-reared *H. gammarus* to find shelter upon release strongly influences survival in the wild (Ball et al., 2001). VIE

tagging did not influence the frequency of shelter-use, with all treatment groups using shelters on a mean of 62-63% of occasions. It would seem that low levels of shelter seeking behaviour amongst all treatment groups might be a result of the differences between the wild and hatchery environments; even adult *H. americanus* only leave the safety of a shelter during the night (Karnofsky et al., 1989), but cultured lobsters have neither the need for, or experience of, extensive shelter-use during hatchery rearing.

For tagging to be an effective tool in monitoring stock enhancement programmes, the tag needs to be retained by the organism and easily detectable by observers (Woods & Martin-Smith, 2004). Rates of tag loss did not vary between single- and double-tagging treatments, and the similarity in survival rates and tag retention between single and double tag treatments supports the potential of different tag combinations being used to create a high number of discrete release cohorts, or multiple tagging to mitigate loss of individual tags.

Calculating an expected rate of tag loss is vital to the reliability of any data generated by mark-recapture sampling, tag loss constitutes a removal from the tagged population that is indistinguishable from mortality or emigration (Gonzalez-Vicente & Diaz, 2012). The rate of tag loss did not seem to decrease during the study, raising concerns for the validity of VIEs as a long-term marker of juvenile *H. gammarus*. Assuming the rate of recorded tag loss (12% in 49 days) continued unchanged in a pool of individuals tagged with a single VIE, less than 40% of tags would remain after 1 year, only 15% would remain after 2 years (around the age that lobsters are first thought to be retained by conventional fishing traps), and just 2% of tags would remain after 4 years (the earliest age at which hatchery-reared lobsters have reached minimum landing sizes – Bannister et al., 1994). Unless this rate of tag loss plateaued within the first year, the relatively rapid loss of tags in this age group clearly presents an insufficient time-frame in which to appraise long-term recruitment via recapture sampling, even when the option of multiple tagging of individuals is considered. Our study period of seven weeks, limited by facility availability at the hatchery, was insufficient to reveal longer term trends in tag retention and detectability.

Tag loss in this study was probably a consequence of the small size of the juveniles; the quantity of elastomer injected was not standardised due to the size variation in the juveniles, and was generally relative to individual size, so smaller juveniles probably received less elastomer. This may have increased the chances of tag loss or migration during moulting. Juvenile size also made tag placement extremely challenging, and misplacement of tags could have exacerbated tag migration or loss; Woods & James' (2003) study on VIE tag retention in adult spiny lobsters found that tag orientation was important to ensure the tag remained intact, and that tags injected transversely across the muscle fibres were more prone to fragmentation. The sizes of the *H. gammarus* juveniles tagged in this study made it unmanageable to ensure this recommended tag-muscle alignment.

Interestingly, tag retention in this study was markedly lower than that experienced by Linnane & Mercer (1998), who achieved 99-100% tag retention over 3 moults with VIE in seven-month-old juveniles of 12-16mm CL. Size was more highly variable among our lobsters, ranging from 7-15mm CL, so it may be that the occurrence of relatively small lobsters in our trial increased the incidence of tag loss; smaller animal size limits the volume of VIE that can be implanted which can affect tag retention and detectability (Dinh et al., 2012). The analysis of tag retention against carapace length was shown to be non-significant but this relationship may have shown greater influence had the animal size groups contained a more even distribution of lobster numbers (CL size groups, from smallest to largest, included 83, 126, 38 and 7 lobsters). Any further investigation of VIE tags' viability for mark-recapture monitoring in hatchery-reared juvenile lobsters should attempt to ascertain the optimal size for VIE insertion by assessing tag retention and ease of detection from different size classes. Growth rates are highly variable in *H. gammarus* (Bannister et al., 1994), so size, rather than age, is likely to be a better indicator of readiness for tagging. We found no significant effect of carapace length on tag retention; future studies would be advised to attempt to standardise elastomer quantity per individual by volume, or volume per individual size (CL), so that this effect can be tested more rigorously.

Of those tags retained throughout this study, red tags were always highly visible

to the naked eye, while yellow tags often needed the addition of UV light to confirm their presence. This conforms to the findings of Buckley *et al* (1994) who found that red tags were more detectable than green or yellow tags in the marine environment. The ventral abdominal musculature in which VIE markers were inserted remains translucent throughout the lobster's life, so the visibility of the tag should be unaffected by the colouration of the lobster, though may be limited by the tag size and any tag migration. While both the tag colours we used were prominently enhanced under UV light, it is debatable whether such small tags would have remained readily identifiable in the adult life stages of lobsters. Long-term trials are required to assess the tag's suitability to assign continuous identification of hatchery origin until fishery minimum landing sizes, and to estimate incidence of tags being lost or becoming otherwise undetectable to ensure recapture datasets may be calibrated accordingly.

Among other technologies, genetic markers offer an alternative to physical tagging as a method of detecting stocked individuals among wild conspecifics. While long generation times and concerns over genetic bottlenecking oppose the development of a genetically marked strain used in some stocked fish (e.g. Atlantic cod – Jørstad et al., 2004b), parentage assignments via genotyping offer more potential. If a sufficient quantity of polymorphic loci were isolated and characterised, these could be used to genotype hatchery broodstock so that subsequent genetic assays among the enhanced population would allow the establishment of hatchery origin to those individuals expressing the genotypes of hatchery parents. So long as monandrous mating was standard in the host population, and assuming brood females had no previous mating events with the male who had fertilised the egg clutch that was spawned in the hatchery, genetic markers could allow the creation of parentage assignments using maternal DNA derived from body tissues and paternal DNA deduced from fertilised eggs (Ferguson, 2002). Genetic markers have important advantages over physical tags; tag loss is effectively eliminated, the release of younger (Stage IV or V) juveniles could facilitate comparative survival analyses to identify the optimum size at release, deleterious genetic effects on the target population can be measured, and the construction of multi-generational lineages provides the opportunity to assess the contribution of stocking over the

longer term. Microsatellite DNA markers in particular have already proven successful in distinguishing cultured individuals from wild equivalents among mixed populations of Steelhead trout, *Oncorhynchus mykiss* (Christie et al., 2012a) and Black seabream, *Spondyliosoma cantharus* (Jeong et al., 2007). For *H. gammarus,* microsatellite DNA profiling has already been successfully used to assign maternal parentage to individuals of both wild and cultured maternal origin (Jørstad et al., 2005a), and twelve polymorphic microsatellite markers developed for the European lobster have been made publicly accessible (André & Knutsen, 2010). But while genetic profiling may increasingly present the most effective option to identify hatchery-reared crustaceans and appraise stocking success, a physical tag of requisite reliability still offers important benefits that genetic markers do not, such as the social and economic advantages to the hatchery of a tag that may be identified directly by fishers.

Conclusions

This study found no negative effects of VIE tags on juvenile lobsters in terms of survival, the physical indicators of growth or moult success and frequency, or in the behavioural indicators of mobility and shelter-use. No effect of VIE tagging was found that suggests it negatively influences lobster survival, nor any attribute by which long-term wild recruitment could be expected to be limited in comparison to untagged conspecifics. Similar studies found comparable results for VIE tags in larger juvenile lobsters, but achieved higher tag retention (Uglem et al., 1996; Linnane & Mercer, 1998). This study suggests that VIE tagging causes no developmental limitations, but that for 5-month old *H. gammarus* juveniles, tag loss could significantly hamper its suitability as a mark-recapture tool in lobster stock enhancement assessment. On the basis of this research, the VIE tag has proven to be suitable for use in five-month-old juvenile lobsters in aspects of physical and behavioural development, which were not found to be decreased by tagging in any attribute that inhibited survival in the short-term or would be expected to do so over the long-term. However, we have also shown that, in this size range (11mm CL, ±4mm), the VIE tag may be unsuitable for long-term identification purposes as a tool for facilitating monitoring of the impact of stocking in lobster populations.

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