Biol Invasions
DOI 10.1007/s10530-012-0279-9

ORIGINAL PAPER

Genetic diversity and reproductive mode in two non-native hydromedusae, *Maeotias marginata* and *Moerisia* sp., in the upper San Francisco Estuary, California

Mariah H. Meek · Alpa P. Wintzer · Nicole Shepherd · Bernie May

Received: 16 September 2011 / Accepted: 3 July 2012

Abstract Reproductive strategy can play a significant role in invasion success and spread. Asexual and sexual reproduction may confer different advantages and disadvantages to a founding population, resulting in varying impacts on genetic diversity and the ability to invade. We investigate the role of reproductive mode in two species of non-native hydromedusae (*Maeotias marginata* and *Moerisia* sp.) in the San Francisco Estuary (SFE). Both species can reproduce asexually and sexually. We employed 7–8 microsatellite markers to determine overall genetic diversity and to investigate contributions of asexual and sexual reproduction to the populations. We found both species had high levels of genetic diversity (Average $H_E = 0.63$ and 0.58, Number individuals sampled = 111 and 277, for *M. marginata* and *Moerisia* sp. respectively) but also detected multiple individuals in clonal lineages. We identified the same clones across sampling locations and time, and the index of asexual reproduction (R) was 0.89 for *M. marginata* and 0.91 for *Moerisia* sp. Our results suggest both species maintain high population genetic diversity through sexual reproduction, in combination with asexual reproduction, which allows rapid propagation. In addition, we conducted genetic sequence analyses at the ribosomal ITS1 marker, using samples of *Moerisia* sp. from the SFE and *M. lyonsi* from Chesapeake Bay. We found 100 % sequence similarity showing that *Moerisia* sp. in the SFE and Chesapeake Bay are the same species. The two hydromedusae studied here possess the means to propagate rapidly and have high genetic diversity, both of which may allow them to successfully adapt to changing environments and expand their invasions.

Keywords Jellyfish · Clonal · Biological invasion · Genetic diversity · San Francisco Bay · Reproduction

M. H. Meek (✉) · N. Shepherd · B. May
Department of Animal Science, University of California, One Shields Ave, Davis, CA 95616, USA
e-mail: mhmeek@ucdavis.edu

A. P. Wintzer
Center for Watershed Sciences, University of California, One Shields Ave, Davis, CA 95616, USA

Introduction

Understanding factors affecting the abundance of invasive species is critical for evaluating their impacts on invaded ecosystems and predicting their ability to spread into new areas. Reproductive strategy can play a significant role in invasion success and spread (Roman and Darling 2007). Asexual reproduction may confer an advantage by allowing invading species to avoid demographic constraints and permitting reproduction and population growth in areas where densities are too low for successful mating, such as at the edge of the invasion (Barrett and Richardson 1986; Lambrinos 2001; Sakai et al. 2001). Additionally, the potential for preservation of co-adapted gene

Published online: 11 July 2012

complexes or "general purpose genotypes" with high phenotypic plasticity and broad tolerance ranges is greater under asexual reproduction (Baker 1965; Lambrinos 2001). In a review of the characteristics of invasive plants, Kolar and Lodge (2001) found significant and positive relationships between the ability to reproduce vegetatively and the completion of an invasion transition (Introduction → Establishment → Invasion). The potential importance of asexual reproduction in invasion success has also been demonstrated in the extensive spread of the New Zealand mud snail (*Potamopyrgus antipodarum)* in North America. This invasion primarily involves one clonal line that has rapidly expanded through apomictic parthenogenesis, demonstrating that entirely asexual lineages can be successful invaders (Dybdahl and Kane 2005). Another example is *Daphnia pulex* in Africa, where a previously diverse population of *D. pulex* has been replaced by a single non-native clonal line from the Americas (Mergeay et al. 2006). However, asexually reproducing invasive species may sometimes be less successful compared to those reproducing sexually (e.g. Lambrinos 2001). Low amounts of genetic variation, due to founder effects, combined with limited recombination and a reduced ability to purge deleterious mutations in asexual reproduction may limit a species' capacity to expand and adapt to heterogeneous environments. This may decrease a species' ability to persist in an ecosystem over space and time (Fisher 1930; Hughes and Stachowicz 2004; Sakai et al. 2001).

Genetic diversity in an introduced population can be retained through the effects of multiple introductions, sexual reproduction, and rapid population expansion (Dlugosch and Parker 2008; Novak and Mack 2005; Roman and Darling 2007; Wares et al. 2005). If a population can reproduce asexually while maintaining high genetic diversity through the introduction of multiple clonal lines, occasional sexual reproduction, or both, it is possible adaptive evolution can occur quickly, increasing the level of invasiveness (Facon et al. 2006). The long life of genotypes due to the presence of multiple individuals per genotype in asexual populations can allow the retention of genetic diversity compared to strictly sexual populations where unique genotypes will be removed through the effects of genetic drift (Burnett et al. 1995; McFadden 1997). Burns (2008) found that the ability to reproduce both asexually and sexually can increase invasibility of some plant families. Novak and Mack (2000) looked at genetic diversity in the invasion of the apomictic vine *Bryonia alba.* They found high clonal diversity within and among invaded populations of *B. alba*, which they attributed to multiple invasions of different clonal lines and the presence of sexual reproduction. Additionally, Roman and Darling (2007) found, in a review of introduced populations, that invasive populations with decreased genetic diversity compared to the native populations were often species that could reproduce asexually. This suggests that asexual reproduction, coupled with occasional sexual reproduction, may ameliorate the negative effects of population bottlenecks experienced at the beginning of an invasion.

We investigate the relationship of reproductive mode and genetic diversity in the invasion of two species of hydromedusae (*Maeotias marginata* and *Moerisia* sp.) in Suisun Marsh in the San Francisco Estuary, CA. Both species are purported to be native to the Ponto-Caspian region and have established additional non-native populations in the Napa and Petaluma Rivers, CA, and the Chesapeake Bay, US (Mills and Rees 2000; Rees 1999; Rees and Gershwin 2000). These species possess a bipartite life cycle, with a free swimming, dioecious medusa phase and a benthic asexually reproducing polyp phase (Mills and Rees 2000; Mills and Sommer 1995; Rees and Gershwin 2000). The medusae are sexually reproducing free-spawners, whose planula larvae settle out in the benthos to develop into polyps. Polyps asexually reproduce juvenile medusae or more polyps. This diverse life history allows the possibility that the populations are entirely clonal, producing both polyps and medusae exclusively through asexual reproduction, or it is possible they employ both reproductive modes for their propagation.

Very little is known about the population dynamics of these species or their impact on the SFE ecosystem. There has been a recent decline in important planktivorous fish species in the San Francisco Estuary (SFE), and competition with invasives is listed as a likely cause (IEP 2008; Sommer et al. 2007). *Maeotias marginata* and *Moerisia* sp. are included in the list of possible competitors, as they prey upon the same food resources as the juvenile fishes (Rees and Gershwin 2000; Wintzer et al. 2011). *Maeotias marginata* was first noticed in the system in the 1950s and *Moerisia* sp. was introduced as late as 1993, both likely by

fouling on ship hulls or ballast water exchange (Mills and Rees 2000; Mills and Sommer 1995; Rees and Gershwin 2000). The identity of *Moerisia* sp. in the SFE has not been determined, due to morphological variation that complicates classification (Rees and Gershwin 2000). The possibility for cryptic species necessitates reliable species identification to provide the foundation for understanding these invasions (Dawson and Jacobs 2001; Holland et al. 2004). Additionally, understanding their life history characteristics and genetic diversity will be important to developing effective control strategies (Burdon and Marshall 1981), in addition to providing insights into the role of genetic diversity and reproductive strategy in an invasion.

In our study, we had three aims:

1. Quantify genetic diversity of non-native *M. marginata* and *Moerisia* sp. populations in Suisun Marsh
2. Determine the level of clonality in each species and compare the polyp and medusa phases within species
3. Determine if the *Moerisia* sp. found in the SFE is the same species as the non-native *Moerisia lyonsi* found in the Chesapeake Bay (Calder and Burrell 1967).

We were able to successfully sample the *Moerisia* sp. polyp and medusa populations, but were only able to find *M. marginata* medusae. *M. marginata* polyps have not yet been found in the field. Therefore, we compare polyp and medusa phases only with *Moerisia* sp.

Methods

Sample collection and processing

We collected 111 medusae of *M. marginata* and 171 polyps and 150 medusae of *Moerisia* sp. from three sites in Suisun Marsh (Fig. 1). Suisun Marsh is a brackish water system covering approximately 34,000 ha in the upper SFE. One-third of the area is formed by a system of tidally influenced sloughs, with margins of tules and reeds, while the rest is a combination of diked marshlands and upland grasslands (Meng and Matern 2001). We sampled *M. marginata* medusae in September 2007 and *Moerisia* sp. in August and September 2007, during the height of the seasonal jellyfish blooms. At each of the three locations, we horizontally towed a conical zooplankton net (230 μm mesh, 0.5 m diameter, 2 m length) from a boat in the middle of the sampled sloughs. We pulled the net at mid-water column depth for 5 min in the shallower Boynton Slough and we towed the net in the bottom and top half of the water column for 2.5 min each in the deeper Suisun and Montezuma sloughs. Additionally, to collect the larger, more mobile *M. marginata,* we towed an otter trawl (1 m × 2.5 m mouth, 5 m length, 35 mm stretch graded to 4 mm stretch mesh at the cod end) for 5 min in Boynton Slough and 10 min in the larger Suisun and Montezuma Sloughs. After collection, all samples were immediately preserved in 95 % ethanol.

To collect polyps, we deployed one settling plate array at each site. We constructed settling plates out of 100 cm^2 sheet PVC that had been roughed on each side with an orbital sander. Each array was made of six plates hung at each of two levels, 0.5 m below the water's surface and 0.5 m above the bottom, with three plates hung vertically and three horizontally per level. In August and September 2007, we collected the plates monthly, preserved them in 95 % ethanol, and redeployed new plates. For both the medusa and polyp samples, we changed the ethanol preservative after 1 week, in order to maintain a high ethanol content and sample integrity.

We examined *Moerisia* sp. medusae and smaller-sized *M. marginata* medusae under a dissecting microscope, removed any debris, inspected the manubrium to ensure no organisms were inside the bell or gut cavity, and pulled off all tentacles. We removed the tentacles to ensure we were sampling single individuals and that tentacles from other individuals were not tangled with the individual we were sampling. Each individual medusa was placed in its own sampling tube containing Qiagen Gentra Puregene Lysis Buffer for future DNA extraction. For the larger-sized *M. marginata* individuals, we used forceps and a scalpel to cut off a portion of the bell, excluding the tentacles and any gut contents, and placed the tissue in a sampling tube containing Qiagen Gentra Puregene Lysis Buffer.

To sample polyps for genetic analyses, we plucked individual polyps from the settling plates and placed each in its own sample tube containing Qiagen Gentra Puregene Lysis Buffer. To ensure a sampling of the

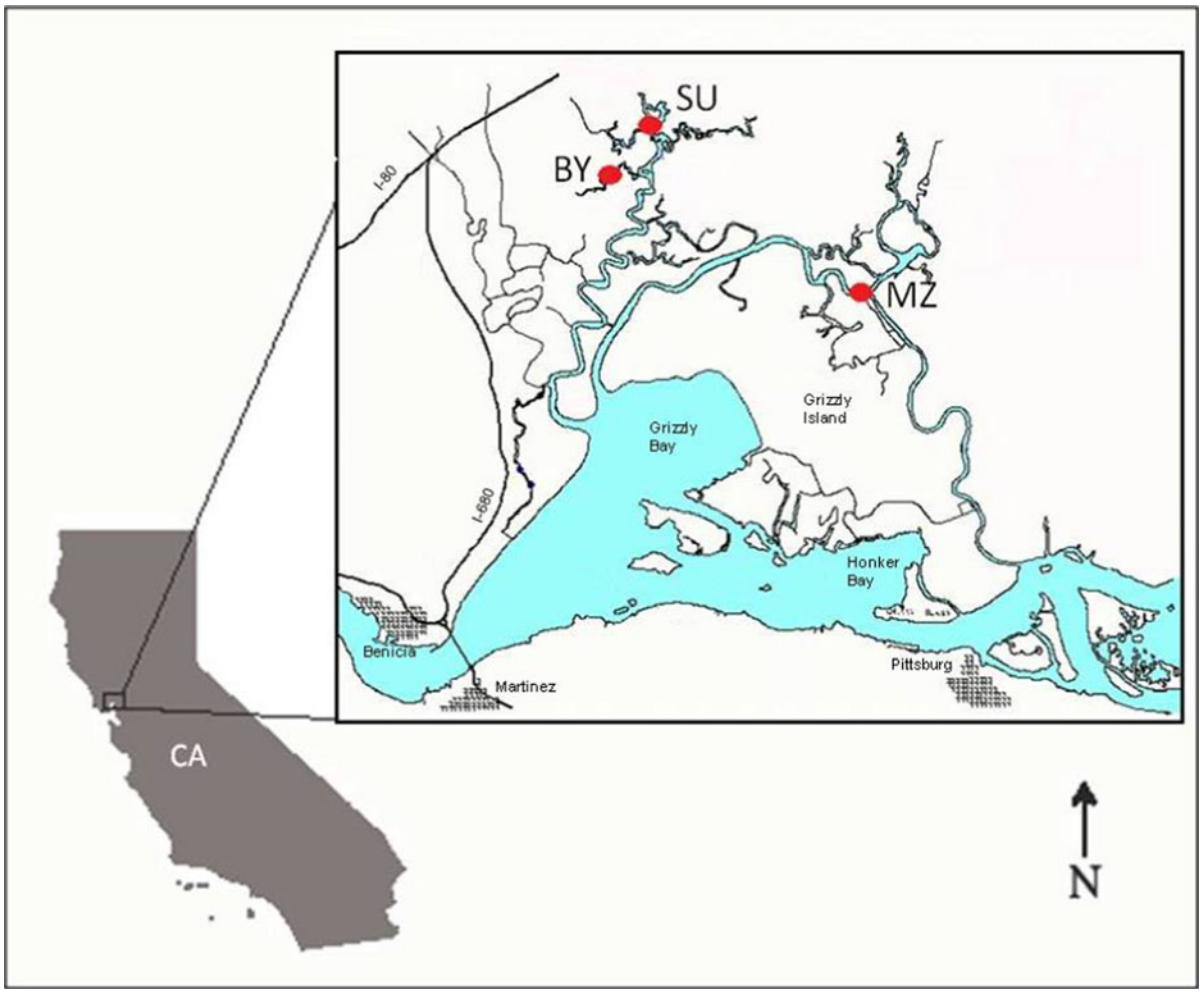

Fig. 1 Location of the three sampling sites in Suisun Marsh, CA (BY-Boynton, SU-Suisun, and MZ-Montezuma sloughs)

entire polyp population and not just the same clones, we employed two methods. Using the microscope, we could see that while many polyps were individually dispersed throughout the plates, there were several patches of many polyps together. To determine if these patches were clone mates, we sampled multiple polyps from six patches (N = 2–16 per patch). We refer to these collections as "patch polyp samples" from this point forward. We then sampled the rest of the polyp population (N = 53 from Boynton slough and N = 74 from Montezuma slough) by collecting polyps that were located far apart from each other, on different settling plates, and unlikely to have occurred from clonal polyp budding. We refer to this sampling as the "polyp population survey."

Additionally, we obtained 4 *Moerisia lyonsi* specimens sampled from the Chesapeake Bay from Dr. Allen Collins at the Smithsonian National Museum of Natural History to compare to our *Moerisia* sp. for species identification.

Molecular methods

We extracted genomic DNA from each individual using Qiagen's Gentra Puregene Kit. Prior to polymerase chain reactions (PCRs), we treated extractions with a Proteinase K cleanup. We added a final concentration of 5 μg/μL Proteinase K to our extractions and then incubated them for 2 h at 60 °C followed by 95 °C for 5 min. This product was used

as template directly in the following PCRs. The *Moerisia* sp. samples were genotyped at 7 species specific polymorphic loci (MmoG115, MmoG123, MmoG167, MmoG181, MmoG109, MmoG133, MmoG194) and *M. marginata* at 8 species specific polymorphic loci (MmaG137-2, MmaG142, MmaG177, MmaG155, MmaG107-2, MmaG139, MmaG154-1, MmaG108), using the primers and protocols described in Meek et al. (2010), with the addition of 1 µl of BSA to each PCR. For many of the individuals, the amplification from the initial PCR was too low to visualize and confidently analyze. Therefore, for these samples we conducted a second PCR, using the original PCR as the template. Using this re-amplification technique has been shown to improve amplification of microsatellites and decrease genotyping error rates (Piggott et al. 2004). In both the original PCR and the re-amplification, we included a negative control to ensure detection of any contamination. We used the negative control from the original PCR as the template for the negative control in the re-amplification. PCR products were visualized with an Applied Biosystems, Inc. (ABI) 3730xl Genetic Analyzer and LIZ600 size standard and were analyzed and scored using ABI Genemapper software. We re-amplified any loci and/or samples that produced peaks that were difficult to call.

Additionally, we performed PCR on DNA extracted from 17 *Moerisia* sp. medusae sampled from Suisun Marsh and the 4 *M. lyonsi* medusae from Chesapeake Bay at the ribosomal Internal Transcribed Spacer 1 (ITS1) marker for sequence analysis using the primers and protocols of Dawson and Jacobs (2001). ITS1 is a rapidly evolving marker and is commonly used for species identification in Cnidarians (e.g. Dawson and Jacobs 2001; Miranda et al. 2010). The PCR products were cleaned using the Agencourt AMPure PCR purification system and sequenced on an ABI 3730xl DNA sequencer.

Data analyses

Population genetic analyses

We analyzed our samples for population level diversity using the individuals genotyped for at least 70 % of the loci (5 + loci in *Moerisia* sp. and 6 + loci in *M. marginata,* Table 1). We removed all repeat multilocus genotypes (MLGs) from the population analyses, leaving only one representative for each unique MLG. We calculated allelic richness for the *Moerisia* sp. medusae, polyp populations, and both combined using the data set lacking clones with the program HP-Rare (Kalinowski 2005). HP-Rare uses a rarefaction method to calculate allelic richness, which corrects for uneven sample sizes. We rarefied the sample to 127 individuals to allow comparison between the medusae and polyp population. We employed the program Genepop (Raymond and Rousset 1995; Rousset 2008) to calculate allelic diversity for *M. marginata* and observed and expected heterozygosities for both species.

Clonal analyses

For all analyses of clonality, we only included those individuals that were genotyped at 100 % of the loci (Table 1). We used the software GenClone 2.0 (Arnaud-Haond and Belkhir 2007) to determine if our sets of loci were appropriate for identifying unique clones. GenClone detects the number of unique MLGs by sampling all the locus combinations, from 1 to n loci, using jackknife subsampling. If the resulting graph of the number of loci used by the number of clones identified reaches an asymptote, then the marker set is sufficient to identify all the unique MLGs in our samples, if not, more loci are needed (Arnaud-Haond et al. 2005). We conducted 5,000 permutations using this method. We then employed

Table 1 Number of each species at each location characterized with microsatellite markers

	Moerisia sp. medusae		*Moerisia* sp. polyp population		*M. marginata* medusae	
% of Loci characterized[a]	100 %	70 %	100 %	70 %	100 %	70 %
Boynton (BY)	56	70	35	53	22	38
Montezuma (MZ)	33	40	47	74	29	35
Suisun (SU)	39	40	–	–	26	38

[a] *Moerisia* sp.: 70 % = 5-7 loci, 100 % = 7 loci; *M. marginata*: 70 % = 6-8 loci, 100 % = 8 loci

GenClone to identify unique MLGs. We re-amplified all individuals that were identified as being only one allele different from each other to reduce the chance of erroneously identifying them as unique MLGs (due to scoring or PCR error) when they were, in fact, clonemates. To investigate the presence of clones, we calculated P_{gen} and P_{sex} using the F_{is} corrected value in Genclone. P_{gen} describes the probability of a particular MLG occurring from the given population and P_{sex} describes the probability of N (N = # individuals with that unique MLG) copies of the genotype occurring through sexual recombination, given the allele frequencies in the population.

To compare clonal diversity between medusae populations of *M. marginata* and *Moerisia* sp., we calculated the Simpson's diversity index (also known as Nei's (1987) genetic diversity) and R, an index of asexual reproduction as described in Dorken and Eckert (2001):

$$R = (G - 1)/(N - 1)$$

where G is the number of unique MLGs in the sample and N is the number of individuals in the sample. This calculation gives a value of 0 when the population consists of a single clone and 1 when each individual possesses a unique MLG, independent of sample size. We also calculated evenness values, as implemented in the program GenClone, to describe the equitability in spread of clonal membership among samples (Arnaud-Haond and Belkhir 2007). This value also ranges from 0 to 1, with 1 indicating that all MLGs have equal abundance.

We analyzed the patch polyp samples separately. We characterized 43 polyps sampled from 6 polyp patches for the entire marker set. The number of individuals sampled from each patch ranged from 2 to 16. To investigate the genetic similarity among patches and within the polyp population samples, we conducted a Factorial Correspondence Analysis in the program Genetix (Belkhir et al. 1996–2004). In this analysis, we coded the individuals from the overall polyp population survey as one population and each unique MLG found in the polyp patches as its own population. This allowed us to visualize inter-patch similarity against the background of the overall population survey.

During our first analysis of the patch polyp data, we found several polyps within the patches were 1 allele different from the rest of the patch. In those instances, the mismatching polyp was missing one of the alleles while the rest of the patch was heterozygous, implying allelic dropout. To investigate this further, we re-amplified, 1–3 times, all individuals sampled (including medusae of both species) that were one allele different from another MLG, using the program Genclone to identify the individuals of interest. Through the re-amplification, we were able to confirm that the polyp samples did exhibit allelic dropout and were indeed identical to the others in their patch. We calculated a 7 % dropout rate by evaluating the dropout in the patch polyps with this equation: $\frac{d}{\sum_1^i n_i s_i}$ where d = the total number of dropped alleles across all patches, i = the number of patches, n_i = the number of individuals in patch i, and s_i = the number of heterozygous sites in patch i. Allelic dropout is common in samples of low quality or quantity DNA (Taberlet et al. 1996). Due to their very small size our polyp samples contained very low quantity DNA (often less than 2 ng/ml), which likely caused the allelic dropout. Therefore, we used a cutoff of greater than 1 allele difference to identify unique MLGs in the polyp samples, which has been shown to be an appropriate method for identifying unique clonal lineages in other studies (Douhovnikoff and Dodd 2003; Schnittler and Eusemann 2010). The observed dropout should not impact our estimates of overall genetic diversity (expected heterozygosity) as the dropout we observed did not preferentially favor some alleles over others.

All individuals in the polyp population survey had a unique MLG. In the medusae sampled, there was only one *Moerisia* sp. MLG pair that was one allele apart and two *M. marginata*. In each of these cases, rather than being Aa heterozygous and the other aa homozygous, implying dropout of the A allele in the second individual, these individuals were Aa and Ab heterozygotes. These genotypes were re-confirmed several times by multiple amplifications and, therefore, analyzed as unique MLGs. We further investigated the possibility of allelic dropout by evaluating the relationships among the sampled individuals genotyped at 100 % of the loci (Table 1). Individuals that were the same clone but experienced allelic dropout, making them appear different, would have high relatedness values. We investigated this by calculating relatedness values for each populations using the program ML-Relate (Kalinowski et al. 2006).

Table 2 Observed (H_O) and expected (H_E) heterozygosity and allelic richness (N_a) for *M. marginata* medusae

Marker	H_O	H_E	N_a
MmaG137-2	0.41	0.49	3
MmaG142	0.63	0.66	8
MmaG177	0.66	0.64	5
MmaG155	0.21	0.29	3
MmaG107-2	0.82	0.72	6
MmaG139	0.78	0.77	8
MmaG154-1	0.85	0.79	9
MmaG108	0.74	0.68	4
Average	0.64	0.63	5.75

Sequence analysis

We analyzed, aligned, and double checked base pair assignment by eye for the ITS1 sequences we obtained using the Sequencher 4.7 software (Gene Codes Corporation, Ann Arbor, MI).

Results

Population genetic diversity

Genetic diversity levels were high, with an average expected heterozygosity across loci of 0.63 in *M. marginata* and 0.58–0.59 in *Moerisia* sp. (Tables 2 and 3). The observed heterozygosity we found in the *Moerisia* sp. polyp population was often lower than the expected heterozygosity. This is likely due to the allelic dropout we observed. This was also observed in locus MmoG167 for *Moerisia* sp. medusae, which is also likely due to allelic dropout as this locus had the highest dropout rate in the polyp patches.

Clonality

We found repeat MLGs, with very high probabilities of arising through asexual reproduction, in both species studied (Table 4). Jackknife analyses reached an asymptote within the number of loci genotyped in both *M. marginata* and *Moerisia* sp., indicating that our marker sets are sufficient for determining clonal identity (Fig. 2). Relatedness values ranged from 0 to 1, with most individuals having very low relatedness values (Fig. 3). We did not see a peak in the number of individuals at the high end of relatedness values ($\sim$0.8–0.99) and, therefore, no evidence for large amounts of allelic dropout causing an underestimation of the number of clones observed.

Table 3 Observed (H_O) and expected (H_E) heterozygosity, and allelic richness (N_a) for the *Moerisia* sp. medusae (N = 150) and polyp populations (N = 127) and both combined

	H_O	H_E	N_a
MmoG 115			
Medusae	0.51	0.53	8.99
Polyps	0.47	0.53	7.00
Both	0.49	0.52	8.84
MmoG 123			
Medusae	0.89	0.81	7.00
Polyps	0.58	0.81	9.00
Both	0.74	0.81	8.28
MmoG 167			
Medusae	0.52	0.86	12.86
Polyps	0.34	0.84	14.00
Both	0.44	0.85	13.99
MmoG 181			
Medusae	0.71	0.76	6.85
Polyps	0.33	0.76	7.00
Both	0.53	0.76	7.39
MmoG 109			
Medusae	0.01	0.01	1.93
Polyps	0.02	0.02	3.00
Both	0.01	0.01	2.52
MmoG 133			
Medusae	0.51	0.47	4.00
Polyps	0.37	0.49	5.00
Both	0.44	0.48	4.75
MmoG 194			
Medusae	0.58	0.64	8.00
Polyps	0.39	0.66	10.00
Both	0.49	0.65	9.52
Average			
Medusae	0.53	0.58	7.09
Polyps	0.36	0.59	7.86
Both	0.45	0.58	7.90

Allelic richness for the polyp and medusae population was calculated through rarefaction to 127 individuals sampled

M. marginata

We detected 69 unique MLGs. Five of the unique MLGs had more than one member, with 2–5 individuals in each repeat MLG. The probability of

Table 4 P_{gen} (probability of a MLG occurring in the population) and P_{sex} (probability of the unique multi-locus genotypes (MLG) reoccurring through sexual reproduction N number of times) values for each repeat MLG detected in *M. marginata* (MA) and *Moerisia* sp. (MO) medusae populations

Species	Locations (# indiv. per location)	P_{gen}	P_{sex}
MA	SU (2)	7.54×10^{-7}	5.80×10^{-5}
MA	SU (2)	3.13×10^{-6}	2.55×10^{-4}
MA	MZ (3) and BY (2)	1.29×10^{-5}	3.73×10^{-14}
MA	MZ (1) and SU (1)	8.86×10^{-8}	6.82×10^{-6}
MA	SU (2)	5.38×10^{-7}	4.14×10^{-5}
MO	BY 8/6 (5), BY 9/20 (2), SU 9/20 (2)	6.18×10^{-8}	1.75×10^{-44}
MO	BY 9/20 (1), SU 9/20 (1)	6.67×10^{-7}	1.40×10^{-4}
MO	BY 9/20 (1), SU 9/20 (2)	7.09×10^{-8}	1.10×10^{-10}
MO	SU 9/20 (2)	1.44×10^{-6}	3.03×10^{-4}

The Locations column describes the location(s) where that repeat MLG was found and how many individuals were included in it (N)

re-encountering these MLGs through sexual recombination ranged from 2.55×10^{-4} to 3.73×10^{-14} (Table 4). Two of the five repeat MLGs were represented by individuals from more than one sampling location.

Moerisia sp.

In the medusae population, we detected 116 unique MLGs with four MLGs containing more than one individual. In the polyp population survey, each polyp sampled represented a unique MLG. We did not find any MLG that was represented in both the medusa and polyp populations. One MLG had nine medusae, one had three individuals, and the others contained two individuals. The P_{sex} values for all repeat MLGs were very low (Table 4). The two largest MLGs contained individuals from both Boynton Slough and Suisun Slough. The nine member MLG contained individuals collected during both sampling months.

In our polyp patch results, the FCA demonstrated that the diversity of polyp patches sampled spanned the overall genetic diversity found through the polyp population survey (Fig. 4). While four of the patches only contained individuals of the same MLG, two of the patches sampled contained more than one clonal lineage. Patch 4 contained two MLGs that occurred in close proximity to each other and Patch 5 contained three clonal lineages (Table 5).

Clonal diversity indices

M. marginata appears to have a slightly higher relative contribution of asexual reproduction than *Moerisia* sp., as shown through the calculated R value, although both species exhibit high clonal diversity as demonstrated by the Simpson's diversity index (Table 6). There was greater evenness in clonal diversity in *M. marginata*. *Moerisia* sp. had one clonal line that was represented by nine individuals where the maximum number of individuals in a single MLG for *M. marginata* was four.

Sequence analysis

We found 100 % sequence agreement at ITS1 between *M. lyonsi* from Chesapeake Bay and *Moerisia* sp. found in the SFE (Genbank accession # HM997 188, GU198209, and GU198210).

Discussion

Genetic diversity and reproductive mode

Our study demonstrates high levels of genetic diversity for invasive populations. In a survey of introduced populations that included a total of 80 species of plants, fungi, and animals, Dlugosch and Parker (2008) found expected heterozygosities ranging between 0.01 and 0.95, and a median value of 0.18, putting the heterozygosity levels in our study in the top 85th percentile of the invasive populations surveyed. Darling and Folino-Rorem (2009) found similarly high levels of expected heterozygosity for an invasive population (0.63–0.75) using microsatellites in a study of another non-native hydrozoan, *Cordylophora caspia,* in the Great Lakes. The level of genetic diversity found herein is unique as it is coupled with asexual reproduction in both invasive hydromedusae populations. These jellies possess life history characteristics that may allow them to thrive through all the stages required for a successful invasion (introduction, establishment, spread) (Sakai et al. 2001). Their robust polyp stage, in which *Moerisia* sp. can undergo dormancy through encystment, likely allows them to withstand harsh conditions during

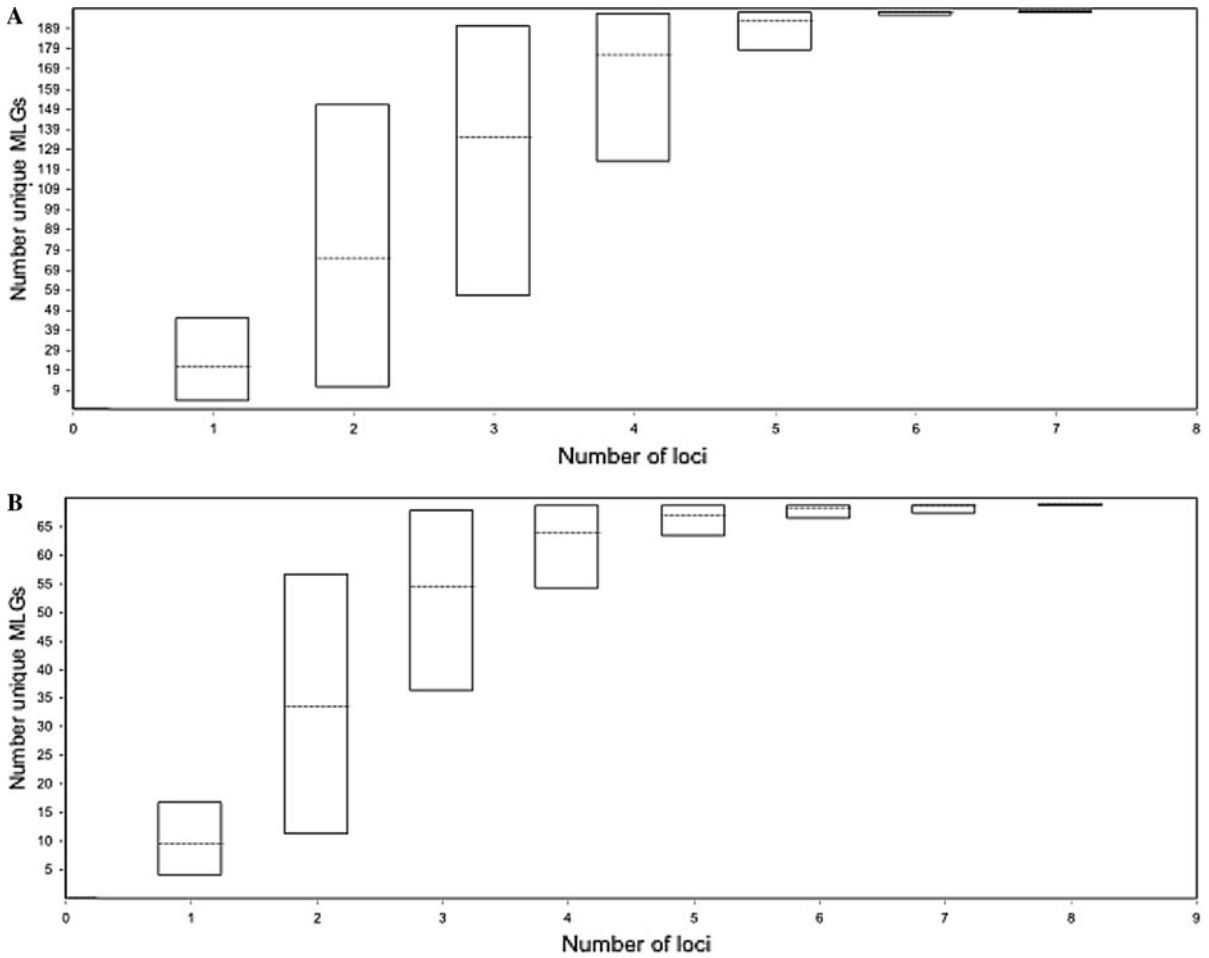

Fig. 2 Jackknife subsampling of number of loci used against the number of unique multi-locus genotypes (MLG) detected for the *Moerisia sp*. (**a**) and *M. marginata* (**b**) datasets. *Box plots* show minimum, maximum, and average (*dashed line*) MLGs detected

transport via ballast water, enabling them to be relocated easily (Purcell et al. 1999). This polyp stage is then able to reproduce rapidly through asexual reproduction, which may confer an advantage during the establishment of the population (Milbau and Stout 2008). The very high genetic diversity observed indicates the occurrence of sexual reproduction is almost certain. The high levels of within population genetic diversity found here may aid in the ability of the introduced populations to adapt to new conditions under climate change and spread to new areas (Roman and Darling 2007). Ting and Geller (2000) conducted a similar study on the Asian sea anemone *Diadumene lineate,* which are also capable of sexual and asexual reproduction. They discovered high clonal diversity within and among invading populations. They found few of the clonal lines occurred in more than one of the invaded populations. The authors concluded that multiple introductions had occurred, giving rise to the high clonal diversity observed, and they stated asexual reproduction in this species likely played a major role in the production of colonizing propagules.

The diversity observed here, combined with the ability to reproduce both sexually and asexually, increases the likelihood that *M. marginata* and *Moerisia* sp. possess the necessary genetic diversity required to continue to thrive in the SFE system. These results suggest that the populations in Suisun Marsh are unlikely to be suffering the negative effects of genetic bottleneck. Hypotheses for causes of the high level of genetic diversity are multiple introductions, sexual reproduction, a large number of founding individuals, and a combination of these factors. Ballast water and hull fouling are very efficient vectors for

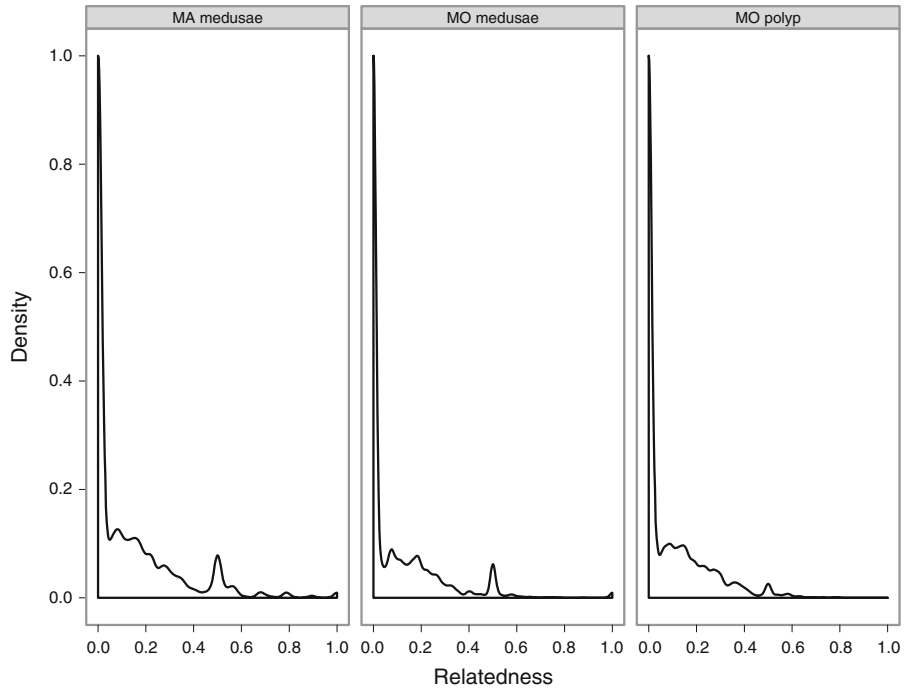

Fig. 3 Distribution of relatedness values for *M. marginata* (MA) medusae and *Moerisia* sp. (MO) medusae and polyp population

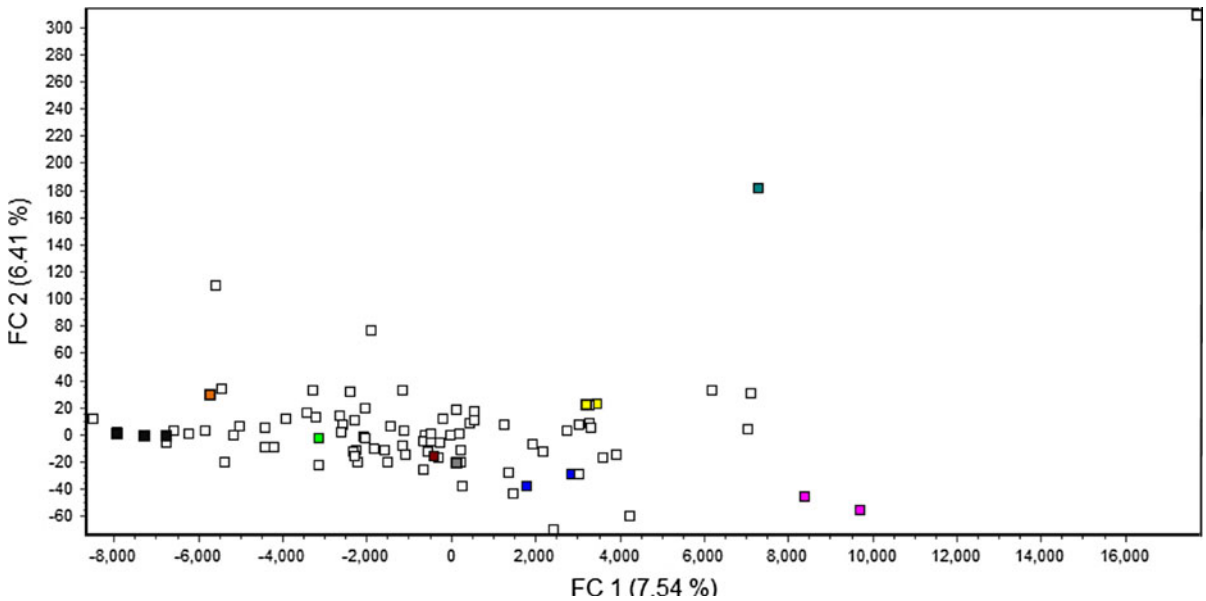

Fig. 4 Factorial correspondence analysis illustrating similarity of polyp patches to overall polyp population. Polyp population samples are in *white*. *Each additional color* represents unique multi-locus genotypes sampled from the patches. Genotypes with more than one of the same *colored box* represent clones with individuals that were one allele apart. Patch 1 = *yellow*, Patch 2 = *blue*, Patch 3 = *orange*, Patch 4 = *pink* and *dark green*, Patch 5 = *grey*, *brown*, and *light green*, Patch 6 = *black*. (Color figure online)

invasion and can provide transport of large propagule pools (Carlton 1985; Carlton and Geller 1993; Fofonoff et al. 2003). An interesting next step to evaluate these hypotheses will be to sample the native populations in the Ponto-Caspian region and the invasive populations in the Chesapeake Bay to compare the

Table 5 The number of unique multi-locus genotypes (MLGs) detected per patch and the number of individuals per MLG in each patch

Patch #	# MLGs	# individuals per MLG
1	1	2
2	1	6
3	1	2
4	2	13, 3
5	3	2,1,3
6	1	12

Table 6 Calculated diversity values for each species

	Simpson's diversity index	R	Evenness
M. marginata medusae	0.995	0.89	0.7
Moerisia sp. medusae	0.995	0.91	0.52
Moerisia sp. polyp population	1.00	1.00	1.00

levels of genetic diversity and presence of clonal lines. This would provide a comparison of the two invasion events to each other and the native range to determine if they exhibit similar patterns.

It is interesting that while we found evidence of polyps asexually reproducing more polyps through the patch polyp analyses, we did not find any repeat MLGs in our polyp population survey. This is likely caused by new polyps arising via sexual reproduction in medusae. Then, once the new polyps have settled, some of them asexually reproduce more polyps, which settle out in the area immediately around the parent polyp. Darling and Folino-Rorem (2009) report similar results in another invasive hydrozoan, *Cordylophora caspia*, in the Great Lakes basin. They discovered this species has high genetic diversity and disperses predominately through sexually produced propagules, with local evidence of asexual reproduction. Other studies have also shown variable input from asexual reproduction in cnidarian populations. Le Goff-Vitry et al. (2004) document that the contribution of asexual propagation was highly variable among sites studied in the coral *Lophelia pertusa* and Henry and Kenchington (2004) found the relative contributions of asexual and sexual reproduction to colonies of the hydroid *Sertularia cupressina* were impacted by substrate and fishing-related disturbance.

We also note that while we found many repeat MLGs in the *Moerisia* sp. medusa population, we did not discover any MLGs that occurred in both the polyp and the medusa populations, despite having detected a single MLG nine times in the medusa population. One explanation could be that there is a large patch of polyps containing this MLG that was not captured by our settling plates. Another explanation is that the number of medusae produced by a single polyp is highly variable. There may be some polyps that are highly successful at producing medusae compared to other polyps. This would result in some MLGs being disproportionately represented in the medusa population as compared to the polyp population. Purcell et al. (1999) found *M. lyonsi* polyps produced polyp buds before medusae buds and that an increase in food availability decreased the time to medusae bud production. Additionally, at low food concentrations, more reproductive output was channeled into medusae production rather than polyp production. The densities of zooplankton prey resources found in Suisun Marsh are quite variable (Wintzer, A.P., unpublished data). It could be that the local conditions experienced by the polyps in Suisun Marsh impact the rate of polyp or medusae production for each polyp individually, making reproduction rates across the polyp population highly variable.

Another interesting finding is the occurrence of individuals of the same MLG across sampling locations. Both *M. marginata* and *Moerisia* sp. had one or more MLG that contained individuals from two sampling locations. The probability of these repeat MLGs arising repeatedly through sexual recombination ranged from 3.73×10^{-14} to 6.82×10^{-6} in *M. marginata* and 1.4×10^{-4} to 1.75×10^{-44} in *Moerisia* sp. There are two additional possible causes of this: (1) polyps of those MLGs are found at both locations and each produced medusae, or (2) the polyp of that MLG is found in one of the locations and the medusae dispersed to the other location, either through active swimming behavior or passive movement from tidal flux. Additionally, in the *Moerisia* sp. sampled, the same MLG was shared between Boynton and Suisun Sloughs, but not between Montezuma Slough and the other sloughs. However, *M. marginata* individuals from Montezuma Slough and Boynton Slough shared the same MLG. It is possible this pattern is explained

by each species' dispersal ability. *Moerisia* sp. is considerably smaller (<8 mm) and likely primarily moves around passively, being strongly affected by tidal flux and river flow (Mills and Rees 2000; Rees and Gershwin 2000). *M. marginata,* however, is larger (bell width < 40 mm) and has greater swimming abilities (pers. obs.) and may be able to actively travel farther distances. This may explain why the same MLG was only found in the closest two locations in *Moerisia* sp., while identical MLGs were found in more distant locations for *M. marginata*.

Species identification

We found the *Moerisia* in the SFE and Chesapeake Bay to be identical at a species-diagnostic marker. Similarly, Dr. Allen Collins at the Smithsonian Institute found 100 % agreement between the *Moerisia* sampled from each location at the mitochondrial 16S marker (A. Collins, pers. comm.). Therefore, we can confidently say they are the same species. While the SFE and Chesapeake Bay specimens agree with Calder and Burrell's (1967) original identification of *M. lyonsi* in North America, it will also be necessary to sequence the type specimen, found in Lake Qurun, Egypt, to confirm that the *Moerisia* sp. found in the SFE and Chesapeake Bay are certainly *M. lyonsi* (Boulenger 1908).

Conclusions

This study provides insights into the role that asexual and sexual reproduction can play in an invasion. The populations studied here demonstrate high levels of genetic diversity for an invasive population, likely maintained through sexual reproduction, while also demonstrating asexual reproduction. The employment of both reproductive modes may allow the species to rapidly propagate and succeed in changing environments and new habitats (Roman and Darling 2007). Suisun Marsh and the SFE are projected to experience higher temperatures, salinity regime changes, and a potential increase in flooding under future scenarios of climate change (Brown 2004). The flexible reproductive capacity and diverse genetic foundation of these invasive populations may provide the building blocks that could be necessary to adapt quickly and successfully to the changing environment. Both the asexual polyp life history phase and the sexual medusae phase are important to the propagation of these populations, which will necessitate the inclusion of both life history stages in any program designed to manage the invasion. These results suggest that these invasive hydromedusae will continue to have a strong presence in the SFE. This may mean further stress is put on local food webs, and is particularly threatening for fish that compete with the hydromedusae for resources.

Acknowledgments We would like to thank Drs. Melinda Baerwald and Peter Moyle for their assistance with this project; Dr. Allen Collins and Genelle Harrison at the Smithsonian National Museum of Natural History for sharing their findings about *Moerisia* sp. in the SFE and Chesapeake Bay; Suisun City Marina and the Suisun Resource Conservation District for their help in gaining access to field sites for settling plates; and Dr. Mike Dawson, William Wetzel, the members of the Genomic Variation Lab, and two anonymous reviewers for helpful comments on the manuscript. This research was supported by funding from CALFED Science Program Grant #1036 (P.I.s: B May and PB Moyle), National Oceanic and Atmospheric Administration Dr. Nancy Foster Scholarship (to MH Meek), University of California-Davis Biological Invasions Integrative Graduate Education and Research Traineeship NSF-DGE#0114432 (to AP Wintzer), University of California-Davis Jastro-Shields Research Scholarship (to MH Meek and AP Wintzer), University of California-Davis Block Grant (to AP Wintzer), and the Giles W. and Elise G. Mead Foundation (to PB Moyle).

References

Arnaud-Haond S, Belkhir K (2007) GENCLONE: a computer program to analyze genotypic data, test for clonality and describe spatial clonal organization. Mol Ecol Notes 7: 15–17

Arnaud-Haond S, Alberto F, Teixeira S, Procaccini G et al (2005) Assessing genetic diversity in clonal organisms: low diversity or low resolution? Combining power and cost efficiency in selecting markers. J Hered 96:434–440

Baker HG (1965) Characteristics and modes of origin of weeds. In: Baker HG, Stebbins GL (eds) The genetics of colonizing species. Academic Press, New York, pp 147–168

Barrett SCH, Richardson BJ (1986) Genetic attributes of invading species. In: Groves RH, Burdon JJ (eds) Ecology of biological invasions. Cambridge University Press, Cambridge, pp 21–33

Belkhir K, Borsa P, Chikhi L, Raufaste N et al (1996–2004) GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Laboratoire Génome, populations, interactions. Laboratoire Génome, populations, interactions, CNRS UMR 5171, Université de Montpellier II, Montpellier, France

Boulenger CL (1908) On *Moerisia lyonsi*, a new hydromedusan from Lake Qurun. Quart J Micro Sci 52:357–378
Brown RL (2004) Summary of 2004 workshop-making science work for Suisun Marsh. Prepared for the San Francisco Bay-delta science consortium, p 125
Burdon JJ, Marshall DR (1981) Biological control and the reproductive mode of weeds. J Appl Ecol 18:649–658
Burnett WJ, Benzie JAH, Beardmore JA, Ryland JS (1995) Patterns of genetic subdivision in populations of a clonal cnidarian, *Zoanthus coppingeri*, from the Great Barrier Reef. Mar Biol 122:665–673
Burns JH (2008) Demographic performance predicts invasiveness of species in the Commelinaceae under high-nutrient conditions. Ecol Appl 18:335–346
Calder DR, Burrell VG (1967) Occurrence of *Moerisia lyonsi* (Limnomedusae, Moerisiidae) in North America. Am Midl Nat 78:540–541
Carlton JT (1985) Trans-oceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. Oceanogr Mar Biol 23:313–371
Carlton JT, Geller JB (1993) Ecological roulette: the global transport of nonindigenous marine organisms. Science 261:78–82
Darling JA, Folino-Rorem NC (2009) Genetic analysis across different spatial scales reveals multiple dispersal mechanisms for the invasive hydrozoan Cordylophora in the Great Lakes. Mol Ecol 18:4827–4840
Dawson MN, Jacobs DK (2001) Molecular evidence for cryptic species of *Aurelia aurita* (Cnidaria, Scyphozoa). Biol Bull (Woods Hole) 200:92–96
Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449
Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). J Ecol 89:339–350
Douhovnikoff V, Dodd RS (2003) Intra-clonal variation and a similarity threshold for identification of clones: application to *Salix exigua* using AFLP molecular markers. Theor Appl Genet 106:1307–1315
Dybdahl MF, Kane SL (2005) Adaptation vs. phenotypic plasticity in the success of a clonal invader. Ecology 86:1592–1601
Facon B, Genton BJ, Shykoff J, Jarne P et al (2006) A general eco-evolutionary framework for understanding bioinvasions. Trends Ecol Evol 21:130–135
Fisher RA (1930) The genetical theory of natural selection. Clarendon, Oxford
Fofonoff PW, Ruiz GM, Steves B, Carlton JT (2003) In ships or on ships? Mechanisms of transfer and invasion for nonnative species to the coasts of North America. In: Ruiz GM, Carlton JT (eds) Invasive species: vectors and management strategies. Island Press, Washington, pp 152–182
Henry LA, Kenchington ELR (2004) Ecological and genetic evidence for impaired sexual reproduction and induced clonality in the hydroid *Sertularia cupressina* (Cnidaria: Hydrozoa) on commercial scallop grounds in Atlantic Canada. Mar Biol 145:1107–1118
Holland BS, Dawson MN, Crow GL, Hofmann DK (2004) Global phylogeography of *Cassiopea* (Scyphozoa : Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. Mar Biol 145:1119–1128
Hughes AR, Stachowicz JJ (2004) Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proc Natl Acad Sci USA 101:8998–9002
IEP (2008) Interagency ecological program 2008 workplan to evaluate the decline of pelagic species in the Upper San Francisco Estuary, p 125
Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189
Kalinowski ST, Wagner AP, Taper ML (2006) ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. Mol Ecol Notes 6:576–579
Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. Trends Ecol Evol 16:199–204
Lambrinos JG (2001) The expansion history of a sexual and asexual species of *Cortaderia* in California, USA. J Ecol 89:88–98
Le Goff-Vitry MC, Pybus OG, Rogers AD (2004) Genetic structure of the deep-sea coral *Lophelia pertusa* in the northeast Atlantic revealed by microsatellites and internal transcribed spacer sequences. Mol Ecol 13:537–549
McFadden CS (1997) Contributions of sexual and asexual reproduction to population structure in the clonal soft coral, *Alcyonium rudyi*. Evolution 51:112–126
Meek MH, Baerwald MR, Wintzer AP, May B (2010) Isolation and characterization of microsatellite loci in two nonnative hydromedusae in the San Francisco Estuary: *Maeotias marginata* and *Moerisia* sp. Conserv Genet Resour 1:205–208
Meng L, Matern SA (2001) Native and introduced larval fishes of Suisun Marsh, California: the effects of freshwater flow. T Am Fish Soc 130:750–765
Mergeay J, Verschuren D, De Meester L (2006) Invasion of an asexual American water flea clone throughout Africa and rapid displacement of a native sibling species. Proc R Soc B 273:2839–2844
Milbau A, Stout JC (2008) Factors associated with alien plants transitioning from casual, to naturalized, to invasive. Conserv Biol 22:308–317
Mills CE, Rees JT (2000) New observations and corrections concerning the trio of invasive hydromedusae *Maeotias marginata*, (*M. inexpectata*), *Blackfordia virginica*, and *Moerisia* sp in the San Francisco Estuary. Sci Mar 64:151–155
Mills CE, Sommer F (1995) Invertebrate introductions in marine habitats: two species of hydromedusae (Cnidaria) native to the Black Sea, *Maeotias inexspectata* and *Blackfordia virginica*, invade San Francisco Bay. Mar Biol 122: 279–288
Miranda LS, Collins AG, Marques AC (2010) Molecules clarify a cnidarian life cycle—the "hydrozoan'' *Microhydrula limopsicola* is an early life stage of the staurozoan *Haliclystus antarcticus*. PLoS One 5:e10182. doi:10.1371/journal.pone.0010182
Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York, NY
Novak SJ, Mack RN (2000) Clonal diversity within and among introduced populations of the apomictic vine *Bryonia alba* (Cucurbitaceae). Can J Bot 78:1469–1481

Novak SJ, Mack RN (2005) Genetic bottlenecks in alien plant species: influence of mating systems and introduction dynamics. In: Sax DF, Stachowicz JJ, Gaines SD (eds) Species invasions: insights into ecology, evolution, and biogeography. Sinauer Associates, Inc., Sunderland, MA, pp 201–228

Piggott MP, Bellemain E, Taberlet P, Taylor AC (2004) A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for faecal DNA in limiting conditions. Conserv Genet 5:417–420

Purcell JE, Båmstedt U, Båmstedt A (1999) Prey, feeding rates, and asexual reproduction rates of the introduced oligohaline hydrozoan *Moerisia lyonsi*. Mar Biol 134:317–325

Raymond M, Rousset F (1995) Genepop (version-1.2)—population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Rees JT (1999) Non-indigenous jellyfish in the upper San Francisco Estuary: potential impacts on zooplankton and fish. Interag Ecol Program Newsl 12:46–50

Rees JT, Gershwin LA (2000) Non-indigenous hydromedusae in California's upper San Francisco Estuary: life cycles, distribution, and potential environmental impacts. Sci Mar 64:73–86

Roman J, Darling JA (2007) Paradox lost: genetic diversity and the success of aquatic invasions. Trends Ecol Evol 22:454–464

Rousset F (2008) GENEPOP ' 007: a complete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Resour 8:103–106

Sakai AK, Allendorf FW, Holt JS, Lodge DM et al (2001) The population biology of invasive species. Annu Rev Ecol Syst 32:305–332

Schnittler M, Eusemann P (2010) Consequences of genotyping errors for estimation of clonality: a case study on *Populus euphratica* Oliv. (Salicaceae). Evol Ecol 24:1417–1432

Sommer T, Armor C, Baxter R, Breuer R et al (2007) The collapse of pelagic fishes in the Upper San Francisco Estuary. Fisheries 32:270–277

Taberlet P, Griffin S, Goossens B, Questiau S et al (1996) Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Res 24:3189–3194

Ting JH, Geller JB (2000) Clonal diversity in introduced populations of an Asian sea anemone in North America. Biol Invasions 2:23–32

Wares JP, Hughes RG, Grosberg RK (2005) Mechanisms that drive evolutionary change: insights from species introductions and invasions. In: Sax DF, Stachowicz JJ, Gaines SD (eds) Species invasions: insights into ecology, evolution, and biogeography. Sinauer Associates, Inc., Sunderland, MA, pp 229–257

Wintzer AP, Meek MH, Moyle PB (2011) Trophic ecology of two non-native hydrozoan medusae in the upper San Francisco Estuary. Mar Freshwater Res 62:952–961