

MOLTOOLS: a workshop on “Molecular tools for monitoring marine invasive species”

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The 2011–2014 European Community project VECTORS (“Vectors of Change in Oceans and Seas-Marine Life, Impact on Economic Sectors”) was an integrated, multidisciplinary European project which aimed to improve our understanding of how environmental anthropogenic drivers are impacting European marine ecosystems now and in the future. VECTORS investigated how these changes may affect marine biodiversity and ecosystem functioning, the range of goods and services provided by the oceans, the resulting socio-economic impacts and some possible future scenarios for mitigation and adaptation.

The VECTORS Work Package WP2.1 (*Mechanisms of Change: Outbreaks and Invasives*) was committed to identifying mechanisms of species outbreaks and invasions and introduction vectors as both

symptoms and drivers of change in European marine ecosystems. VECTORS particularly focused on native outbreak-forming species (OFS) and invasive alien species (IAS) (Box 1) to deliver new information on (1) IAS and OFS distribution in European seas, (2) stochastic and deterministic drivers of invasions and ecophysiological features boosting regime shifts of marine communities, (3) sources and vectors of bioinvasions, and (4) consequences of bioinvasions, in terms of impact on ecosystem functioning and services. These studies provided a substantive contribution to the understanding of the ecological conditions in which outbreaks and alien invasions are more likely to occur, and the determination of critical thresholds/tipping points that mark alternative regime shifts in community structure and organization.

The progressive reduction in the inherent costs of molecular data over the past 20 years, along with rapid recent technological and analytical developments, has resulted in molecular methods becoming an inescapable component of investigation on biological invasions, from simple biodiversity assessment to historical reconstruction of invasion histories. Within this context, 60 participants from 14 nations gathered in Lecce, Italy on 12–14th September 2012 for MOLTOOLS (Fig. 1), a dedicated workshop to discuss and promote the use of molecular tools for monitoring and understanding marine invasive species. The workshop was designed to address several key questions regarding mechanisms and management

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Box 1 Outbreak-forming species and invasive alien species

Outbreak forming species (OFS) are indigenous or alien species with invasive potential, undergoing pulse-like, periodic exponential population growth (usually days to months) during which they have an impact on biological diversity, ecosystem functioning, socio-economic values or human health. Non-indigenous species (i.e. alien, exotic, non-native) (NIS) are introduced organisms outside their natural (past or present) range of distribution, and outside their natural dispersal potential, which might survive and subsequently reproduce, threatening biodiversity

Species of unknown origin that cannot be ascribed as being native or alien are termed *cryptogenic* species. In many cases, non-indigenous species do not harm the regional ecology and economics. However, in certain cases, non-indigenous species can become “invasive” species and have enormous and long-lasting impacts on the region. The European Union’s Marine Strategy Framework Directive (2008/56/EC) lists NIS as Descriptor 2 for the evaluation of Good Environmental Status (GES) of marine ecosystems, stating that NIS should be at levels that do not adversely alter the ecosystem. Invasive alien species (IAS) are a subset of established NIS able to spread in invaded regions and impact biological diversity and/or ecosystem functioning, socio-economic values, or human health

of outbreaks and invasions: (1) what species are there, and where do they originate from? (2) What are the main vectors of invasions and the connectivity among invading populations? (3) How can evolutionary adaptation be recognized? (4) What are the best available early warning tools to detect and keep on control the spread and success of IAS and OFS species?

In all, the workshop offered the opportunity to bring together world experts to jointly review scientific issues, existing methods, technical difficulties associated with development of sophisticated tools, and to consider the usefulness of molecular technologies for various applications of monitoring biological invasions and outbreaks. As a corollary, the workshop encouraged the usage of molecular markers and protocols across international research groups engaged in monitoring OFS and IAS in the marine environment. The workshop included 2 days of invited oral presentations, a poster session and several group discussions. The entire workshop was recorded on a DVD produced by CONISMA—Italian National Consortium for Marine Sciences, for distribution to VECTORS participants and other European scientific institutions. The workshop organization also included a young scientist support program, which promoted



Fig. 1 Banner and logo of the MOLTOOLS workshop. Artwork: Alberto Gennari. Concept and design: Fabio Tresca and Stefano Piraino

participation of 20 international students/young scientists by covering their registration and accommodation fees.

This special section contains nine keynote articles stemming from the workshop. Already published elsewhere, a tenth paper (Rius and Darling 2014) is rooted in discussions that occurred during the MOLTOOLS workshop. In all, these papers reflect the main themes of the symposium, in forms of large-scale reviews, perspectives, or experimental methodological papers, and they illustrate how molecular tools are being utilized to address a number of pressing questions associated with marine invasions, from

identifying invaders and understanding invasion histories to measuring their potential impacts.

Three papers in the current issue capture some of the most widely adopted contemporary applications of molecular tools. Broadly speaking, these applications involve the reconstruction of invasion history across multiple spatial and temporal scales—determination or confirmation of the identity of invasive species, discovery of the likely sources of invasive populations, and inference of demographic patterns associated with establishment and spread of those populations. For instance, Azzurro et al. (2015) describe a genetic study of the dusky sweeper *Pempheris rhomboidea*, a Lessepsian invader of the Mediterranean Sea. The authors demonstrate how molecular genetic tools can be applied to clarify the identity of morphologically cryptic invasive populations, and further conduct phylogenetic reconstructions of the historical range of *P. rhomboidea* and its congeners. Bayha et al. (2015) combine similar phylogenetic analyses with population genetics to examine the phylogeography of the globally distributed invasive jellyfish *Mnemiopsis leidyi*. This study reveals the power of extensive global datasets (large sample sizes and multiple genetic mitochondrial and nuclear genetic markers) to resolve broad-scale patterns of introduction. Such comprehensive genetic studies of high profile invaders may provide invaluable information to managers regarding risks posed by particular vectors and pathways of introduction. The results of Bayha et al. further reinforce the importance of utilizing multiple genetic markers, particularly given the common observation of discordance between markers with different evolutionary histories (e.g. mitochondrial vs. nuclear markers). Finally, Dawson et al. (2015) offer an even broader illustration of the utility of phylogeographic and population genetic analyses for understanding invasion dynamics. By looking at genetic patterns across multiple species of scyphozoan jellyfish, the paper critically examines attempts to infer the environmental drivers of bloom events. The authors observe that recent investigations of global environmental patterns have largely not been conducted on the spatial scales appropriate for understanding the population dynamics of jellyfish blooms (as indicated by genetic analyses), thus leaving critical gaps in the knowledge and assumptions underpinning our understanding of these events and consequently hampering our ability to manage them effectively.

Two other papers in this issue attempt to expand on these traditional applications of molecular tools. Rius et al. (2015) note that despite considerable attention paid to marine invasions in European seas, genetic research has generally been limited to descriptive analyses of genetic variation and inference of invasion history, with relatively little attention paid to evolutionary mechanisms that may drive the success (or failure) of invasive populations. In reviewing recent genetic studies of marine invasive species they recognize the challenges associated with employing readily available genetic information to ask questions more directly relevant to the outcomes of introduction events: Can populations adapt rapidly to novel environmental pressures? What role does phenotypic plasticity play in establishment success? What is the link between standing genetic variation, population bottlenecks, and fitness? Rius et al. relate these challenges, in part, to the notoriously tenuous association between neutral genetic diversity and functional traits. Nevertheless, they describe an increasingly sophisticated analytical toolbox that may enable researchers to begin addressing such questions. Elsewhere in the current issue, Tepolt (2015) describes how the adoption of such tools has begun to elucidate the genetic patterns associated with adaptive responses by invasive populations. While studies of adaptation in the course of marine invasions remain relatively rare, Tepolt offers a comprehensive review of evolutionary studies in marine systems that have employed genetic and molecular analyses to understand adaptation, and illustrates the promise of such analyses for better describing the mechanisms underlying the success of marine invasive species. Noting several recent studies revealing local adaptation by invasive populations, Tepolt cautions against the assumption that all populations of a single invasive species will respond similarly to novel environmental conditions, suggesting that management strategies may need to recognize shifting risk profiles associated with complex evolutionary trajectories. Explicit consideration of evolutionary processes in the assessment of invasion risk or the design of management approaches may be particularly important given wide recognition of the strong selective forces imposed by changing climatic conditions.

Both Rius et al. and Tepolt recognize the important role that new technologies—specifically high throughput sequencing (HTS, also known as next-generation sequencing of NGS)—will likely play in advancing our

understanding of the evolutionary drivers of invasion success. Two other papers in the current issue also investigate the importance of those technologies, though in slightly different contexts. Comtet et al. (2015) describe how HTS enables the expansion of DNA barcoding—the widely utilized identification of organisms based on a short “barcode” segment of their genome—to DNA “metabarcoding,” which rapidly and inexpensively characterizes entire biotic communities. In contrast to the functional analyses described by Rius et al. and Tepolt, metabarcoding provides unique opportunities for describing, potentially in great detail, changes in marine biodiversity associated with biological invasions. Comtet et al. review the promise of metabarcoding at multiple stages of the invasion process, from early detection of introduced propagules of high risk marine pests, to monitoring expanding invasion fronts and even tracking the effectiveness of control strategies. While it recognizes the potential utility of HTS for understanding marine invasions, Comtet et al. is also a cautionary tale. A number of significant challenges exist before HTS can be seamlessly and confidently applied in decision-making contexts. Technological and analytical solutions are still being sought to problems such as the interpretation of rare sequences, the correlation of positive DNA detections with distributions of live organisms, and the inference of relative or absolute organism abundance. Despite these issues, Comtet et al. offer convincing evidence for the role of metabarcoding approaches in future management scenarios, particularly given the overlapping interests of managers in tracking invasions and more broadly understanding changes in marine biodiversity. Manzari et al. (2015) offer a slightly different perspective on the metabarcoding approach, describing a study utilizing HTS to illustrate changes to the marine microbiome associated with a massive invasive jellyfish bloom in a coastal lagoon. The application of genetic tools for microbial community analysis has a long history, and avoids some of the challenges facing similar applications for metazoan communities. But Manzari et al. reminds us that paying attention to microbiota may offer crucially important insights regarding invasion processes in the sea; the authors not only reveal the potential power of HTS for understanding broad-scale ecological impacts of marine invasions, but also illustrate the important role that microbial communities may play in mediating such impacts.

Mollo et al. (2015) provide us with another important reminder: while nucleic acids (DNA and RNA) may dominate discussions of molecular tools, there are a host of other informative biomolecules worthy of our attention. Secondary metabolites may play critically important roles in determining establishment success by mediating interactions between introduced organisms and novel environmental conditions or native biota. In many cases these molecules may also directly underpin the major impacts of marine invasions, from negative effects on resident species to public health hazards. Mollo et al. review the central, but often neglected, importance of chemical ecology in understanding these dynamics and liken the approach to waging “chemical warfare” to combat particularly damaging marine invasions. They illustrate how, by exploiting information on marine natural products (MNPs), chemical ecologists may offer novel alternative management options, including even the possibility of control measures based on commercial harvesting for desirable bioactive compounds.

A final paper in this special issue (Darling 2015) explores the complex interactions between scientists (particularly geneticists) and managers of marine and aquatic invasions, highlighting some of the persistent challenges to translating research outcomes into effective management and policy. Through both investigation of recent literature on genetic studies of aquatic invasive species and solicitation of expert opinion on the role of genetic information in decision-making, Darling reveals a number of obstacles contributing to the “knowing-doing gap” in invasion biology. Such a gap has been shown to plague numerous disciplines in conservation science, and Darling attempts to trace the roots of this problem in the specific context of the molecular tools discussed elsewhere in this issue, offering perspectives on approaches for improving the ability of those tools to inform management decisions.

It is clear from the papers in this special issue, alongside a burgeoning literature in this journal and elsewhere, that molecular approaches will remain a critical component of the invasion biology toolkit, and will likely only grow in utility as technologies and analytical methods continue to improve. Beyond illustrating the existing and potential applications of these tools, the contributors to this issue further remind us of the importance of developing research efforts, generating information and communicating science in

ways that enable decision-makers to better design management and policy strategies for reducing the risks posed by invasive species. The success of efforts such as the VECTORS-supported workshop from which these papers were developed will ultimately be reflected in the degree to which the molecular tools described in these pages contribute to more effective management of marine invasions.

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