# FINAL PROJECT REPORT

**PROJECT TERM:** April 1, 2007 – June 30, 2011
**PROJECT TITLE:** Predicting the effects of invasive Hydrozoa (jellyfish) on pelagic organisms under changing salinity and temperature regimes
**AMOUNT FUNDED: $430,870.00**

## CONTRACTOR/GRANT RECIPIENT CONTACT INFORMATION

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## PROJECT SUMMARY

Pelagic organisms are in serious decline in the San Francisco Estuary (SFE). A potentially important, yet understudied, factor in this decline is the invasion of four predatory hydrozoan species (*Maeotias marginata, Moerisia sp., Blackfordia virginica, and Cordylophora caspia)*. Our current level of knowledge regarding the basic biology and ecology of these organisms is alarmingly poor in light of both their possibly negative effect on the SFE ecosystem and the increasing trends in jellyfish blooms around the globe. Our proposed research aimed to investigate the potential effects of these species on the SFE ecosystem and to determine the key factors allowing successful establishment and spread of these species. This multi-tiered research project involved three independent tasks utilizing genetic analyses, field surveys, and controlled laboratory investigations. First, through the use of genetic analyses, we identified the species present in all life history forms via the development of molecular tools. In the second portion of this task, we used molecular techniques to evaluate clonal diversity and mode of reproduction. Hydrozoans are novel predators in that they can reproduce both asexually and sexually. The capacity to reproduce asexually may confer a strong advantage on these rapidly expanding invasives, therefore it is important to gain a clear understanding of both the nature of clonal diversity and how they are reproducing and spreading. The second task was two-fold and was accomplished via directed field surveys focused in Suisun Marsh. In the first portion, we established a reliable estimation of the current distributions and abundances of invasive hydrozoans and related these trends to water quality and habitat data. The remainder of this task involved detailed gut contents analysis to determine patterns of prey selectivity (including predation on larval fishes), temporal feeding behavior, and dietary overlap with planktivorous fishes. The third task involved a suite of laboratory studies, designed to determine ecophysiological characteristics of these species. We quantified feeding rates upon zooplankton for both the medusae and polyp life history stages, as well as evaluated salinity and temperature tolerances and their effect on survival and reproduction. The outcome of this work is a clearer understanding of the effects of several abundant and novel hydrozoan predators in the system, as well as information that will be useful for predicting trends and patterns of the populations in response to a changing SFE environment. This body of work addressed the CALFED Priority Research Topics of aquatic invasive species, trends and patterns of populations and system response to a changing environment, and habitat availability and response to change. It also is an important research component in elucidating the cause of pelagic organism decline in SFE.

**PRELIMINARY BUDGET SUMMARY**

**Please note this is not a final budget summary as there are still charges pending to clear the account.

| EXPENSE | AMOUNT INVOICED 2006-2007 | AMOUNT INVOICED 2007-2008 | AMOUNT INVOICED 2008-2009 | AMOUNT INVOICED 2009-2010 | AMOUNT INVOICED 2010-2011 | AMOUNT INVOICED TO DATE |
|---|---|---|---|---|---|---|
| Salaries and Wages | $ 0 | $ 16,614.89 | $ 52,128.62 | $ 41,547.96 | $ 77,441.51 | $187,732.98 |
| Employee Benefits | $0 | $ 5,928.57 | $ 11,445.13 | $ 16,080.03 | $ 13,645.58 | $ 47,099.31 |
| Equip-ment & Facilities | $0 | | | | | |
| Supplies, Materials & Services | $0 | $ 28,182.80 | $ 19,207.51 | $ 5,308.76 | $ 37,253.11 | $ 89,952.18 |
| Travel | $0 | $ 1,363.83 | $ 1,700.58 | $ 1,378.00 | $ 3,532.14 | $ 7,974.55 |
| Sub-contracts | $0 | | | | | |
| Indirect Costs | | | | | | |
| Overhead | $0 | $ 12,419.48 | $ 19,450.39 | $ 13,905.72 | $ 30,022.39 | $ 75,797.98 |
| Totals | $0 | $64,509.57 | 103,932.23 | 78,220.47 | 161,894.73 | 408,557.00 |

**PROJECT STATUS TO DATE (BY TASK)**

TASK 2: Genetic Studies

**November 2010-June 2011: Activities, tasks, and milestones performed and/or completed:**

- Completed draft manuscript on genetic diversity and reproduction of *M. marginata* and *Moerisia* sp.
- Submitted manuscript on genetic diversity and reproduction of *M. marginata* and *Moerisia* sp. to *Molecular Ecology*

**June 2010-November 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed data analyses to investigate genetic diversity and asexual and sexual reproduction in *M. marginata* and *Moerisia* sp.
- Began draft manuscript on genetic diversity and reproduction of *M. marginata* and *Moerisia* sp.
- Presented findings of genetic and clonal diversity of *M. marginata* and *Moerisia* sp. at the Bay Delta Science Conference in Sacramento, CA
- Presented findings on the invasion biology of *Moerisia* sp. at the Ecological Society of America Annual Meeting in Pittsburgh, PA

**November 2009-May 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed genotyping of *M. marginata* and *Moerisia* sp. samples using microsatellite markers
- Began data analyses to investigate genetic diversity and asexual and sexual reproduction in *M. marginata* and *Moerisia* sp.

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Completed and published microsatellite marker development manuscript (Meek et al. *in press*)
- Continued genotyping of *M. marginata* and *Moerisia* sp. samples using microsatellite markers
- Presented preliminary findings of genetic and clonal diversity of *Moerisia* sp. at the Coastal and Estuarine Research Federation Biennial Conference in Portland, OR

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Identified and sorted samples collected for genetic analyses
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Writing methods note on microsatellite marker development
- Developing Taqman assay for rapid species identification

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Collected hydrozoan samples through Dept. of Water Resources sampling programs within Suisun Bay
- Identified and sorted samples collected for genetic analyses
- Completed initial development of molecular markers for use in species identification
- Identified polyps collected to species using developed molecular markers
- Developed further microsatellite markers for analyses of genetic diversity and reproductive mode
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Presented research at the 8$^{th}$ Biennial State of the San Francisco Estuary Conference

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Began monthly field collection of *Maeotias marginata, Cordylophora caspia,* and *Moerisia* sp. in Suisun Marsh and the Napa and Petaluma Rivers in May 2007
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh and the Napa and Petaluma Rivers in June 2007
- Developed 7 polymorphic microsatellite markers each for *M. marginata* and *Moerisia* sp.
- Extracted DNA from 20-100 individuals of each species, collected throughout the study area, for genetic analyses with developed microsatellite markers
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 3—Ecological Field Studies

**November 2010-June 2011: Activities, tasks, and milestones performed and/or completed:**

- Published polyp ecology manuscript in *Aquatic Ecology*
- Submitted *Moerisia* sp. population dynamics manuscript to *Estuarine, Coastal and Shelf Science*
- Hydrozoan-fish trophic interactions manuscript accepted for publication in *Marine and Freshwater Research*

**June 2010-November 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed draft manuscript on of hydrozoan-fish trophic interactions
- Presented findings of *Moerisia* sp. population dynamics at the Bay Delta Science Conference in Sacramento, CA

**November 2009-May 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed processing fish gut content samples
- Completed draft of *Moerisia* sp. population dynamics manuscript for publication
- Writing draft of hydrozoan-fish trophic interactions manuscript

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Completed processing polyp samples, zooplankton samples, and jellyfish gut content samples
- Completed draft of polyp ecology manuscript for publication
- Began processing fish gut content samples
- Writing draft of *Moerisia* sp. population dynamics manuscript
- Presented polyp research at Coastal and Estuarine Research Federation Biennial Conference in Portland, OR and CALFED Science Conference in Sacramento, CA

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Continued analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)

- Began analyzing polyp gut contents
- Completed digestion rate experiments with polyps and medusae
- Presented research at American Society of Ichthyologists and Herpetologists Society Annual Meeting (Montreal, Canada)

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Began analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Began collecting monthly fish and non-gelatinous zooplankton samples in May 2007 throughout Suisun Marsh
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh and the Napa and Petaluma Rivers in June 2007
- Began monthly collections of hydrozoans in Suisun Marsh and the Napa and Petaluma Rivers June 2007
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 4: Ecological Laboratory Studies

**November 2010-June 2011: Activities, tasks, and milestones performed and/or completed:**

- Completed revisions of draft *C. caspia* temperature and salinity tolerance and trophic studies manuscript

**June 2010-November 2010: Activities, tasks, and milestones performed and/or completed:**

- Revising draft *C. caspia* temperature and salinity tolerance and trophic studies manuscript for publication

**November 2009-May 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed draft *C. caspia* temperature and salinity tolerance and trophic studies manuscript for publication

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Analyzed data from temperature and salinity tolerance experiments and feeding rates experiments with *M. marginata, Moerisia* sp., and *C. caspia*
- Writing feeding rates and tolerance level draft manuscripts
- Presented feeding rates research at CALFED Science Conference in Sacramento, CA

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Collected individuals from field and developed methods for culture of all four hydrozoan species
- Completed 10 day temperature and salinity tolerance experiment with *M. marginata* (medusae), *Moerisia* sp. (medusae), and *C. caspia* (polyps).
- Completed feeding rates experiments with *B. virginica* (medusae), *M. marginata* (medusae), *Moerisia* sp. (medusae and polyps), and *C. caspia* (polyps).

**September 2007-April 2008:**

- Procured facilities in which experiments/culture will take place Summer 2008
- Contacted experts in jellyfish culture for husbandry advice

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Collected live *M. marginata* and *Moerisia* sp. from Suisun Marsh
- Developed methods for culture of live *M. marginata* and *Moerisia* sp. in the laboratory for future temperature and salinity tolerance level and feeding rate experiments
- Developed methods for culture of various copepod species to be used in feeding rate experiments

## LIST THE ACHIEVED OBJECTIVES, FINDINGS, AND MAJOR CONTRIBUTIONS

- Molecular marker development - We characterized 10 new microsatellite markers in each of two species of hydromedusae, *Maeotias marginata* and *Moerisia* sp. Genetic diversity was estimated using 20-41 individuals collected from Suisun Marsh within the San Francisco Estuary, CA. Allelic richness ranged from 5-9 in *M. marginata* and 2-10 in *Moerisia* sp. with average expected heterozygosities of 0.71 and 0.57 respectively. One locus in *M. marginata and* two in *Moerisia* sp. deviated from Hardy-Weinberg equilibrium expectations, likely due to null alleles. For more information, please see Appendix C.

- Analysis of genetic diversity and reproductive mode - 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 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 for *M. marginata* and *Moerisia* sp. respectively) but also detected multiple individuals (2-9) 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 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. For more information, please see Appendix D.

- Feeding rate and temp and salinity tolerance analyses - *Cordylophora caspia,* a hydrozoan from the Ponto-Caspian region, has become established in the brackish water habitats of the San Francisco Estuary (SFE). It is important to understand what factors influence the spread of *C. caspia* and how it may impact the food web, particularly given the severe decline of pelagic fishes in the SFE. In this study, we asked four questions: 1. What are the effects of temperature and salinity on *C. caspia* colony growth? 2. How do exposure time for feeding, temperature, and salinity impact feeding rate? 3. What is the diet of *C. caspia?* and 4. What is the digestion rate of prey? We experimentally investigated the effects of temperature and salinity on colony growth and feeding rates, and analyzed gut contents from wild specimens to determine diets. Warmer temperatures resulted in higher growth rates, with the maximum growth at 25 C, but salinity did not impact growth. Further work is needed to parse out the influence of exposure time, temperature, and salinity on feeding. We found *C. caspia* eats a wide range of mostly planktonic prey items, proportional to prey availability, and digestion times for one prey item ranged from 48-69 minutes. This work is a valuable step in understanding the potential impact of this abundant non-native on the SFE ecosystem. For more information, please see Appendix E.

- Polyp ecology – The populations of several invasive jellyfish appear to be increasing around the globe. While data on non-native hydromedusae in the San Francisco Estuary have been accumulating in recent years, little is known regarding their polyp phase. The goal of this study was to gather the first field-derived ecological data for polyp stages of *Blackfordia virginica*, *Moerisia* sp., and *Cordylophora caspia* in the estuary. Monthly fouling plates were deployed at five sites during 2007 and 2008. Settlement data indicate a seasonal presence of *B. virginica* and *Moerisia* sp., with both distribution and abundance correlated with a combination of water quality and physical parameters. *Cordylophora caspia* appeared to be present beyond the time period sampled and may be active in the system year-round. The ability of polyps to persist month to month was low, likely due to predation by other non-native species and competition for space. For more information, please see Appendix F.

- *Moerisia* sp. population dynamics - Some populations of gelatinous zooplankton appear to be increasing and causing ecological disruptions during large bloom events. The goal of this study was to investigate the life history and population dynamics of the small, invasive *Moerisia* sp. and its potential ecological impacts in the upper San Francisco Estuary, California. Medusae and polyps were collected from 8 and 2 sites, respectively, during 2007 and 2008. Polyps emerged from a resting stage during June, at a minimum temperature of approximately 14ºC. Asexual reproduction of medusae buds was positively correlated with temperature and negatively related to salinity. The production of polyp buds was positively correlated with dissolved oxygen and water transparency

levels. Sexual reproduction, defined by the presence of eggs, was related to the size of medusae. Cessation of reproduction of both polyps and medusae occurred in October, when temperatures dropped below 17ºC. This life history is similar to other hydrozoans and allows *Moersia* sp. to reach large numbers seasonally in the San Francisco Estuary, possibly contributing to the recent declines of pelagic fish and zooplankton. For more information, please see Appendix G.

- Medusa trophic ecology - Blooms of some gelatinous zooplankton are increasing worldwide, often disrupting foodwebs. Invasions of non-native jellyfish are a growing problem in many estuaries, including the San Francisco Estuary, where at least two species of Ponto-Caspian hydrozoans, *Maeotias marginata* and *Moerisia* sp., are abundant. This study investigates their trophic ecology, testing the hypotheses: 1) diets over the bloom and at the diel scale are comprised of a variety of prey items; 2) hydrozoans are generalist feeders; 3) hydrozoans feed on the larvae of declining fish species; and 4) the potential for prey competition exists between the hydrozoans and two declining planktivorous fishes, striped bass (*Morone saxatilis*) and threadfin shad (*Dorosoma petenense*). Both hydrozoans ate a variety of crustaceans, most notably calanoid copepods, which were found in greater proportion in the guts than the environment. The only fish larvae consumed were gobies. Density of *Moerisia* sp. was negatively correlated to gut fullness for both fishes, and diet overlap was high between shad and hydrozoans, but low for bass. Because of strong spatial and temporal overlap between hydrozoans and shad, competition for zooplankton may be occurring. These hydrozoans have invaded other systems, and should be monitored to assess potential ecological interactions in these locations. For more information, please see Appendix H.

## DISCUSS THE MANAGEMENT IMPLICATIONS OF PROJECT FINDINGS

- Molecular marker development - The brackish water hydrozomedusae studied herein are novel predators in the SFE and, thus, have an especially high likelihood of impacting this system and competing with juvenile fishes. Very little is known about the basic biology and life history of these invaders. The microsatellite markers characterized for these species provide necessary tools to answer questions about their life history, which will aid in determining how best to manage for their potential impacts. The development of these markers allowed us to investigate the genetic diversity of these populations, as well as determine reproductive strategies and life history characteristics of these potentially important non-natives.

- Analysis of genetic diversity and reproductive mode – It is important to determine what factors affect the abundance of invasive species to understand their potential impacts on invaded ecosystems and their ability to spread into new areas. Knowledge of the life history traits that aid range expansion may help predict future invasions, as well as the ecological and evolutionary consequences of invasion. 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. *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. Very little is known about the basic life histories of these non-natives or their potential impact on the system. Understanding the life history characteristics and genetic diversity will be important to developing effective control strategies. If we know which life history stage (polyp or medusa) is primarily responsible for reproduction, it may be possible to target the most prolific life history stage for control. The populations studied here demonstrate high rates of sexual reproduction and genetic diversity, in addition to abundant asexual reproduction in through the polyp phase. This may allow the species to adapt and evolve in their new environment and spread to new environments through the dispersive medusa stage. These results suggest that these invasive hydromedusae will continue to have a strong presence in the SFE, with potential to expand their populations. This may mean further stress is put on local food webs, and is particularly threatening for those fish that compete with hydromedusae for resources. Additionally, the high genetic diversity and ability to reproduce both asexually and sexually may allow these species to quickly adapt to future climate change. Suisun Marsh and the SFE are projected to experience higher temperatures, salinity regime changes, and a potential increase in flooding. The flexible reproductive capacity and diverse genetic foundation of these invasive populations make it more likely they will adapt quickly and successfully to the changing environment.

- Feeding rate and temp and salinity tolerance analyses - Given *C. caspia's* capabilities for high growth and feeding rates, the population of *C. caspia* in the SFE has the potential to have a considerable impact on the zooplankton community. This impact may be detrimental to native fish populations, which also depend on zooplankton as a food source. Additionally, our experiments found increasing growth rates with increasing temperature and we did not reach an upper limit temperature where growth rate decreased. Under projections of climate change, Suisun Marsh will experience between 2-6 C warming in the next decade. As the recorded average summer temperature between 1980-2008 in Suisun Marsh hasn't exceeded 23 C, it is possible that *C. caspia* will continue to thrive in the area, and may increase its range, spreading into new habitats as conditions change.

- Polyp ecology - The reduced ecological health of the heavily modified San Francisco Estuary has resulted in plans for habitat restoration. A knowledge of general habitat use of this suite of hydrozoans, as well as environmental factors that increase populations, will be advantageous to restoration efforts, in order to minimize creation of conditions beneficial to these unwanted species. An avoidance of the "optimal" abiotic conditions given in Table 2 (within appendix F) will negatively influence polyp survival. However, for a more effective restoration strategy, these studies should be paired with field studies on medusae that give an understanding of population dynamics of these species.

- *Moerisia* sp. population dynamics - The flexible and rapid reproductive capacities, coupled with the ability to form resting stage "seed banks" during unsuitable conditions, may allow *Moerisia* sp. to have a very large seasonal impact on other pelagic species in the upper San Francisco Estuary, especially via trophic interactions. Perhaps the greatest concern relates to recent precipitous declines in the local populations of four planktivorous fish species, delta smelt, longfin smelt, age-0 striped bass, and threadfin

shad. Large reductions in the number of calanoid copepods, a major prey source for these fishes, have also been documented in the system. *Moerisia* sp. consumes a wide variety of zooplankton, especially calanoid copepods. Although individuals of this species are small, diet data demonstrate that it rarely has an empty gut and is capable of digesting multiple items at once. Additionally, laboratory culture has revealed that *Moerisia* sp. leaves many prey items dead, but uneaten, likely increasing the level of competition with fishes for food resources.

- Medusa trophic ecology - *Maeotias marginata* and *Moerisia* sp. are predators on a variety of small crustaceans in the San Francisco Estuary. Evidence suggests that they may employ some selectivity during feeding, but this requires further study to confirm. While their ability to consume the larvae of imperiled fishes is reduced by limited spatial and temporal overlap, the medusae and some planktivorous fishes do consume a similar prey base. Threadfin shad and delta smelt populations, in particular, may be affected by both hydrozoans due to their dietary overlap of copepods with these fish species. These findings are troubling, especially in conjunction with evidence of increasing medusae populations, declining zooplankton populations, and the presence of additional well-established invasive zooplanktivores, such as the overbite clam (*Corbula amurensis*).

## PROJECT DELIVERABLES

MANUSCRIPTS AND REPORTS:

- Summary—Appendix A
- Semi-Annual Reports #1-6—Appendix B
- Manuscript: Isolation and characterization of microsatellite loci in two non-native hydromedusae in the San Francisco Estuary: *Maeotias marginata* and *Moerisia* sp.—Appendix C
- Draft manuscript: Genetic diversity and reproductive mode in two non-native hydromedusae, *Maeotias marginata* and *Moerisia* sp., in the Upper San Francisco Estuary, California--Appendix D
- Draft manuscript: Trophic ecology and effects of temperature and salinity on growth in the non-native hydrozoan, *Cordylophora caspia*, in the upper San Francisco Estuary, California—Appendix E
- Manuscript: Ecological insights into the polyp stage of non-native hydrozoans in the San Francisco Estuary—Appendix F
- Draft manuscript: Life history and population dynamics of *Moerisia* sp., a non-native hydrozoan, in the upper San Francisco Estuary—Appendix G
- Manuscript: Trophic ecology of two non-native hydrozoan medusa in the Upper San Francisco Estuary—Appendix H

PRESENTATIONS:

- Wintzer, A., M. Meek and Moyle, P. Life history and population dynamics of *Moerisia* sp., a non-native hydrozoan in the upper San Francisco Estuary. Bay-Delta Science Conference 2010

- Wintzer, A., M. Meek, P. Moyle, and May, B. Ecological insights into the benthic stages of non-native jellyfish in the San Francisco Estuary. Coastal and Estuarine Research Federation Biennial Conference 2009

- Wintzer, A., M. Meek, P. Moyle, and May, B. The Secret Lives of Polyps: Ecological insights into the benthic stages of non-native jellyfish in the San Francisco Estuary. CALFED Science Conference 2008

- Wintzer, A. and P. Moyle. Interactions between fishes and invasive jellyfish in the San Francisco Estuary (USA). American Society of Ichthyologists and Herpetologists Society Annual Meeting 2008

- Wintzer, A. and P. Moyle. Interactions between fishes and invasive jellyfishes in the upper San Francisco Estuary, CA. State of the Estuary Conference 2008

- Wintzer, A. and P. Moyle. Interactions between fishes and invasive jellyfishes in the upper San Francisco Estuary, USA. American Fisheries Society Annual Meeting 2007

- Wintzer, A. and P. Moyle. Interactions between fishes and invasive jellyfishes in the upper San Francisco Estuary, USA. Second International Jellyfish Blooms Symposium 2007

- Meek, M., A. Wintzer, N. Elen, B. May, and P. Moyle. Genetic diversity and population dynamics of Moerisia sp., an invasive hydromedusa in the San Francisco Estuary. Ecological Society of America Annual Meeting 2010

- Meek, M., A. Wintzer, N. Elen, and B. May. Sex, clones, and Suisun Marsh: Genetic diversity and reproductive mode in two species of invasive hydromedusae in the upper San Francisco Estuary. Bay Delta Science Conference. 2010

- Meek, M., A. Wintzer, and B. May. To Clone or Not to Clone? Genetic analyses of clonal diversity and sexual reproduction in an invasive hydrozoan jelly in the San Francisco Estuary. Coastal and Estuarine Research Federation Biennial Conference. 2009

- Meek, M., A. Wintzer, and B. May. Feeding rates of an invasive hydrozoan jellyfish in Suisun Marsh. CALFED Science Conference 2008

- Meek, M., M. Baerwald, A. Wintzer, and B. May. Clonal diversity, reproductive mode, and physiological tolerances in three species of invasive hydrozoans. State of the Estuary Conference 2007

- Meek, M., M. Baerwald, A. Wintzer, and B. May. Characterization of microsatellite markers for three species of invasive hydrozoans in the San Francisco Estuary. American Fisheries Society Annual Meeting 2007

- Meek, M., M. Baerwald, and B. May. The Secrets to Successful Invasions: Clonal diversity, reproductive mode and physiological tolerances in three species of invasive hydrozoans. International Jellyfish Blooms Symposium 2007

APPENDIX A

Predicting the Effects of Invasive Hydrozoa (Jellyfish) on Pelagic Organisms Under Changing Salinity and Temperature Regimes

Pelagic organisms are in serious decline in the San Francisco Estuary (SFE). A potentially important, yet understudied, factor in this decline is the invasion of three predatory hydrozoan species (*Maeotias marginata, Moerisia sp.,* and *Blackfordia virginica)*. Our current knowledge of the basic biology and ecology of these organisms is alarmingly poor in light of both their possible negative effect on the SFE ecosystem and the increasing trends in jellyfish blooms around the globe. Our research program will investigate the potential effects of these species on the SFE ecosystem to determine factors allowing their successful establishment and spread and to predict future effects and spread. This research project involves three independent tasks: genetic analyses, field surveys, and laboratory investigations. Hydrozoans are novel predators in that they can reproduce both asexually and sexually. The capacity to reproduce asexually may confer a strong advantage for rapid population growth; therefore it is important to gain a clear understanding of both the nature of clonal diversity and how they are reproducing and spreading. In the genetic portion of this research, we will use molecular techniques to evaluate clonal diversity and mode of reproduction. Additionally, we will identify the species present in all life history forms via the development of molecular tools. The second task is two-fold and will be accomplished via directed field surveys in Suisun Marsh. In the first portion, we will establish a reliable estimation of the current distributions and abundances of invasive hydrozoans and relate these trends to water and habitat quality. The remainder of this task will involve detailed dietary analyses to determine patterns of prey selectivity (including predation on larval fishes), day/night feeding behavior, and diet overlap with planktivorous fishes. The third task will involve a suite of laboratory studies, designed to determine physiological characteristics of these species. We will quantify feeding rates upon zooplankton and larval fish prey, evaluate salinity and temperature tolerances, and determine the effects of diet and tolerances on survival and reproduction. The expected outcome of this research will be a clearer understanding of the effects of several abundant and novel hydrozoan predators on the SFE ecosystem, as well as providing information to predict trends and patterns of the populations in response to the changing SFE environment.

CALFED Science Grant
B.P. May and P.B. Moyle
$430,870

APPENDIX B



**September 2007**

**SAMPLE SEMI-ANNUAL PROJECT REPORT SEMI-ANNUAL REPORT #: 1**

**CALFED SCIENCE PROGRAM PROJECT NUMBER:** 1036

**PROJECT TERM:** April 1, 2007 – March 30, 2010

**PROJECT TITLE:** Predicting the effects of invasive Hydrozoa (jellyfish) on pelagic organisms under changing salinity and temperature regimes

## CONTRACTOR/GRANT RECIPIENT CONTACT INFORMATION

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***Lead Investigator***

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## GRANTING PROGRAM CONTACT INFORMATION

***Technical Contact***

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***Grant/Administrative Contact***

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E-MAIL: jmcgee@calwater.ca.gov

**Funding Source:** CALFED Science Program

**Project Location:** Upper San Francisco Estuary

**Brief Description of Project:** Field surveys and laboratory investigations of invasive hydrozoans in the upper San Francisco Estuary.

**Primary Objective to be Achieved:** To better understand to ecology of invasive hydrozoan species in the Upper San Francisco Estuary and their potential impact on declining fish populations.

**BUDGET SUMMARY**

| TASK/SUBTASK | % COMPLETE (BY DOLLARS) | AMOUNT INVOICED (CURRENT FISCAL YEAR) | AMOUNT INVOICED TO DATE (ALL YEARS) | PROJECTED EXPENDITURES (NEXT 6 MONTHS) |
|---|---|---|---|---|
| TASK 1 | 0% | $0 | $0 | $11,540.00 |
| TASK 2 | 6.10% | $14,400.00 | $14,400.00 | $66,336.25 |
| TASK 3 | 2.18% | $2,503.91 | $2,503.91 | $31,453.59 |
| TASK 4 | .005% | $218.00 | $218.00 | $13,290.75 |
| TOTAL | 8.285% | $17,121.91 | $17,121.91 | $122,620.59 |

**PROJECT STATUS TO DATE (BY TASK)**

TASK 2: Genetic Studies

**Activities, tasks, and milestones performed and/or completed:**

- Began monthly field collection of *Maeotias marginata, Cordylophora caspia,* and *Moerisia* sp. in Suisun Marsh (Fig. 1) and the Napa and Petaluma Rivers in May 2007
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Developed 7 polymorphic microsatellite markers each for *M. marginata* and *Moerisia* sp.
- Extracted DNA from 20-100 individuals of each species, collected throughout the study area, for genetic analyses with developed microsatellite markers
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 3—Ecological Field Studies

**Activities, tasks, and milestones performed and/or completed:**

- Began collecting monthly fish and non-gelatinous zooplankton samples in May 2007 throughout Suisun Marsh (Fig. 3)

- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Began monthly collections of hydrozoans in Suisun Marsh (Fig. 3) and the Napa and Petaluma Rivers June 2007
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 4: Ecological Laboratory Studies

**Activities, tasks, and milestones performed and/or completed:**

- Collected live *M. marginata* and *Moerisia* sp. from Suisun Marsh
- Developing methods for culture of live *M. marginata* and *Moerisia* sp. in the laboratory for future temperature and salinity tolerance level and feeding rate experiments
- Developing methods for culture of various copepod species to be used in feeding rate experiments

**PROJECT-WIDE STATUS**

ACHIEVED OBJECTIVES, FINDINGS, AND CONTRIBUTIONS:

- Sample collection

PROBLEMS OR DELAYS ENCOUNTERED: Continued concerns about low numbers of Delta smelt have imposed limits on the collection of this species. Therefore, we will not be able to include Delta smelt in the analysis of medusae diet overlap with fishes.

DELIVERABLES:

- Public Summary
- Semi-Annual Report #1

PERSONNEL CHANGES: none

CONTRACT MODIFICATIONS: none

NOTES/OTHER:

# April 2008

## SAMPLE SEMI-ANNUAL PROJECT REPORT SEMI-ANNUAL REPORT #: 2

**CALFED SCIENCE PROGRAM PROJECT NUMBER:** 1036

**PROJECT TERM:** April 1, 2007 – March 30, 2010

**PROJECT TITLE:** Predicting the effects of invasive Hydrozoa (jellyfish) on pelagic organisms under changing salinity and temperature regimes

## CONTRACTOR/GRANT RECIPIENT CONTACT INFORMATION

***Program Administrator***

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***Lead Investigator***

NAME: Dr. Bernie May
INSTITUTION: UC Davis
ADDRESS: Department of Animal Sciences, 1 Shields Avenue Davis, CA 95616
PHONE: 530 754 8123
EMAIL: bpmay@ucdavis.edu

## GRANTING PROGRAM CONTACT INFORMATION

***Technical Contact***

NAME: Ladd Lougee
TITLE: Staff Environmental Scientist
AGENCY: CALFED Science Program
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***Grant/Administrative Contact***

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AGENCY: CALFED Science Program
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E-MAIL: jmcgee@calwater.ca.gov

**Funding Source:** CALFED Science Program

**Project Location:** Upper San Francisco Estuary

**Brief Description of Project:** Field surveys and laboratory investigations of invasive hydrozoans in the upper San Francisco Estuary.

**Primary Objective to be Achieved:** To better understand to ecology of invasive hydrozoan species in the Upper San Francisco Estuary and their potential impact on declining fish populations.

**BUDGET SUMMARY**

| TASK/SUBTASK | % COMPLETE (BY DOLLARS) | AMOUNT INVOICED (CURRENT FISCAL YEAR) | AMOUNT INVOICED TO DATE (ALL YEARS) | PROJECTED EXPENDITURES (NEXT 6 MONTHS) |
|---|---|---|---|---|
| TASK 1 | 1.6% | $183.40 | $183.40 | $7000 |
| TASK 2 | 28.7% | $23189.56 | $23189.56 | $20000 |
| TASK 3 | 4.9% | $5573.13 | $5573.13 | $20000 |
| TASK 4 | 3% | $272.55 | $272.55 | $10000 |
| TOTAL | 6.8% (% of entire grant) | $29218.64 | $29218.64 | $57000 |

**PROJECT STATUS TO DATE (BY TASK)**

TASK 2: Genetic Studies

**September 2008-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Collected hydrozoan samples through Dept. of Water Resources sampling programs within Suisun Bay
- Identified and sorted samples collected for genetic analyses
- Completed initial development of molecular markers for use in species identification
- Identified polyps collected to species using developed molecular markers
- Developed further microsatellite markers for analyses of genetic diversity and reproductive mode
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Began monthly field collection of *Maeotias marginata, Cordylophora caspia,* and *Moerisia* sp. in Suisun Marsh (Fig. 1) and the Napa and Petaluma Rivers in May 2007
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Developed 7 polymorphic microsatellite markers each for *M. marginata* and *Moerisia* sp.

- Extracted DNA from 20-100 individuals of each species, collected throughout the study area, for genetic analyses with developed microsatellite markers
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 3—Ecological Field Studies

**September 2008-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Began analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Obtained hydrozoan samples from CDFG collected within Suisun Bay during 2007
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Began collecting monthly fish and non-gelatinous zooplankton samples in May 2007 throughout Suisun Marsh (Fig. 3)
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Began monthly collections of hydrozoans in Suisun Marsh (Fig. 3) and the Napa and Petaluma Rivers June 2007
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 4: Ecological Laboratory Studies

**September 2008-April 2008:**

- Procured facilities in which experiments/culture will take place Summer 2008
- Contacted experts in jellyfish culture for husbandry advice

**April 2007-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Collected live *M. marginata* and *Moerisia* sp. from Suisun Marsh
- Developed methods for culture of live *M. marginata* and *Moerisia* sp. in the laboratory for future temperature and salinity tolerance level and feeding rate experiments
- Developed methods for culture of various copepod species to be used in feeding rate experiments

## PROJECT-WIDE STATUS

ACHIEVED OBJECTIVES, FINDINGS, AND CONTRIBUTIONS:

- Sample collection

PROBLEMS OR DELAYS ENCOUNTERED:

**September 2008-April 2008:** Strict collection regulations of longfin smelt during their candidacy period for Federal listing have imposed limits on the collection of this species for the

2008 field season. We have contacted Mr. Chuck Armor (CDFG) for permission to collect a limited number of longfin smelt and are awaiting a reply. Pending the outcome of this interaction, we may not be able to include longfin smelt in the analysis of medusae diet overlap with fishes during 2008.

**April 2007-September 2008:** Continued concerns about low numbers of Delta smelt have imposed limits on the collection of this species. Therefore, we will not be able to include Delta smelt in the analysis of medusae diet overlap with fishes.

DELIVERABLES:

- Semi-Annual Report #2
- Summary
- Semi-Annual Report #1

PERSONNEL CHANGES: none

CONTRACT MODIFICATIONS: none

NOTES/OTHER:

**September 2008**

**SEMI-ANNUAL PROJECT REPORT SEMI-ANNUAL REPORT #: 3**

**CALFED SCIENCE PROGRAM PROJECT NUMBER:** 1036

**PROJECT TERM:** April 1, 2007 – March 30, 2010

**PROJECT TITLE:** Predicting the effects of invasive Hydrozoa (jellyfish) on pelagic organisms under changing salinity and temperature regimes

## CONTRACTOR/GRANT RECIPIENT CONTACT INFORMATION

***Program Administrator***

NAME: Kimberly Lamar
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PHONE: 530 747 3924
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***Lead Investigator***

NAME: Dr. Bernie May
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## GRANTING PROGRAM CONTACT INFORMATION

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***Grant/Administrative Contact***

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E-MAIL: jmcgee@calwater.ca.gov

**Funding Source:** CALFED Science Program

**Project Location:** Upper San Francisco Estuary

**Brief Description of Project:** Field surveys and laboratory investigations of invasive hydrozoans in the upper San Francisco Estuary.

**Primary Objective to be Achieved:** To better understand to ecology of invasive hydrozoan species in the Upper San Francisco Estuary and their potential impact on declining fish populations.

**BUDGET SUMMARY**

| TASK/SUBTASK | % COMPLETE (BY DOLLARS) | AMOUNT INVOICED (CURRENT FISCAL YEAR) | AMOUNT INVOICED TO DATE (ALL YEARS) | PROJECTED EXPENDITURES (NEXT 6 MONTHS) |
|---|---|---|---|---|
| TASK 1 | 1.46% | $352.00 | $535.00 | $7,000 |
| TASK 2 | 24.67% | $35,049.00 | $58,239.00 | $20,000 |
| TASK 3 | 26.54% | $24,867.00 | $30,440.00 | $20,000 |
| TASK 4 | 28.02% | $272.55 | $12,199.00 | $10,000 |
| TOTAL | 23.5% (% of entire grant) | $60,540.55 | $101,413.00 | $57,000 |

**PROJECT STATUS TO DATE (BY TASK)**

TASK 2: Genetic Studies

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Identified and sorted samples collected for genetic analyses
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Writing methods note on microsatellite marker development
- Developing Taqman assay for rapid species identification

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Collected hydrozoan samples through Dept. of Water Resources sampling programs within Suisun Bay
- Identified and sorted samples collected for genetic analyses
- Completed initial development of molecular markers for use in species identification
- Identified polyps collected to species using developed molecular markers

- Developed further microsatellite markers for analyses of genetic diversity and reproductive mode
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Began monthly field collection of *Maeotias marginata, Cordylophora caspia,* and *Moerisia* sp. in Suisun Marsh (Fig. 1) and the Napa and Petaluma Rivers in May 2007
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Developed 7 polymorphic microsatellite markers each for *M. marginata* and *Moerisia* sp.
- Extracted DNA from 20-100 individuals of each species, collected throughout the study area, for genetic analyses with developed microsatellite markers
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 3—Ecological Field Studies

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Continued analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Began analyzing polyp gut contents
- Completed digestion rate experiments with polyps and medusae
- Presented research at American Society of Ichthyologists and Herpetologists Society Annual Meeting (Montreal, Canada)

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Began analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Obtained hydrozoan samples from CDFG collected within Suisun Bay during 2007
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Began collecting monthly fish and non-gelatinous zooplankton samples in May 2007 throughout Suisun Marsh (Fig. 3)
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Began monthly collections of hydrozoans in Suisun Marsh (Fig. 3) and the Napa and Petaluma Rivers June 2007
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 4: Ecological Laboratory Studies

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Collected individuals from field and developed methods for culture of all four hydrozoan species
- Completed 10 day temperature and salinity tolerance experiment with *M. marginata* (medusae), *Moerisia* sp. (medusae), and *C. caspia* (polyps).
- Completed feeding rates experiments with *B. virginica* (medusae), *M. marginata* (medusae), *Moerisia* sp. (medusae and polyps), and *C. caspia* (polyps).

**September 2007-April 2008:**

- Procured facilities in which experiments/culture will take place Summer 2008
- Contacted experts in jellyfish culture for husbandry advice

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Collected live *M. marginata* and *Moerisia* sp. from Suisun Marsh
- Developed methods for culture of live *M. marginata* and *Moerisia* sp. in the laboratory for future temperature and salinity tolerance level and feeding rate experiments
- Developed methods for culture of various copepod species to be used in feeding rate experiments

## PROJECT-WIDE STATUS

ACHIEVED OBJECTIVES, FINDINGS, AND CONTRIBUTIONS:

- Sample collection
- Feeding rates experiments
- Digestion rate experiments

PROBLEMS OR DELAYS ENCOUNTERED:

**April 2008-September 2008:** We attempted to successfully complete the 20-day temperature and salinity tolerance experiment using *M. marginata* medusae, *Moerisia* sp. medusae, and *C. caspia* polyps. We found the medusae did not respond well in the long term to the culture containers, despite our attempts to design them in an appropriate fashion (based on the guidance of consulted jellyfish experts). Medusae held in the long term developed 'bell rot' and their condition deteriorated quickly. Therefore, we halted the experiment after 10 days. *C. caspia* did well during the experiment and produced usable data. Time and resources permitting, we will attempt a short term critical tolerance experiment during the summer of 2009 to test the temperature and salinity tolerance levels of these species.

**September 2007-April 2008:** Strict collection regulations of longfin smelt during their candidacy period for Federal listing have imposed limits on the collection of this species for the 2008 field season. We have contacted Mr. Chuck Armor (CDFG) for permission to collect a limited number of longfin smelt and are awaiting a reply. Pending the outcome of this interaction, we may not be able to include longfin smelt in the analysis of medusae diet overlap with fishes during 2008.

**April 2007-September 2007:** Continued concerns about low numbers of Delta smelt have imposed limits on the collection of this species. Therefore, we will not be able to include Delta smelt in the analysis of medusae diet overlap with fishes.

DELIVERABLES:

- Semi-Annual Report #3
- Semi-Annual Report #2
- Summary
- Semi-Annual Report #1

PERSONNEL CHANGES: none

CONTRACT MODIFICATIONS: none

NOTES/OTHER:

# November 2009

**SEMI-ANNUAL PROJECT REPORT SEMI-ANNUAL REPORT #: 4**
**CALFED SCIENCE PROGRAM PROJECT NUMBER:** 1036
**PROJECT TERM:** April 1, 2007 – December 31, 2010
**PROJECT TITLE:** Predicting the effects of invasive Hydrozoa (jellyfish) on pelagic organisms under changing salinity and temperature regimes

## CONTRACTOR/GRANT RECIPIENT CONTACT INFORMATION

***Program Administrator***
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***Lead Investigator***
NAME: Dr. Bernie May
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## GRANTING PROGRAM CONTACT INFORMATION

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***Grant/Administrative Contact***
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PHONE: 916-445-7690
E-MAIL: jmcgee@calwater.ca.gov

**Funding Source:** CALFED Science Program

**Project Location:** Upper San Francisco Estuary

**Brief Description of Project:** Field surveys and laboratory investigations of invasive hydrozoans in the upper San Francisco Estuary.

**Primary Objective to be Achieved:** To better understand to ecology of invasive hydrozoan species in the Upper San Francisco Estuary and their potential impact on declining fish populations.

**BUDGET SUMMARY**

| TASK/SUBTASK | % COMPLETE (BY DOLLARS) | AMOUNT INVOICED (CURRENT FISCAL YEAR) | AMOUNT INVOICED TO DATE (ALL YEARS) | PROJECTED EXPENDITURES (NEXT 6 MONTHS) |
|---|---|---|---|---|
| TASK 1 | 30.86% | $0.00 | $11,293.39 | $10,000.00 |
| TASK 2 | 37.96% | $0.00 | $89,617.91 | $60,000.00 |
| TASK 3 | 31.53% | $0.00 | $36,167.77 | $30,000.00 |
| TASK 4 | 35.27% | $0.00 | $15,351.98 | $15,000.00 |
| TOTAL | 35.38% | $0.00 | $152,431.05 | $115,000.00 |

**PROJECT STATUS TO DATE (BY TASK)**

TASK 2: Genetic Studies

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Completed and published microsatellite marker development manuscript (Meek et al. *in press*)
- Continued genotyping of *M. marginata* and *Moerisia* sp. samples using microsatellite markers
- Presented preliminary findings of genetic and clonal diversity of *Moerisia* sp. at the Coastal and Estuarine Research Federation Biennial Conference in Portland, OR

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Identified and sorted samples collected for genetic analyses
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Writing methods note on microsatellite marker development
- Developing Taqman assay for rapid species identification

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Collected hydrozoan samples through Dept. of Water Resources sampling programs within Suisun Bay
- Identified and sorted samples collected for genetic analyses
- Completed initial development of molecular markers for use in species identification
- Identified polyps collected to species using developed molecular markers
- Developed further microsatellite markers for analyses of genetic diversity and reproductive mode
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Began monthly field collection of *Maeotias marginata, Cordylophora caspia,* and *Moerisia* sp. in Suisun Marsh (Fig. 1) and the Napa and Petaluma Rivers in May 2007
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Developed 7 polymorphic microsatellite markers each for *M. marginata* and *Moerisia* sp.
- Extracted DNA from 20-100 individuals of each species, collected throughout the study area, for genetic analyses with developed microsatellite markers
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 3—Ecological Field Studies

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Completed processing polyp samples, zooplankton samples, and jellyfish gut content samples
- Completed draft of polyp ecology manuscript for publication
- Began processing fish gut content samples
- Writing draft of *Moerisia* sp. population dynamics manuscript
- Presented polyp research at Coastal and Estuarine Research Federation Biennial Conference in Portland, OR and CALFED Science Conference in Sacramento, CA

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Continued analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Began analyzing polyp gut contents
- Completed digestion rate experiments with polyps and medusae
- Presented research at American Society of Ichthyologists and Herpetologists Society Annual Meeting (Montreal, Canada)

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh

- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Began analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Obtained hydrozoan samples from CDFG collected within Suisun Bay during 2007
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Began collecting monthly fish and non-gelatinous zooplankton samples in May 2007 throughout Suisun Marsh (Fig. 3)
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- Began monthly collections of hydrozoans in Suisun Marsh (Fig. 3) and the Napa and Petaluma Rivers June 2007
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 4: Ecological Laboratory Studies

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Analyzed data from temperature and salinity tolerance experiments and feeding rates experiments with *M. marginata, Moerisia* sp., and *C. caspia*
- Writing feeding rates and tolerance level draft manuscripts
- Presented feeding rates research at CALFED Science Conference in Sacramento, CA

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Collected individuals from field and developed methods for culture of all four hydrozoan species
- Completed 10 day temperature and salinity tolerance experiment with *M. marginata* (medusae), *Moerisia* sp. (medusae), and *C. caspia* (polyps).
- Completed feeding rates experiments with *B. virginica* (medusae), *M. marginata* (medusae), *Moerisia* sp. (medusae and polyps), and *C. caspia* (polyps).

**September 2007-April 2008:**

- Procured facilities in which experiments/culture will take place Summer 2008
- Contacted experts in jellyfish culture for husbandry advice

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Collected live *M. marginata* and *Moerisia* sp. from Suisun Marsh
- Developed methods for culture of live *M. marginata* and *Moerisia* sp. in the laboratory for future temperature and salinity tolerance level and feeding rate experiments
- Developed methods for culture of various copepod species to be used in feeding rate experiments

## PROJECT-WIDE STATUS

ACHIEVED OBJECTIVES, FINDINGS, AND CONTRIBUTIONS:

- Sample collection
- Feeding rates experiments
- Digestion rate experiments
- Microsatellite marker development

PROBLEMS OR DELAYS ENCOUNTERED:
**July 2009-November 2009:** None to report

**April 2008-September 2008:** We attempted to successfully complete the 20-day temperature and salinity tolerance experiment using *M. marginata* medusae, *Moerisia* sp. medusae, and *C. caspia* polyps. We found the medusae did not respond well in the long term to the culture containers, despite our attempts to design them in an appropriate fashion (based on the guidance of consulted jellyfish experts). Medusae held in the long term developed 'bell rot' and their condition deteriorated quickly. Therefore, we halted the experiment after 10 days. *C. caspia* did well during the experiment and produced usable data. Time and resources permitting, we will attempt a short term critical tolerance experiment during the summer of 2009 to test the temperature and salinity tolerance levels of these species.

**September 2007-April 2008:** Strict collection regulations of longfin smelt during their candidacy period for Federal listing have imposed limits on the collection of this species for the 2008 field season. We have contacted Mr. Chuck Armor (CDFG) for permission to collect a limited number of longfin smelt and are awaiting a reply. Pending the outcome of this interaction, we may not be able to include longfin smelt in the analysis of medusae diet overlap with fishes during 2008.

**April 2007-September 2007:** Continued concerns about low numbers of Delta smelt have imposed limits on the collection of this species. Therefore, we will not be able to include Delta smelt in the analysis of medusae diet overlap with fishes.

DELIVERABLES:

- Semi-Annual Report #4
- Microsatellite marker development manuscript
- Semi-Annual Report #3
- Semi-Annual Report #2
- Summary
- Semi-Annual Report #1

PERSONNEL CHANGES: none

CONTRACT MODIFICATIONS: temporal extension granted until December 31, 2010

NOTES/OTHER:

**April 2010**

**SEMI-ANNUAL PROJECT REPORT SEMI-ANNUAL REPORT #: 5**

**CALFED SCIENCE PROGRAM PROJECT NUMBER:** 1036

**PROJECT TERM:** April 1, 2007 – December 31, 2010

**PROJECT TITLE:** Predicting the effects of invasive Hydrozoa (jellyfish) on pelagic organisms under changing salinity and temperature regimes

## CONTRACTOR/GRANT RECIPIENT CONTACT INFORMATION

***Program Administrator***

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## GRANTING PROGRAM CONTACT INFORMATION

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***Grant/Administrative Contact***

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**Funding Source:** CALFED Science Program

**Project Location:** Upper San Francisco Estuary

**Brief Description of Project:** Field surveys and laboratory investigations of invasive hydrozoans in the upper San Francisco Estuary.

**Primary Objective to be Achieved:** To better understand to ecology of invasive hydrozoan species in the Upper San Francisco Estuary and their potential impact on declining fish populations.

**BUDGET SUMMARY**

| TASK/SUBTASK | % COMPLETE (BY DOLLARS) | AMOUNT INVOICED (CURRENT FISCAL YEAR) | AMOUNT INVOICED TO DATE (ALL YEARS) | PROJECTED EXPENDITURES (NEXT 6 MONTHS) |
|---|---|---|---|---|
| TASK 1 | 30.86% | $0.00 | $11,293.39 | $10,000.00 |
| TASK 2 | 37.96% | $0.00 | $89,617.91 | $60,000.00 |
| TASK 3 | 31.53% | $0.00 | $36,167.77 | $30,000.00 |
| TASK 4 | 35.27% | $0.00 | $15,351.98 | $15,000.00 |
| TOTAL | 35.38% | $0.00 | $152,431.05 | $115,000.00 |

**PROJECT STATUS TO DATE (BY TASK)**

TASK 2: Genetic Studies

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Completed and published microsatellite marker development manuscript (Meek et al. *in press*)
- Continued genotyping of *M. marginata* and *Moerisia* sp. samples using microsatellite markers
- Presented preliminary findings of genetic and clonal diversity of *Moerisia* sp. at the Coastal and Estuarine Research Federation Biennial Conference in Portland, OR

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Identified and sorted samples collected for genetic analyses
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Writing methods note on microsatellite marker development
- Developing Taqman assay for rapid species identification

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Collected hydrozoan samples through Dept. of Water Resources sampling programs within Suisun Bay
- Identified and sorted samples collected for genetic analyses
- Completed initial development of molecular markers for use in species identification
- Identified polyps collected to species using developed molecular markers
- Developed further microsatellite markers for analyses of genetic diversity and reproductive mode
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Began monthly field collection of *Maeotias marginata, Cordylophora caspia,* and *Moerisia* sp. in Suisun Marsh (Fig. 1) and the Napa and Petaluma Rivers in May 2007
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Developed 7 polymorphic microsatellite markers each for *M. marginata* and *Moerisia* sp.
- Extracted DNA from 20-100 individuals of each species, collected throughout the study area, for genetic analyses with developed microsatellite markers
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 3—Ecological Field Studies

**November 2009-April 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed processing fish gut content samples
- Completed draft of *Moerisia* sp. population dynamics manuscript for publication
- Writing draft of hydrozoan-fish trophic interactions manuscript

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Completed processing polyp samples, zooplankton samples, and jellyfish gut content samples
- Completed draft of polyp ecology manuscript for publication
- Began processing fish gut content samples
- Writing draft of *Moerisia* sp. population dynamics manuscript
- Presented polyp research at Coastal and Estuarine Research Federation Biennial Conference in Portland, OR and CALFED Science Conference in Sacramento, CA

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Continued analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Began analyzing polyp gut contents
- Completed digestion rate experiments with polyps and medusae

- Presented research at American Society of Ichthyologists and Herpetologists Society Annual Meeting (Montreal, Canada)

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
- Continued collection of polyp settling plate arrays monthly plate in Suisun Marsh and the Napa and Petaluma Rivers
- Began analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Obtained hydrozoan samples from CDFG collected within Suisun Bay during 2007
- Presented research at the 8th Biennial State of the San Francisco Estuary Conference

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Began collecting monthly fish and non-gelatinous zooplankton samples in May 2007 throughout Suisun Marsh (Fig. 3)
- Deployed polyp settling plate arrays and began monthly plate collection in Suisun Marsh (Fig. 2) and the Napa and Petaluma Rivers in June 2007
- Began monthly collections of hydrozoans in Suisun Marsh (Fig. 3) and the Napa and Petaluma Rivers June 2007
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 4: Ecological Laboratory Studies

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Analyzed data from temperature and salinity tolerance experiments and feeding rates experiments with *M. marginata, Moerisia* sp., and *C. caspia*
- Writing feeding rates and tolerance level draft manuscripts
- Presented feeding rates research at CALFED Science Conference in Sacramento, CA

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Collected individuals from field and developed methods for culture of all four hydrozoan species
- Completed 10 day temperature and salinity tolerance experiment with *M. marginata* (medusae), *Moerisia* sp. (medusae), and *C. caspia* (polyps).
- Completed feeding rates experiments with *B. virginica* (medusae), *M. marginata* (medusae), *Moerisia* sp. (medusae and polyps), and *C. caspia* (polyps).

**September 2007-April 2008:**

- Procured facilities in which experiments/culture will take place Summer 2008
- Contacted experts in jellyfish culture for husbandry advice

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Collected live *M. marginata* and *Moerisia* sp. from Suisun Marsh
- Developed methods for culture of live *M. marginata* and *Moerisia* sp. in the laboratory for future temperature and salinity tolerance level and feeding rate experiments
- Developed methods for culture of various copepod species to be used in feeding rate experiments

**PROJECT-WIDE STATUS**

ACHIEVED OBJECTIVES, FINDINGS, AND CONTRIBUTIONS:

- Sample collection
- Feeding rates experiments
- Digestion rate experiments
- Microsatellite marker development

PROBLEMS OR DELAYS ENCOUNTERED:
**July 2009-November 2009:** None to report

**April 2008-September 2008:** We attempted to successfully complete the 20-day temperature and salinity tolerance experiment using *M. marginata* medusae, *Moerisia* sp. medusae, and *C. caspia* polyps. We found the medusae did not respond well in the long term to the culture containers, despite our attempts to design them in an appropriate fashion (based on the guidance of consulted jellyfish experts). Medusae held in the long term developed 'bell rot' and their condition deteriorated quickly. Therefore, we halted the experiment after 10 days. *C. caspia* did well during the experiment and produced usable data. Time and resources permitting, we will attempt a short term critical tolerance experiment during the summer of 2009 to test the temperature and salinity tolerance levels of these species.

**September 2007-April 2008:** Strict collection regulations of longfin smelt during their candidacy period for Federal listing have imposed limits on the collection of this species for the 2008 field season. We have contacted Mr. Chuck Armor (CDFG) for permission to collect a limited number of longfin smelt and are awaiting a reply. Pending the outcome of this interaction, we may not be able to include longfin smelt in the analysis of medusae diet overlap with fishes during 2008.

**April 2007-September 2007:** Continued concerns about low numbers of Delta smelt have imposed limits on the collection of this species. Therefore, we will not be able to include Delta smelt in the analysis of medusae diet overlap with fishes.

DELIVERABLES:

- Semi-Annual Report #5
- Semi-Annual Report #4
- Microsatellite marker development manuscript
- Semi-Annual Report #3
- Semi-Annual Report #2
- Summary
- Semi-Annual Report #1

PERSONNEL CHANGES: none

CONTRACT MODIFICATIONS: temporal extension granted until December 31, 2010

NOTES/OTHER:

# November 2010

**SEMI-ANNUAL PROJECT REPORT SEMI-ANNUAL REPORT #: 6**
**CALFED SCIENCE PROGRAM PROJECT NUMBER:** 1036
**PROJECT TERM:** April 1, 2007 – June 30, 2011
**PROJECT TITLE:** Predicting the effects of invasive Hydrozoa (jellyfish) on pelagic organisms under changing salinity and temperature regimes

## CONTRACTOR/GRANT RECIPIENT CONTACT INFORMATION

***Program Administrator***
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***Lead Investigator***
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***Grant/Administrative Contact***
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E-MAIL: jmcgee@calwater.ca.gov

**Funding Source:** CALFED Science Program

**Project Location:** Upper San Francisco Estuary

**Brief Description of Project:** Field surveys and laboratory investigations of invasive hydrozoans in the upper San Francisco Estuary.

**Primary Objective to be Achieved:** To better understand to ecology of invasive hydrozoan species in the Upper San Francisco Estuary and their potential impact on declining fish populations.

**BUDGET SUMMARY**

| TASK/SUBTASK | % COMPLETE (BY DOLLARS) | AMOUNT INVOICED (CURRENT FISCAL YEAR) | AMOUNT INVOICED TO DATE (ALL YEARS) | PROJECTED EXPENDITURES (NEXT 6 MONTHS) |
|---|---|---|---|---|
| TASK 1 | 60.15% | $ 9,412.77 | $ 25,803.79 | $ 17,094.05 |
| TASK 2 | 91.73% | $ 62,458.89 | $ 198,069.56 | $ 17,866.40 |
| TASK 3 | 69.84% | $ 8,523.55 | $ 82,354.29 | $ 35,563.86 |
| TASK 4 | 60.72% | $ 7,399.15 | $ 32,857.97 | $ 21,260.08 |
| TOTAL | 78.70% | $ 87,794.35 | $ 339,085.61 | $ 91,784.39 |

**PROJECT STATUS TO DATE (BY TASK)**

TASK 2: Genetic Studies

**June 2010-November 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed data analyses to investigate genetic diversity and asexual and sexual reproduction in *M. marginata* and *Moerisia* sp.
- Began draft manuscript on genetic diversity and reproduction of *M. marginata* and *Moerisia* sp.
- Presented findings of genetic and clonal diversity of *M. marginata* and *Moerisia* sp. at the Bay Delta Science Conference in Sacramento, CA
- Presented findings on the invasion biology of *Moerisia* sp. at the Ecological Society of America Annual Meeting in Pittsburgh, PA

**November 2009-May 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed genotyping of *M. marginata* and *Moerisia* sp. samples using microsatellite markers
- Began data analyses to investigate genetic diversity and asexual and sexual reproduction in *M. marginata* and *Moerisia* sp.

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Completed and published microsatellite marker development manuscript (Meek et al. *in press*)
- Continued genotyping of *M. marginata* and *Moerisia* sp. samples using microsatellite markers
- Presented preliminary findings of genetic and clonal diversity of *Moerisia* sp. at the Coastal and Estuarine Research Federation Biennial Conference in Portland, OR

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Continued monthly field collections of invasive hydrozoans
- Identified and sorted samples collected for genetic analyses
- Genotyped individuals of *M. marginata* and *Moerisia* sp. using microsatellite markers
- Writing methods note on microsatellite marker development
- Developing Taqman assay for rapid species identification

**September 2007-April 2008: Activities, tasks, and milestones performed and/or completed:**

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- Developed 7 polymorphic microsatellite markers each for *M. marginata* and *Moerisia* sp.
- Extracted DNA from 20-100 individuals of each species, collected throughout the study area, for genetic analyses with developed microsatellite markers
- Presented research at the 2$^{nd}$ International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 3—Ecological Field Studies

**June 2010-November 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed draft manuscript on of hydrozoan-fish trophic interactions
- Presented findings of *Moerisia* sp. population dynamics at the Bay Delta Science Conference in Sacramento, CA

**November 2009-May 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed processing fish gut content samples
- Completed draft of *Moerisia* sp. population dynamics manuscript for publication
- Writing draft of hydrozoan-fish trophic interactions manuscript

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Completed processing polyp samples, zooplankton samples, and jellyfish gut content samples
- Completed draft of polyp ecology manuscript for publication
- Began processing fish gut content samples
- Writing draft of *Moerisia* sp. population dynamics manuscript
- Presented polyp research at Coastal and Estuarine Research Federation Biennial Conference in Portland, OR and CALFED Science Conference in Sacramento, CA

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- Continued collecting monthly fish, hydrozoan and non-gelatinous zooplankton samples throughout Suisun Marsh
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- Continued analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Began analyzing polyp gut contents
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- Began analyzing samples of polyps (ID and enumeration), zooplankton (ID and enumeration), and jellyfish (ID, enumeration)
- Obtained hydrozoan samples from CDFG collected within Suisun Bay during 2007
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**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

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- Began monthly collections of hydrozoans in Suisun Marsh (Fig. 3) and the Napa and Petaluma Rivers June 2007
- Presented research at the 2nd International Jellyfish Blooms Symposium and the American Fisheries Society Annual Meeting

TASK 4: Ecological Laboratory Studies

**June 2010-November 2010: Activities, tasks, and milestones performed and/or completed:**

- Revising draft *C. caspia* temperature and salinity tolerance and trophic studies manuscript for publication

**November 2009-May 2010: Activities, tasks, and milestones performed and/or completed:**

- Completed draft *C. caspia* temperature and salinity tolerance and trophic studies manuscript for publication

**July 2009-November 2009: Activities, tasks, and milestones performed and/or completed:**

- Analyzed data from temperature and salinity tolerance experiments and feeding rates experiments with *M. marginata, Moerisia* sp., and *C. caspia*
- Writing feeding rates and tolerance level draft manuscripts
- Presented feeding rates research at CALFED Science Conference in Sacramento, CA

**April 2008-September 2008: Activities, tasks, and milestones performed and/or completed:**

- Collected individuals from field and developed methods for culture of all four hydrozoan species
- Completed 10 day temperature and salinity tolerance experiment with *M. marginata* (medusae), *Moerisia* sp. (medusae), and *C. caspia* (polyps).
- Completed feeding rates experiments with *B. virginica* (medusae), *M. marginata* (medusae), *Moerisia* sp. (medusae and polyps), and *C. caspia* (polyps).

**September 2007-April 2008:**

- Procured facilities in which experiments/culture will take place Summer 2008
- Contacted experts in jellyfish culture for husbandry advice

**April 2007-September 2007: Activities, tasks, and milestones performed and/or completed:**

- Collected live *M. marginata* and *Moerisia* sp. from Suisun Marsh
- Developed methods for culture of live *M. marginata* and *Moerisia* sp. in the laboratory for future temperature and salinity tolerance level and feeding rate experiments
- Developed methods for culture of various copepod species to be used in feeding rate experiments

## PROJECT-WIDE STATUS

ACHIEVED OBJECTIVES, FINDINGS, AND CONTRIBUTIONS:

- Sample collection
- Feeding rates experiments
- Digestion rate experiments
- Microsatellite marker development
- Microsatellite characterization of samples
- Gut content analyses

PROBLEMS OR DELAYS ENCOUNTERED:

**June 2010-Novebmer 2010:** None to report

**November 2009-May 2010:** None to report

**July 2009-November 2009:** None to report

**April 2008-September 2008:** We attempted to successfully complete the 20-day temperature and salinity tolerance experiment using *M. marginata* medusae, *Moerisia* sp. medusae, and *C. caspia* polyps. We found the medusae did not respond well in the long term to the culture containers, despite our attempts to design them in an appropriate fashion (based on the guidance of consulted jellyfish experts). Medusae held in the long term developed 'bell rot' and their condition deteriorated quickly. Therefore, we halted the experiment after 10 days. *C. caspia* did well during the experiment and produced usable data. Time and resources permitting, we will attempt a short term critical tolerance experiment during the summer of 2009 to test the temperature and salinity tolerance levels of these species.

**September 2007-April 2008:** Strict collection regulations of longfin smelt during their candidacy period for Federal listing have imposed limits on the collection of this species for the 2008 field season. We have contacted Mr. Chuck Armor (CDFG) for permission to collect a limited number of longfin smelt and are awaiting a reply. Pending the outcome of this interaction, we may not be able to include longfin smelt in the analysis of medusae diet overlap with fishes during 2008.

**April 2007-September 2007:** Continued concerns about low numbers of Delta smelt have imposed limits on the collection of this species. Therefore, we will not be able to include Delta smelt in the analysis of medusae diet overlap with fishes.

DELIVERABLES:

- Semi-Annual Report #6

- Draft manuscript: Trophic ecology and effects of temperature and salinity on growth in the non-native hydrozoan, *Cordylophora caspia*, in the upper San Francisco Estuary, California
- Semi-Annual Report #5
- Draft manuscript: Ecological insights into the polyp stage of non-native hydrozoans in the San Francisco Estuary
- Draft manuscript: Population dynamics of *Moerisia* sp., a non-native hydrozoan in the upper San Francisco Estuary
- Semi-Annual Report #4
- Manuscript: Isolation and characterization of microsatellite loci in two non-native hydromedusae in the San Francisco Estuary: *Maeotias marginata* and *Moerisia* sp.
- Semi-Annual Report #3
- Semi-Annual Report #2
- Summary
- Semi-Annual Report #1

PERSONNEL CHANGES: none

CONTRACT MODIFICATIONS: temporal extension granted until June 30, 2010

NOTES/OTHER:

Conservation Genet Resour
DOI 10.1007/s12686-009-9050-7

APPENDIX C

TECHNICAL NOTE

# Isolation and characterization of microsatellite loci in two non-native hydromedusae in the San Francisco Estuary: *Maeotias marginata* and *Moerisia* sp.

**Mariah H. Meek · Melinda R. Baerwald · Alpa P. Wintzer · Bernie May**

Received: 18 June 2009 / Accepted: 25 June 2009
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**Abstract** We characterized 10 new microsatellite markers in each of two species of hydromedusae, *Maeotias marginata* and *Moerisia* sp. Genetic diversity was estimated using 20–41 individuals collected from Suisun Marsh within the San Francisco Estuary, CA. Allelic richness ranged from 5–9 in *M. marginata* and 2–10 in *Moerisia* sp. with average expected heterozygosities of 0.71 and 0.57 respectively. One locus in *M. marginata and* two in *Moerisia* sp. deviated from Hardy–Weinberg equilibrium expectations, likely due to null alleles.

**Keywords** Hydrozoa · Jellyfish · Oligos · San Francisco Estuary

Two species of hydromedusae, *Maeotias marginata* and *Moerisia* sp., native to the Ponto-Caspian region, have become established in the San Francisco Estuary, CA (SFE). Non-native jellyfish and other hydroids can have severe effects on the ecosystems they invade as many are voracious predators, consuming large amounts of prey and disrupting planktivorous food webs (Purcell and Arai 2001). Additionally, jellyfish blooms are increasing globally (Mills 2001) and can directly affect fish populations by devouring massive quantities of eggs and larvae and decreasing fish survival through competition for resources (Purcell and Arai 2001; Purcell et al. 2001; Purcell 2003; Lynam et al. 2005). The brackish water hydrozomedusae studied herein are novel predators in the SFE and, thus, have an especially high likelihood of impacting this system and competing with juvenile fish (Moyle and Light 1996). Very little is known about the basic biology and life history of these invaders. Microsatellite markers characterized for these species will allow us to investigate population structure and genetic diversity, as well as determine reproductive strategies and life history characteristics of these potentially important non-natives.

We collected the medusae phase of *M. marginata* and *Moerisia* sp. from Suisun Marsh in the SFE. We extracted whole genomic DNA from bell tissue of *M. marginata* and entire individuals of *Moerisia* sp., due to their small size, using Qiagen's Gentra Puregene Kit protocol. Genetic Identification Services constructed, screened, and sequenced four libraries enriched with the following repeat motifs: (1) GATA, (2) CCAT and CTGT, (3) ATG, AAC, and ATT, and (4) a mix of all tetra-nucleotide repeats available, using pooled DNA from both species and according to the procedures of Meredith and May (2002). A total of 381 clones were sequenced.

We analyzed sequences using SEQUENCHER version 4.7 (Gene Codes Corporation) and used MREPS version 2.5 (Kolpakov et al. 2003) to identify repeat regions. Once repeat loci were located, we employed PRIMER 3 (Rozen and Skaletsky 2000) to create primer pairs flanking each region of interest. We then tested primer pairs on 2–10 individuals of each species to assess microsatellite amplification and level of polymorphism.

Some primer pairs worked best with Promega GoTaq Flexi DNA polymerase, while others worked better with Roche FastStart Taq DNA polymerase. The recipe for those with Promega Taq was 2 µl 5× Promega Buffer, 0.8 µl of 10 µM dNTPs, 0.6 µl of 25 µM Mg, 0.08 µl

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Published online: 07 July 2009

**Table 1** Characterization of new microsatellite loci in hydromedusae from the San Francisco Estuary

| Locus | Sp. | GenBank Accession no. | Primer sequence (5′–3′) forward and reverse | Primer 5′ label | *N* | Repeat motif | Annealing temp °C (Taq used) | *A* (size range in bp) | $H_O$ | $H_E$ | $HWE_{pv}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MmaG105 | MA | GQ281247 | F: AACGGATCCACCACTAGCAC<br>R: TTTGTTCATTGTTCGTTTGTGA | PET | 26 | $(TACA)_{35}$ | 58 (P) | 9 (280–367) | 0.92 | 0.83 | 0.85 |
| MmaG107-2 | MA | GQ281248 | F: GGTAGAACTCTGGATACATGCAA<br>R: TCTATGCTAACTGGGATGGAGA | PET | 27 | $(TCAG)_9$…$(GTCT)_{21}$ | 56 (F) | 5 (213–234) | 0.85 | 0.74 | 0.80 |
| MmaG108 | MA | GQ281249 | F: ACTCCGCTCGTCTGTCAGTT<br>R: GAGTCCGTGGCGAAGTTATG | VIC | 30 | $(AGTC)_6$…$(CAGT)_{16}$ | 60 (P) | 5 (171–184) | 0.83 | 0.76 | 0.08 |
| MmaG137-2 | MA | GQ281250 | F: CATCAGCAAGACGAAGGTGA<br>R: GCGCCGCATATTATGTAACC | 6-FAM | 20 | $(AGAT)_8$ | 60 (P) | 5 (140–201) | 0.60 | 0.58 | 1.00 |
| MmaG139 | MA | GQ281251 | F: AATTGTCCACCCTCAGTTCG<br>R: TACTCTGCCAGACTGCTTGC | VIC | 25 | $(AGAC)_{14}$ | 58 (f) | 5 (374–398) | 0.92 | 0.80 | 0.87 |
| MmaG142 | MA | GQ281252 | F: TGGAACAGGTTGAACGACTG<br>R: AAACTGGTCAGGGATGTTCG | NED | 36 | $(ATAG)_{13}$ | TD[a] (F) | 6 (210–230) | 0.64 | 0.66 | 0.16 |
| MmaG154-1 | MA | GQ281253 | F: GACGTACGTGCGAAGTACCC<br>R: AGAGACGGACCGACTGAAGA | NED | 20 | $(TCTG)_{15}$…$(CTGT)_{18}$ | 60 (P) | 6 (227–308) | 0.90 | 0.80 | 0.39 |
| MmaG155 | MA | GQ281254 | F: GATGGCTTCTCGTACATGACC<br>R: TACTGAGCAGGCGTACATGG | PET | 41 | $(ATCTTT)_4$ | TD[a] (F) | 4 (303–329) | 0.56 | 0.54 | 0.90 |
| MmaG157 | MA | GQ281255 | F: TGAGTCCCGACTTGAAGTGA<br>R: GTTTTCCCAGTCACGACGTT | VIC | 30 | $(ACA)_{19}$ | 50 (F) | 5 (209–581) | 0.60 | 0.77 | 0.00* |
| MmaG177 | MA | GQ281256 | F: TGTTTGGAAAACCGAAAAGC<br>R: ACAAGCAAGCCATGCACATA | 6-FAM | 25 | $(AGAT)_{13}$ | 50 (F) | 4 (250–262) | 0.60 | 0.61 | 0.52 |
| MmoG109 | MO | GQ281257 | F: CAATACATTCGCAAATAAACAAACT<br>R: CCCCGCTGTATTGGTGTTA | PET | 24 | $(AAC)_4$ | 50 (P) | 3 (270–292) | 0.04 | 0.12 | 0.02 |
| MmoG115 | MO | GQ281258 | F: CCATTGTGCTGCAACGTATT<br>R: ATTGTTACTTTTCGTTGTTATGTCCT | NED | 25 | $(ACA)_{16}$…$(AAC)_4$ | 50 (P) | 10 (237–263) | 0.64 | 0.60 | 0.50 |
| MmoG123 | MO | GQ281259 | F: AGGTGCTTCTTGTGGGATTCT<br>R: ACCAACAGGAAACGAACCAA | PET | 22 | $(TTG)_{20}$ | 50 (P) | 6 (311–346) | 0.73 | 0.72 | 0.73 |
| MmoG133 | MO | GQ281260 | F: TCATCATGACGCCACTTGTT<br>R: TGATGTTGATCGTGGTTGTTTT | 6-FAM | 25 | $(AAAC)_9$ | 50 (P) | 4 (307–329) | 0.64 | 0.53 | 0.66 |
| MmoG138 | MO | GQ281261 | F: TTTGCACGAATGTTTGGTGT<br>R: CCTCATGCTTAAGTGGTGCTC | VIC | 28 | $(AGAAGGAA)_2$ | 58 (F) | 4 (173–183) | 0.64 | 0.69 | 0.04 |
| MmoG167 | MO | GQ281262 | F: AGCTTCATTTGGACGCAAAG<br>R: ACATTTTGTCGGCGATTACC | VIC | 24 | $(AAC)_{23}$ | 58 (F) | 8 (336–367) | 0.50 | 0.87 | 0.00* |
| MmoG181 | MO | GQ281263 | F: CGAAGCTAGCTAATGAAATGACC<br>R: GCTCTTATGGATGGGTTTGC | VIC | 29 | $(AAC)_{17}$ | 50 (F) | 5 (277–303) | 0.76 | 0.73 | 0.21 |

**Table 1** continued

| Locus | Sp. | GenBank Accession no. | Primer sequence (5′–3′) forward and reverse | Primer 5′ label | *N* | Repeat motif | Annealing temp °C (Taq used) | *A* (size range in bp) | $H_O$ | $H_E$ | $HWE_{pv}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MmoG183 | MO | GQ281264 | F: ACAAAGTTTAGGTGAACTGTGCTC<br>R: ACGCCAGTGTGGTATGTGAT | 6-FAM | 29 | $(TA)_9$ | 50 (P) | 2 (147–149) | 0.24 | 0.22 | 1.00 |
| MmoG194 | MO | GQ281265 | F: TTTGCGCACGTATGACAAAT<br>R: TGAGGTATTTTGTTGAAGGTTGG | VIC | 24 | $(AATG)_8$…$(TGAA)_5$ | 50 (P) | 6 (376–436) | 0.79 | 0.71 | 0.94 |
| MmoG196 | MO | GQ281266 | F: TCTTACAACTTTGCCCACGA<br>R: CATGCCCGCCACAAT | PET | 24 | $(CAGA)_4$ | 50 (F) | 4 (147–340) | 0.04 | 0.49 | 0.00* |

Shown are locus name, species (Sp.) (MA, *Maeotias marginata*; MO, *Moerisia* sp.), GenBank accession number, forward and reverse primer sequences, primer dye label, repeat motif, PCR annealing temperature and Taq polymerase used (P = Promega, F = Roche FastStart), number alleles observed (*A*) and allele size range, observed and expected heterozygosities ($H_O$ and $H_E$, respectively), and *P* values for tests for deviation from Hardy–Weinberg Equilibrium ($HWE_{pv}$)

[a] *TD* Touch down PCR conditions: 95°C for 5 min, 15 cycles of 94°C for 30 s, 65°C for 30 s (decreasing by 1°C with each cycle), and 72°C for 1 min, 15 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min, and hold at 10°C

* Significant after Bonferroni correction

Promega Taq, and 0.4–0.7 µl of each 1 µM primer. The recipe for PCRs run with FastStart Taq was 1 µl 10× FastStart Buffer (with $MgCl_2$), 0.8 µl of 10 µM dNTPs, 0.3 µl FastStart Taq, and 0.4–0.7 µl of each 1 µM primer, with the exception of primers MmoG167 which were used at 5 µM. For all reactions, enough water was added to bring the total reaction volume up to 10 µl. All PCRs were performed using a Bio-Rad DNA Engine Dyad thermal cycler using the following cycling parameters: 95°C for 5 min, and 30 cycles of 94°C for 30 s, various annealing temperatures for 30 s (See Table 1), and 72°C for 1 min, followed by 60°C for 45 min, and held at 10°C. Loci were screened by diluting PCR products 1:1 with 98% formamide loading buffer, denaturing at 95°C for 3 min, and separating PCR products on a 5% denaturing polyacrylamide gel at 50 W. We visualized the gel by overlaying with SYBR-Green-agarose following the protocol of Rodzen et al. (1998) and scanning with a GE Healthcare Fluorimager 595.

Ten for each species of the 71 loci screened were polymorphic and well-resolved in the initial screening. These loci were then characterized with additional individuals to bring the total to a minimum of 20 individuals screened (Table 1). This characterization was completed by adding a 5′ fluorescent label (NED, VIC, and PET from Applied Biosystems, 6-FAM from Integrated DNA Technologies) to the forward primer. One ml of labeled PCR product was added to 8.8 µl of highly deionized formamide (Gel Company) and 0.2 µl of LIZ600 size standard (ABI) and run on an ABI 3130xl Genetic Analyzer. We used GENEMAPPER version 4.0(ABI) to visualize and score fragments. None of the loci amplified for both species.

Expected and observed heterozygosities were calculated using GENETIC DATA ANALYSIS (Lewis and Zaykin 2001) and Genepop (Raymond and Rousset 1995) was used to evaluate for Hardy–Weinberg equilibrium (HWE) using Fisher's exact test with 100,000 permutations and removal of missing data. Summary of the microsatellite loci are presented in Table 1. Number of alleles per locus ranged from 5–9 in *M. marginata* and 2–10 in *Moerisia* sp. with average expected heterozygosities of 0.71 and 0.57, respectively. One locus in *M. marginata* and two in *Moerisia* sp. deviated from HWE expectations. This is likely due to the presence of null alleles since observed heterozygosities were lower than expected for all three loci.

The microsatellite loci described here will be used to conduct future genetic studies investigating the invasion biology of these hydrozoans.

**Acknowledgements** We thank CALFED Science Program Grant #1036, NOAA Dr. Nancy Foster Scholarship, UC Davis Jastro-

Shields Research Scholarship, and US National Science Foundation grant NSF-DGE #0114432 IGERT UC Davis for financial support. We are grateful to Dr. Peter Moyle, Nicole Elen, Jessica Petersen, and John Durand for assistance and encouragement during this project.

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APPENDIX D

# GENETIC DIVERSITY AND REPRODUCTIVE MODE IN TWO NON-NATIVE HYDROMEDUSAE, *MAEOTIAS MARGINATA* AND *MOERISIA* SP., IN THE UPPER SAN FRANCISCO ESTUARY, CALIFORNIA

Running Title: Diversity and reproduction in invasive species

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Keywords: jellyfish, clonal, biological invasion, genetic diversity, San Francisco Bay, reproduction

**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 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 for *M. marginata* and *Moerisia* sp. respectively) but also detected multiple individuals (2-9) 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 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.

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**INTRODUCTION**

Biotic invasions are altering natural communities at an alarming rate (Mack *et al.* 2000; Vitousek *et al.* 1996). 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 & 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 & Richardson 1986; Lambrinos 2001; Sakai *et al.* 2001). Additionally, the potential for preservation of co-adapted gene 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 conducted by Kolar and Lodge (2001), the authors found a positive relationship in plants 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 & 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 & 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 & Parker 2008; Novak & Mack 2005; Roman & 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

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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 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 & Rees 2000; Rees 1999; Rees & Gershwin 2000). These species possess a bipartite life cycle, with a free swimming, dioecious medusa phase and a benthic asexually reproducing polyp phase (Mills & Rees 2000; Mills & Sommer 1995; Rees & 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 basic life histories of these species or their impact on the SFE system. 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

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

In our study, we had four 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 population
3. Investigate diversity and clonal propagation in both the polyp and medusa phases
4. 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 & 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 address objectives #1 and #2 with both species, and objective #3 with only *Moerisia* sp.

## METHODS

### Sample collection and processing

We collected the medusa stage of *M. marginata* and the polyp and medusa stage 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

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and upland grasslands (Meng & 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 (230µm mesh, 0.5m diameter, 2m length) from a boat in the middle of the sampled sloughs. We pulled the net at mid-water column depth for five minutes in the shallower Boynton Slough and we towed the net in the bottom and top half of the water column for 2.5 minutes each in the deeper Suisun and Montezuma sloughs. Additionally, to collect the larger, more mobile *M. marginata,* we towed an otter trawl (1m x 2.5m mouth, 5m length, 35mm stretch graded to 4mm stretch mesh at the cod end) for 5 minutes in Boynton Slough and 10 minutes 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, 0.5m below the water's surface and 0.5m 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 one 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.

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149 To sample polyps for genetic analyses, we plucked individual polyps from the settling plates and
150 placed each in its own sample tube containing Qiagen Gentra Puregene Lysis Buffer. To ensure a
151 sampling of the entire polyp population and not just the same clones, we employed two methods. Using
152 the microscope, we could see that while many polyps were individually dispersed throughout the plates,
153 there were several patches of many polyps together. To determine if these patches were clone mates, we
154 sampled multiple polyps from 6 patches (N = 2-16 per patch). We refer to these collections as "patch
155 polyp samples" from this point forward. We then sampled the rest of the polyp population (N = 53 from
156 Boynton slough and N = 74 from Montezuma slough) by collecting polyps that were located far apart
157 from each other and unlikely to have occurred from clonal polyp budding. We refer to this sampling as
158 the "polyp population survey."
159 Additionally, we obtained 4 *Moerisia lyonsi* specimens sampled from the Chesapeake Bay from
160 Dr. Allen Collins at the Smithsonian National Museum of Natural History to compare to our *Moerisia* sp.
161 for species identification.
162 Molecular methods
163 We extracted genomic DNA from each individual using Qiagen's Gentra Puregene Kit protocol.
164 Prior to polymerase chain reactions (PCRs), we treated extractions with a Proteinase K cleanup. We
165 added a final concentration of 5 µg/µL Proteinase K to our extractions and then incubated them for 2
166 hours at 60 C followed by 95 C for 5 minutes. This product was used as template directly in the following
167 PCRs. The *Moerisia* sp. samples were genotyped at 7 polymorphic loci (MmoG115, MmoG123,
168 MmoG167, MmoG181, MmoG109, MmoG133, MmoG194) and *M. marginata* at 8 loci (MmaG137-2,
169 MmaG142, MmaG177, MmaG155, MmaG107-2, MmaG139, MmaG154-1, MmaG108), using the
170 primers and protocols described in Meek *et al*. (2010), with the addition of 1 µl of BSA to each PCR. For
171 many of the individuals, the amplification from the initial PCR was too low to visualize and confidently
172 analyze. Therefore, for these samples we conducted a second PCR, using the original PCR as the
173 template. Using this re-amplification technique has been shown to improve amplification of

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174 microsatellites and decrease genotyping error rates (Piggott *et al.* 2004). In both the original PCR and the
175 re-amplification, we included a negative control to ensure detection of any contamination. We used the
176 negative control from the original PCR as the template for the negative control in the re-amplification.
177 PCR products were visualized with an Applied Biosystems, Inc. (ABI) 3730xl Genetic Analyzer and
178 LIZ600 size standard and were analyzed and scored using ABI Genemapper software.

179 Additionally, we performed PCR on DNA extracted from 17 *Moerisia* sp. medusae sampled from
180 Suisun Marsh and the 4 *M. lyonsi* medusae from Chesapeake Bay at the ribosomal Internal Transcribed
181 Spacer 1 (ITS1) marker for sequence analysis using the primers and protocols of Dawson and Jacobs
182 (2001). ITS1 is a rapidly evolving marker and is commonly used for species identification in Cnidarians
183 (ex. (Dawson & Jacobs 2001; Miranda *et al.* 2010). The PCR products were cleaned using the Agencourt
184 AMPure PCR purification system and sequenced on an ABI 3730xl DNA sequencer.

185 Data analyses

186 ***Clonal Analyses*** For all analyses of clonality, we only included those individuals that were
187 genotyped at 100% of the loci (Table 1). We used the software GenClone 2.0 (Arnaud-Haond & Belkhir
188 2007) to determine if our sets of loci were appropriate for identifying unique clones. GenClone detects the
189 number of unique multilocus genotypes (MLGs) by sampling all the loci combinations, from 1 through n
190 loci, using jackknife subsampling. If the resulting graph of the number of loci used by number of clones
191 identified reaches an asymptote, then the marker set is sufficient to identify all the unique MLGs in our
192 samples, if not, more loci are needed (Arnaud-Haond *et al.* 2005). We conducted 5000 permutations using
193 this method. We then employed GenClone to identify unique MLGs. We re-amplified all individuals
194 that were identified as being only one allele different from each other to reduce the chance of erroneously
195 identifying them as unique MLG (due to scoring or PCR error) when they were, in fact, clonemates. To
196 investigate the presence of clones, we calculated $P_{gen}$ and $P_{sex}$ using the $F_{is}$ corrected value in Genclone.
197 $P_{gen}$ describes the probability of a particular MLG occurring from the given population and $P_{sex}$ is the

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probability of N copies of the genotype occurring through sexual recombination (N= # individuals with that unique MLG), 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 & Belkhir 2007). This value also ranges from 0-1, with 1 indicating that all MLGs have equal abundance.

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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 2-16. To investigate the genetic similarity among patches and with 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 were able to confirm that the polyp samples did exhibit allelic dropout and

223 were indeed identical to the others in their patch. Allelic dropout is common in samples of low quality or
224 quantity DNA (Taberlet *et al.* 1996). Due to their very small size our polyp samples contained very low
225 quantity DNA, which likely caused the allelic dropout. Therefore, we used a cutoff of greater than 1 allele
226 difference to identify unique MLGs in the polyp samples, which has been shown to be an appropriate
227 method for identifying unique clonal lineages in other studies (Douhovnikoff & Dodd 2003; Schnittler &
228 Eusemann 2010).

229 All MLGs found in the polyp population survey were more than one allele apart. In the medusae
230 sampled, there was only one *Moerisia* sp. MLG pair that was one allele apart and two *M. marginata*. In
231 each of these cases, rather than being Aa heterozygous and the other aa homozygous, implying dropout of
232 the A allele in the second individual, these individuals were Aa and Ab heterozygotes. These genotype
233 were re-confirmed several times by multiple amplifications and, therefore, analyzed as unique MLGs.

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

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

246 **RESULTS**

247 Clonality

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We found repeat MLGs, with very high probabilities of arising through asexual reproduction, in both species studied (Table 2). 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).

***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 re-encountering these MLGs through sexual recombination ranged from $2.55 \times 10^{-4}$ to $3.73 \times 10^{-14}$ (Table 2). 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 2). 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 analysis, the FCA demonstrates that the diversity of polyp patches sampled spans the overall genetic diversity found through the polyp population survey (Fig 3). 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 3).

***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 4). There was greater evenness in clonal diversity in *M. marginata*. *Moerisia* sp. had one clonal line that was

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represented by nine individuals where the maximum number of individuals in a single MLG for *M. marginata* was four.

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. (Table 5 and 6).

Species assignment

It is highly likely the *Moerisia* found in the SFE is the same species as found in the Chesapeake Bay. We found 100% sequence agreement at ITS1 between *M. lyonsi* from Chesapeake Bay and *Moerisia* sp. found in the SFE (Genbank accession # HM997188, GU198209, and GU198210). 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.).

**DISCUSSION**

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Genetic diversity and reproductive mode

Our study demonstrates surprisingly high levels of genetic diversity for invasive populations. In a survey of introduced populations across taxa, Dlugosch and Parker (2008) found expected heterozygosities ranging between 0.01-0.95, and a median value of 0.18, putting the heterozygosity levels found in our study in the top 85$^{th}$ percentile of those surveyed. The level of genetic diversity found herein is especially unusual as it is coupled with abundant asexual reproduction in both invasive hydromedusae population. These jellies possess life history characteristics that 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 transport via ballast water, enabling them to be relocated easily. This polyp stage is then able to reproduce rapidly through asexual reproduction, conferring an advantage during the establishment of the population (Milbau & 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 likely increase the ability of the introduced populations to adapt and spread to new areas (Roman & Darling 2007). Ting and Geller (2000) conducted a similar study on the Asian sea anemone *Diadumene lineate,* which also are 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 state 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 and adapt in the SFE system and to expand their range. These results suggest that the populations in Suisun Marsh are unlikely to be suffering the negative effects of genetic bottleneck. This level of diversity is likely a result of multiple introductions, sexual reproduction, and/or a large number of founding individuals. Ballast water is a very efficient vector for invasion and can provide transport of large propagule pools (Carlton 1985; Carlton & Geller 1993; Fofonoff *et al.* 2003). Many invasions caused by ballast water transport show higher or similar levels of genetic diversity compared to their native populations (Fofonoff *et al.* 2003).

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

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cnidarian populations. 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 are two 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.*

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*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 & Rees 2000; Rees & 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 species diagnostic markers. 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). This finding would be additionally supported by sequencing other Moerisiid species at the ITS1 marker. As noted by Rees and Gershwin (2000), there is much work to be done to resolve the phylogeny and taxonomic status of members of this genus. Several species have been named and described separately, but it is quite possible there is high phenotypic plasticity and actually fewer species than named. It will be greatly beneficial to collect specimens from all species and regions where they are described and use molecular markers to compare levels of sequence divergence and create a phylogeny.

Another interesting next step in this body of work will be to sample the native populations in the Ponto-Caspian region and the invasive populations in the Chesapeake Bay to compare the levels of genetic diversity. This would allow us to determine if the level of diversity in the invasive populations is the same, higher, or lower than those found in the native population and will provide a comparison of the two invasion events to determine if they both exhibit similar patterns. This would aid in further understanding the role of diversity and reproductive mode in successful invasions.

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## 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, likely maintained through sexual reproduction, while also demonstrating large amounts of asexual reproduction. The employment of both reproductive modes may allow the species to rapidly propagate as they adapt in changing environments and new habitats. 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 make it more likely they will 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, with potential to expand their populations. This may mean further stress is put on local food webs, and is particularly threatening for fish that compete with the hydromedusae for resources.

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## AKNOWLEDGEMENTS

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, Will Wetzel, and the members of the Genomic Variation Lab 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), NOAA Dr. Nancy Foster Scholarship (to MH Meek), UC Davis Biological Invasions IGERT NSF-DGE#0114432 (to AP Wintzer), UC Davis Jastro-Shields Research Scholarship

(to MH Meek and AP Wintzer), UC Davis Block Grant (to AP Wintzer), and the Giles W. and Elise G. Mead Foundation (to PB Moyle).

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**DATA ACCESSIBILITY:**

-DNA sequences: Genbank accessions # HM997188, GU198209, and GU198210

**FIGURE CAPTIONS:**

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

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

Figure 3: 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. 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

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Figure 1:

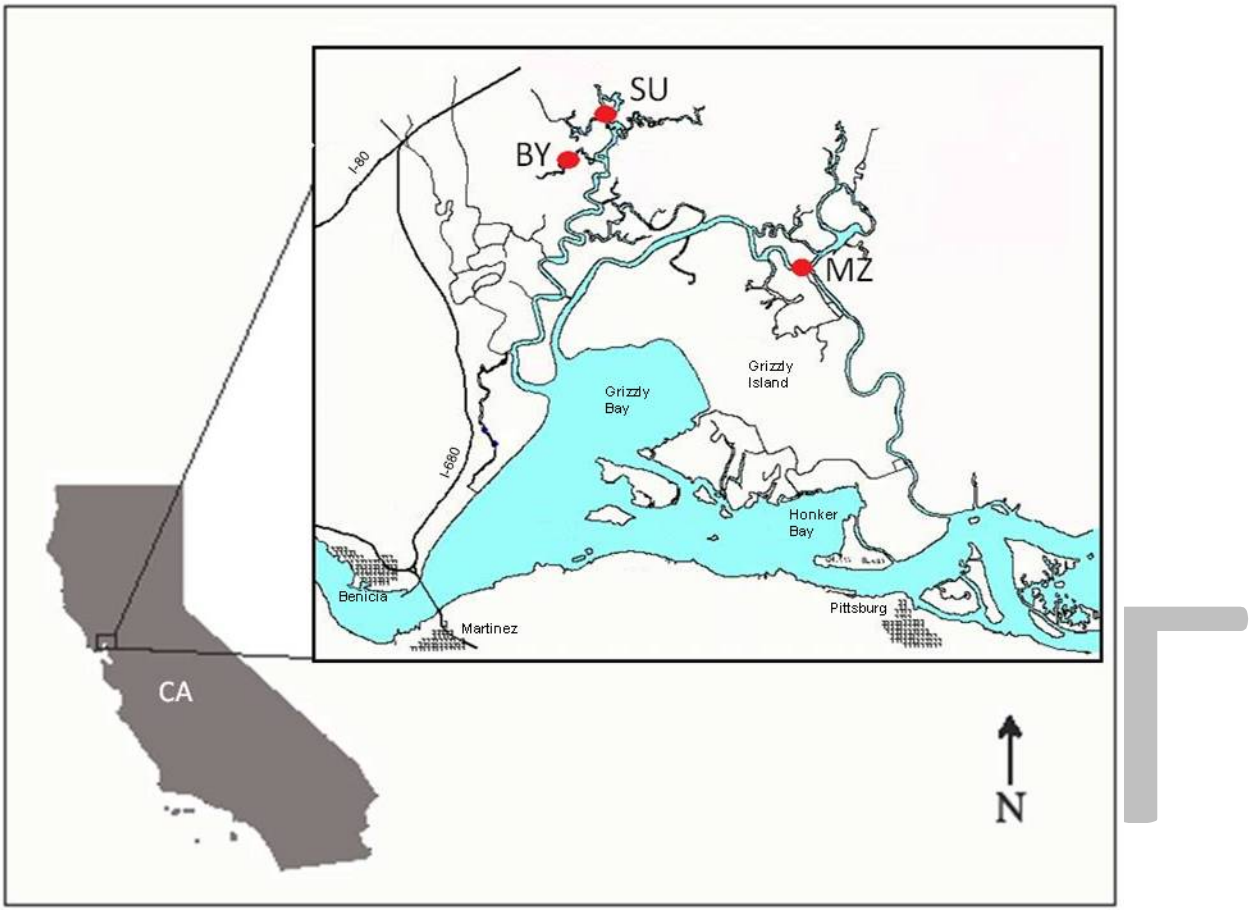
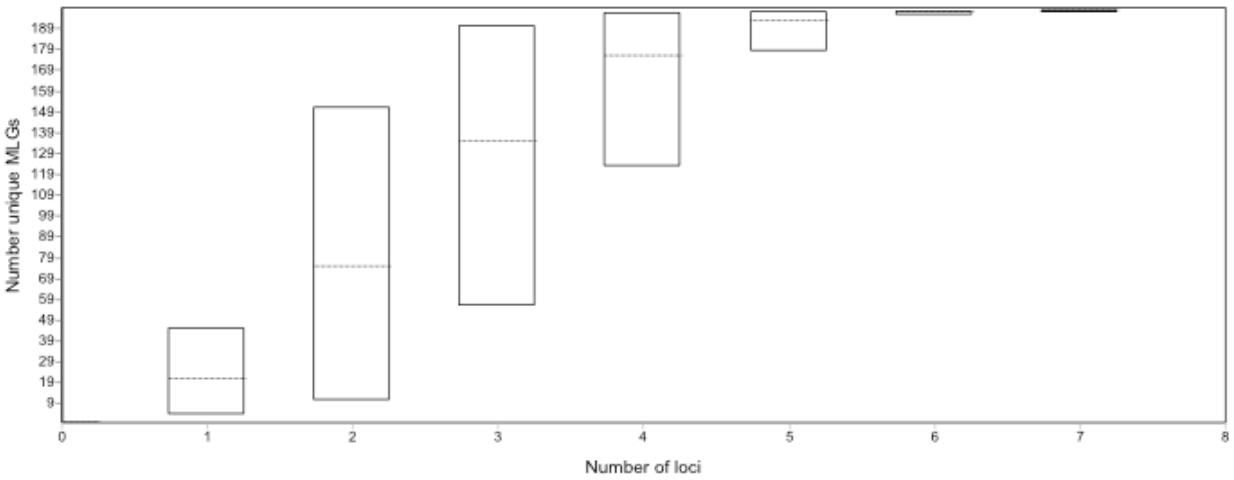

SU
BY
MZ
I-80
I-680
Grizzly Bay
Grizzly Island
Honker Bay
Benicia
Martinez
Pittsburg
CA
N

Figure 2:

A.




B.

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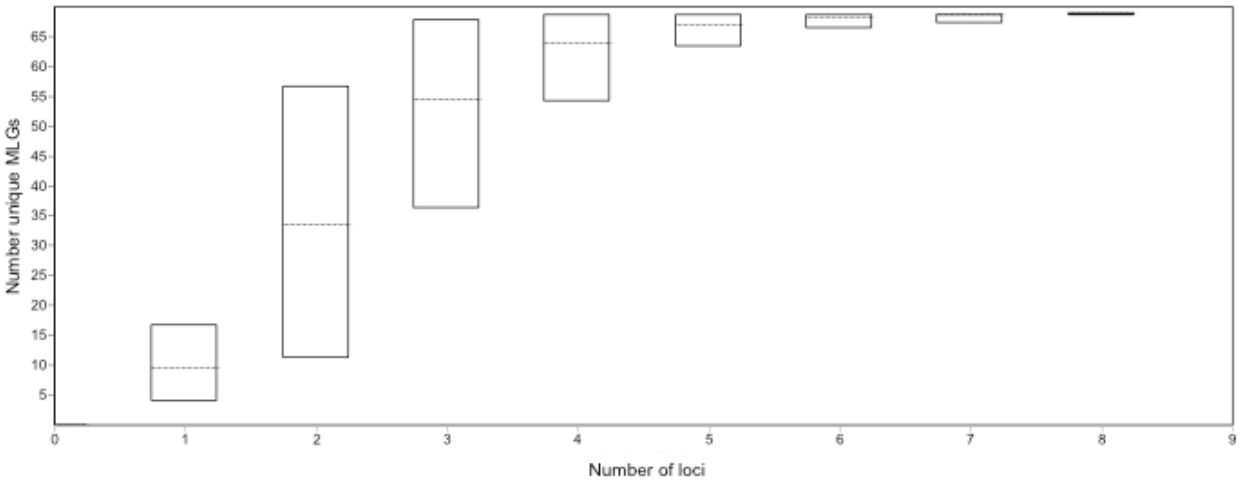
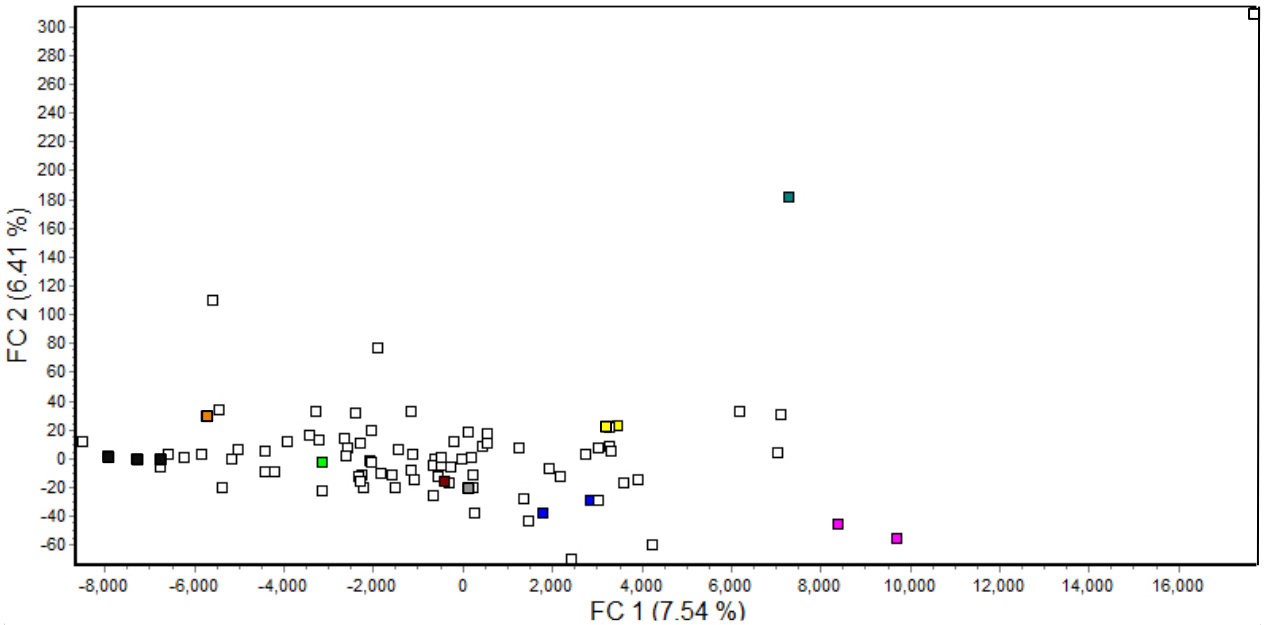


Figure 3:

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568 Table 1: Number of each species characterized with microsatellite markers.

| Species | # characterized at 70% of loci* | # characterized at 100% of loci |
|---|---|---|
| ***M. marginata* medusae** | 111 | 77 |
| ***Moerisia* sp. medusae** | 150 | 128 |
| ***Moerisia* sp. polyp population** | 127 | 82 |

569 **Moerisia* sp. = 5 or more loci, *M. marginata*= 6 or more loci

570 Table 2: $P_{gen}$ (probability of a MLG occurring in the population) and $P_{sex}$ (probability of the MLG
571 reoccurring through sexual reproduction N number of times) values for each repeat MLG detected in *M.*
572 *marginata* (MA) and *Moerisia* sp. (MO) medusae populations. The Locations column describes the
573 location(s) where that repeat MLG was found and how many individuals were included in it (N).

| Species | Locations (# indiv. per location) | $P_{gen}$ | $P_{sex}$ |
|---|---|---|---|
| **MA** | SU (2) | $7.54 \times 10^{-7}$ | $5.80 \times 10^{-5}$ |
| **MA** | SU (2) | $3.13 \times 10^{-6}$ | $2.55 \times 10^{-4}$ |
| **MA** | MZ (3) and BY (2) | $1.29 \times 10^{-5}$ | $3.73 \times 10^{-14}$ |
| **MA** | MZ (1) and SU (1) | $8.86 \times 10^{-8}$ | $6.82 \times 10^{-6}$ |
| **MA** | SU (2) | $5.38 \times 10^{-7}$ | $4.14 \times 10^{-5}$ |
| **MO** | BY 8/6 (5), BY 9/20 (2), SU 9/20 (2) | $6.18 \times 10^{-8}$ | $1.75 \times 10^{-44}$ |
| **MO** | BY 9/20 (1), SU 9/20 (1) | $6.67 \times 10^{-7}$ | $1.40 \times 10^{-4}$ |
| **MO** | BY 9/20 (1), SU 9/20 (2) | $7.09 \times 10^{-8}$ | $1.10 \times 10^{-10}$ |
| **MO** | SU 9/20 (2) | $1.44 \times 10^{-6}$ | 3.03 x 104 |

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575 Table 3: The number of unique multi-locus genotypes (MLGs) detected per patch and the number of
576 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 |

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579 Table 4: 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 |

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581 Table 5: Observed ($H_O$) and expected ($H_E$) heterozygosity and allelic richness ($N_a$) for *M. marginata*
582 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 |

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585 Table 6: Observed ($H_O$) and expected ($H_E$) heterozygosity, and allelic richness ($N_a$) for the *Moerisia* sp.
586 medusae (N = 150) and polyp populations (N = 127) and both combined. Allelic richness for the polyp
587 and medusae population was calculated through rarefaction to 127 individuals sampled.

| | | $H_O$ | $H_E$ | $N_a$ |
|---|---|---|---|---|
| **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 |

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APPENDIX E

# TROPHIC ECOLOGY AND EFFECTS OF TEMPERATURE AND SALINITY ON GROWTH IN THE NON-NATIVE HYDROZOAN, *CORDYLOPHORA CASPIA,* IN THE UPPER SAN FRANCISCO ESTUARY, CALIFORNIA

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KEYWORDS

Hydrozoa; San Francisco Estuary; predation rate; temperature and salinity tolerance; digestion time; population growth

This paper has not been submitted elsewhere in identical or similar form, nor will it be during the first three months after its submission to Hydrobiologia.

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ABSTRACT

*Cordylophora caspia,* a hydrozoan from the Ponto-Caspian region, has become established in the brackish water habitats of the San Francisco Estuary (SFE). It is important to understand what factors influence the spread of *C. caspia* and how it may impact the food web, particularly given the severe decline of pelagic fishes in the SFE. In this study, we asked four questions: 1. What are the effects of temperature and salinity on *C. caspia* colony growth? 2. How do exposure time for feeding, temperature, and salinity impact feeding rate? 3. What is the diet of *C. caspia?* and 4. What is the digestion rate of prey? We experimentally investigated the effects of temperature and salinity on colony growth and feeding rates, and analyzed gut contents from wild specimens to determine diets. Warmer temperatures resulted in higher growth rates, with the maximum growth at 25 C, but salinity did not impact growth. Further work is needed to parse out the influence of exposure time, temperature, and salinity on feeding. We found *C. caspia* eats a wide range of mostly planktonic prey items, proportional to prey availability, and digestion times for one prey item ranged from 48-69 minutes. This work is a valuable step in understanding the potential impact of this abundant non-native on the SFE ecosystem.

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INTRODUCTION

The importance of hydroids in the transfer of energy from pelagic to benthic systems is often overlooked due to their relatively small size and low abundance; however estimates have found that hydroid species composing less than 0.5% of the benthic community biomass can capture approximately 10% of the annual algal production (Gili et al., 1998a). Hydroids can also play very strong roles as predators, with some estimates placing their ingestion rates at some of the highest among suspension feeding organisms, along with bivalves and tunicates (Gili et al., 1998b).The presence of hydroids can disrupt foodweb processes if they become abundant in areas where they are not native members of the trophic community (Purcell et al., 2001).

The San Francisco Estuary (SFE) is one of the most invaded ecosystems in the world, with over 200 non-native species present (Cohen et al., 1998). One of the more abundant non-native species is the hydroid *Cordylophora caspia* (Pallas 1771), with estimates in some areas up to 626,104 hydranths/$m^2$ (Wintzer, AP, unpublished data). *C. caspia* in the SFE feeds largely on zooplankton and where populations are dense it may affect zooplankton abundance. This is of concern in the SFE because in recent years many planktivorous fishes have shown severe declines, with record low numbers of delta smelt (*Hypomesus transpacificus*, ESA threatened species), longfin smelt (*Spirinchus thaleichthys*, California threatened species), age-0 striped bass (*Morone saxatilis,* a non-native sport fish), and threadfin shad (*Dorosoma petenense,* a non-native planktivore) (IEP, 2008; Sommer et al., 2007). The negative impact of competition with *C. caspia* and other invasive species is cited as a potential cause for these declines (IEP, 2008; Sommer et al., 2007).

In spite of their potential importance, relatively little is known about the ecology of *C. caspia* in the SFE. Wintzer et al. (2010) sampled *C. caspia* using fouling plates in Suisun Marsh and the Napa and Petaluma Rivers from June to November, and found them present during all months sampled. Matern and Brown (2005) found evidence of *C. caspia* year round in gut contents of fish from the SFE. Wintzer et al.(2010) analyzed the environmental conditions preferred by *C. caspia* and found its abundance is

associated with low salinity, transparency, temperature, and depth, and mid-levels of dissolved oxygen. They also found the optimal temperature range for *C. caspia* growth and reproduction was 18.1-23.5 C, based on the 5th and 95th percentile for daily polyp recruitment.

In this study, we investigated the ecology of *C. caspia* in the SFE through a combination of laboratory experiments and field collections. We addressed four questions: 1. What are the effects of temperature and salinity on colony growth? 2. How do time for feeding, temperature, and salinity impact feeding rate? 3. What is the diet of *C. caspia?* and 4. What is the digestion rate of prey? Our overall purpose was to elucidate the potential impact of the *C. caspia* invasion on local foodwebs.

## METHODS

### Study area

Suisun Marsh is a brackish water system covering approximately 34,000 ha in the upper San Francisco Estuary. 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 seasonal pools and upland grasslands (Meng et al., 2001). *C. caspia* was introduced into this system, likely by ballast water exchange or ship hulls, as early as the 1920s (Folino-Rorem et al., 2009; Folino-Rorem, 2000).

### Temperature x salinity experiment

On June 12, 2008, we collected *C. caspia* polyps from Suisun Marsh and brought them back to a laboratory at the University of California-Davis. Here, we maintained the polyps in 6.5 ppt salinity water in an aerated tank and fed them to satiation daily with 48 hour old Selco enriched *Artemia* nauplii. On July 27, 2008, we settled the collected polyps in new petri dishes (3 per dish) by gently plucking them off the settling plate with forceps and placing them in the petri dish containing 6.5 ppt water held at 20 C. We allowed polyps 3 days to settle in the petri dish, providing them with *Artemia* nauplii every other day. After settlement, we recorded the number of successfully settled polyps per petri dish and then randomly placed 1-2 petri dishes each in one of 36 5-gallon tanks holding 6.5 ppt water at 20 C, for a total of 57 petri dishes containing *C. caspia* polyps. All 5-gallon tanks were contained inside large circular tanks that

acted as water baths to maintain the desired experimental temperature. There were two sets of large circular tanks per temperature treatment. For each temperature treatment, a large holding tank was used to either cool the water to 15 C with an aquarium chiller, hold the water at 20 C ambient temperature, or heat the water to 25 C with aquarium heaters. From the holding tank, the water flowed into both circular tanks. Water circulated around the circular tanks and then back into the large holding tank. Salinity treatments were achieved by adding Liquid Ocean to fresh water to obtain salinities of 2, 6.5 and 11 ppt. Each of the six 5-gallon tanks in the large circular tank was randomly assigned to one of the salinity treatments, with two tanks per salinity treatment per circular tank. Each tank was aerated with an aquarium aeration system.

On July 31, 2008 we began to adjust the salinities to reach the desired level. To avoid salinity shock, we altered the salinities by only 1 ppt every 4-12 hours. Once the polyps had a chance to acclimate to their new salinities, we began the temperature adjustment. We achieved the desired temperatures by slowly dropping or rising by 1 C every 1-3 hours over 24 hours. We fed the polyps to satiation every day on 48 hour old Selco enriched *Artemia* nauplii and executed 1/3 water changes of the tanks every other day to decrease fouling. At the completion of the experiment, on August 13, 2008, we removed all the dishes from their experimental tanks and recorded the number of live hydranths (a feeding individual) in each dish using a dissecting microscope. After data collection we continued to culture many of the polyps in their given salinity x temperature treatment for use in feeding experiments.

### Feeding experiment

After the temperature x salinity reproduction experiment, we plucked individual polyps from their original petri dishes and placed 3 each in new petri dishes inside 1000 ml plastic containers containing clean water at the salinity to which they were acclimated. We then floated these 1000 ml containers inside the large circular tanks to maintain the acclimated temperature. The polyps were allowed to settle and acclimate to their new containers for eleven days. Polyps then were not provided food for 24 hours before starting the experiment. The food levels provided were determined by volume, rather than by absolute

number of *Artemia*. *Artemia* were cultured in 2 L culture cones by putting ½ tsp *Artemia* eggs into 2 L of 25 ppt water at 25 C and allowed to hatch for 24 hours. Prior to the experiment, all *Artemia* were filtered out of the cone and put in 2 L of clean 25 ppt water to avoid fouling the experimental containers with *Artemia* waste. Nauplii were then sampled from this water for the experiment by allowing the water to continually mix vigorously via aeration, and then sampling from the water using 1 mL plastic pipettes. Polyps were given 1 ml of 24 hr old *Artemia* nauplii and allowed to feed for 1, 2, or 4 hours. At the end of the appropriate feeding time, all dead *Artemia* in the bottom of the container were collected using a plastic 1 ml pipette and preserved in 5% Lugols and the remaining live, unconsumed *Artemia* were preserved in the 1000 ml containers with 5% Lugols for future enumeration.

### Digestion time and diet

We conducted two additional studies to determine digestion times for *Artemia* nauplii and the diets of field-collected individuals. We took single polyps, collected on June 12, 2007 from Suisun Marsh, and placed each in a 20mL culture plate well with clean 6 ppt water at room temperature (21 C). We allowed the polyps to acclimate to their new environment for one hour. After an hour, we gave each polyp a single 24 hour old *Artemia* nauplius by placing the nauplius on the tentacles with forceps. We then watched the polyps under a dissecting microscope and noted the time at which the polyp placed the nauplius in its mouth. Once in the gut, the nauplius was visible under magnification through the polyp gut walls. Using the dissecting microscope, we checked every 10 minutes to see if the nauplius was still in the polyp gut. Near the completion of digestion, we checked every minute, to ensure a precise recording of digestion time. Once the nauplius was completely digested, we noted the time. The completion of digestion was determined when we could no longer see any part of the nauplius through the gut wall. This process was completed for twelve *C. caspia* polyps.

In order to evaluate what the *C. caspia* polyps are eating in the wild, we collected individuals from Suisun Marsh using settling plate arrays deployed monthly between June-October (methods described in Wintzer et al. 2010) and preserved them in 95% Ethanol. Preservation in Ethanol caused

some polyps to expel their gut contents, therefore; we evaluated polyps for fullness using a dissecting microscope and then dissected the gut contents of 22 full hydranths under the microscope using fine needles. We identified prey items to the lowest feasible taxonomic level.

## Data analyses

### *Temperature and salinity experiment*

Data were log-transformed and analyzed using a mixed model ANOVA to determine the effects of temperature and salinity on polyp colony growth. Due to the variation around the number of polyps that settled for the growth experiment (between 1-3 polyps successfully settled per petri dish) we used the proportional increase in polyps (final number of polyps/initial number of polyps) per dish as the dependent variable. To describe population growth, we calculated the intrinsic growth rate ($r_m$) which describes the population's potential for instantaneous increase with unlimited resources, finite rate of increase ($\lambda$), which characterizes the relative daily change in hydranth numbers, and doubling time ($D_t$) using the following equations:

$$r_m=\ln(N_t/N_0)/T$$

$$\lambda=e^{rm}$$

$$D_t=\ln(2)/r_m$$

where $N_{(t)}$ is the number of hydranths at time T, $N_{(0)}$ is the initial number of hydranths, and T is the time in days.

### *Feeding experiment*

Data were analyzed using a mixed model ANOVA to determine the effects of temperature and salinity on predation rates of polyps. Additionally, we examined the effect of polyp presence on the number of dead *Artemia* in the feeding experiment and the effect of predation time on feeding rate. There was variation in growth of the polyp colonies over the 12 days that the newly settle polyps were acclimated (1-14 hydranths), so all rates were standardized to the number of feeding hydranths in the

experimental container. To determine predation rates, we first calculated the instantaneous mortality rate (Z, hours$^{-1}$) owing to predation:

$$Z=((\ln N_i/N_p)-(\ln N_i/N_c))/T$$

where $N_i$ is the initial number of prey, $N_p$ is the number of live prey recovered from the containers with polyps, $N_c$ is the average number of live prey recovered from the control containers, and T is the amount of time for feeding (de Lafontaine et al., 1988; Elliott et al., 1996; Elliott et al., 1997). We then calculated predation rates (prey ingested per predator per hour):

$$I =(N_i (1- \exp(-Z)))/P$$

where P = number of hydranths in the container. All dependent variables were log transformed to satisfy the assumptions of ANOVA.

## RESULTS

### Temperature and salinity experiment

We found a significant effect of temperature on growth of the polyp colonies (p=0.0007), but no effect of salinity (p=0.51). The average growth rates at the increasing temperature levels were 3.94, 32.94, and 45.09 hydranths per polyp per day (standard error ± 1.04, 6.52, 8.38 respectively) (Figure 1). The maximum number of hydranths grown per polyp per day was 109 in the 25 C treatment. There was also a significant effect of temperature on population growth parameters (Table 1). Population growth was significantly higher at 20 and 25 C than 15 C. The doubling time at the warmer temperatures was approximately 3 days, while it was 7 days at the cold temperature. The intrinsic growth rates ranged from 0.11-0.27/day and the finite rate of increase ranged from 1.12-1.13 hydranths/day.

### Feeding experiment

We first looked at the effect of polyp presence, temperature, salinity, and exposure time on the number of live *Artemia* found at the end of the experiment. We found there was a highly significant effect of polyp presence (p<0.0001). No other factor was significant in affecting number of live *Artemia*. Therefore, we can confidently say that polyp presence drove number of live *Artemia* in the experiment.



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However, when we evaluated the effect of polyp presence on the number of dead *Artemia*, we found no significant effect of hydranths (p=0.40). Therefore, we can be confident the effect of polyp presence on the number of live *Artemia* was driven by polyp consumption, rather than just polyps killing and not eating the *Artemia*.

Evaluation of predation rates was hindered by the large variation in number of *Artemia* nauplii present in each container, as determined from the control counts (106 nauplii, ± 4 s.e.). Due to this variation, we calculated Z and I using the mean number *Artemia* fed, as calculated from the control data. Using this number, we found no significant effect of time, temperature or salinity on feeding rate. The average feeding rate across all treatments was 4.06 ± 0.52 nauplii/polyp/hr (Table 2). Further work is needed to resolve the interactions of time, temperature, and salinity on feeding rate.

### Diet and digestion time

The average number of prey items per hydranth was 2.23 ± 0.32. Prey items included the calanoid copepod *Pseudodiaptomus forbesi*, copepod nauplii, cyclopoid copepods, *Daphnia* sp., and an unidentified nematode (Table 3). Nauplii were the most common prey item, followed by *P. forbesi* and cyclopoid copepods. However, all cyclopoid copepods were found in the samples from October, indicating that they were possibly not available as a resource in the other months. Nauplii were found in the guts across all sampling times, except November. We were only able to sample two polyps in November. These polyps contained only *Daphnia* sp. The maximum number of prey items in a gut was 6 (2 polyps, containing either 6 cyclopoid copepods or 5 cyclopoid copepods and 1 copepod nauplii).

We found the mean rate of digestion for one *Artemia* nauplii was 62 ± 1.6 minutes (minimum 48 m, maximum 69 m).

## DISCUSSION

### Temperature and salinity experiment

Our results support previous work showing *C. caspia* can achieve high growth rates. Wintzer et al. (2010) determined a high value recruitment estimate of 22,998 hydranths per day per $m^2$ in some areas

of Suisun Marsh. We found a population doubling time ranging from 3-7 days, which is consistent with that found by Kinne (1958) (as reviewed in Gili & Hughes, 1995) and Fulton (1962) who found doubling times of 4-5 and 3 days, respectively. Fulton (1962) also found colonies could reach sizes of approximately 2,000 hydranths and grow exponentially. Given estimates of up to 626,104 hydranths/$m^2$ in the SFE (Wintzer, AP, unpublished data) and the growth rates described herein, it is clear that the population of *C. caspia* has the potential to rapidly propagate and expand its population.

We found, within the ranges tested, temperature but not salinity affects colony growth rate. Consistent with our findings, Wintzer et al. (2010) also found salinity did not control seasonal presence in the SFE. Wintzer et al. (2010) found *C. caspia* recruitment in brackish water habitats of the SFE during all months sampled. This suggests that temperature and salinity in the SFE remain within tolerance limits. The mean monthly salinity of Suisun Marsh from 1980-2008 ranged between 1-6 ppt and temperature ranged between 9-23 C (O'Rear et al., 2009). While this temperature range is below the optimal found by both our study and Wintzer et al. (2010), the low does not appear to be lethal to *C. caspia*.

Other studies have also found *C. caspia* has a wide range of salinity and temperature tolerance limits, but the actual limits vary with the populations studied and in the field versus lab. Studies have found *Cordylophora* species in other regions at salinities of 0-30 ppt (reviewed in Folino-Rorem et al., 2005) and optimal temperature and salinity conditions of 11-30 C and 0.2-16.7 ppt, respectively (reviewed in Roos, 1979). Kinne (1958) found differences in salinities required for peak production of new hydranths from *C. caspia* at Helgoland (24 ppt) and off Kiel Canal (15 ppt), geographically close populations off northern Germany. Kinne (1958) also found there is a difference in tolerance ranges for salinity within different tissue types, with gonozooids and tentacles having the lowest tolerance limits. Roos (1979), however, found that salinity did not appear to dictate the presence of *C. caspia* in the Netherlands. Kinne (1964) reported *C. caspia* undergoes structural acclimation to changes in salinity, including changes in hydranth and colony structure, as well as cellular adjustments. Fulton (1962) found

the optimal temperature for *C. caspia* growth was 18-26 C, with little effect on growth rate within this range.

Interestingly, we found the greatest colony growth rate at the highest temperature tested (25° C), which is above the optimal temperature for polyp recruitment in the field found by Wintzer et al. (2010). This inconsistency is likely due to complex ecological and/or biological interactions occurring in the field, driving species distribution and abundance. Possible explanations for field conditions that caused this incongruence include lower concentrations of prey species in the field at high water temperatures or decreased dissolved oxygen at high temperatures, limiting colony growth. Both of these factors were removed from our laboratory experiment as each treatment was given the same amount of food and the water was well aerated. This experiment demonstrates that *C. caspia* has the potential to maintain high growth rates at high temperatures, provided its other needs are met.

We found growth rate was significantly decreased, but still positive, in the 15 C treatment. An informative extension of this work will be to determine at what low temperature the colony goes dormant. At extreme salinities (Kinne, 1971) and temperatures (Fulton, 1962), *C. caspia* colonies die back with portions of the ceonosarc lying dormant inside the perisarc, allowing the hydroid to withstand unfavorable conditions. Once conditions improve, the colony is able to regrow and resume reproduction (Roos, 1979). This ability makes *C. caspia* a very hearty colonizer and is likely what has allowed it to spread to the SFE via ship hulls or ballast water (Folino-Rorem, 2000). Kinne (1956) found *C. caspia* colonies become dormant below 4 C. These low temperatures, however, are not often reached in Suisun Marsh (O'Rear et al., 2009), making this habitat an ideal location for the success of this invader.

### Feeding and diet studies

Hydroids can be a strong force in trophic communities (Gili et al., 1995). Von Holle and Ruiz (1997) suggest that *C. caspia* acts as a restructuring force in a fouling community in Baltimore Harbor, Maryland, through negative interactions with ciliates and bryozoans and positive interactions with barnacles, amphipods, and polychaetes. Colonies of *Eudendrium racemosum,* another colonial hydroid,

must consume prey equivalent to 7.2% of their own biomass daily to meet their metabolic requirements (Gili et al., 1995). Given the densities of *C. caspia* found in the SFE (Wintzer et al. 2010) and the potential feeding rates described here, it is likely that *C. caspia* acts as a powerful predatory force on zooplankton in Suisun Marsh.

Other studies have shown that *C. caspia* hydranths capture *Artemia* nauplii to repletion within the first few minutes of feeding (Fulton, 1962). This is consistent with our findings that show the highest predation rates in the first 1 hour and lower rates in the following 3 hours, likely due to satiation. This is also consistent with our finding that digestion time for a single *Artemia* nauplii is approximately 1 hour. However, given that the average number of prey items found in *C. caspia* guts was over 2, further work is needed to determine how number and size of prey items in the digestive system affect digestion time.

Continued investigations are needed to further elucidate the role of prey exposure time, temperature, and salinity have in determining feeding rates. Kinne and Paffenhofer (1965) found digestion time in *Clava multicornis* is reduced with increasing temperature and that salinity also impacted digestion times. They found that temperature and salinity had interacting effects on hydranth dimensions and tentacle number, potentially also impacting feeding rates. Food consumption by *Moerisia lyonsi* polyps is significantly reduced under low temperatures and high salinity conditions (Ma et al., 2005). It is likely that salinity and temperature interact in a complex way to affect predation rates in *C. caspia*.

Additionally, water flow rates can affect capture rate (Gili et al., 1995). High flow rates will result in lower capture rates due to too much flow pressure, while low flow rates will decrease capture rate due to decreased delivery of prey items to the hydranth. Therefore, our predation rate estimates are conservative due to a lack of water flow in our experimental containers.

Our preliminary diet study indicates that *C. caspia* consumes a wide variety of prey items in Suisun Marsh, taking advantage of seasonal shifts in dominant zooplankton. The diets reflect that *P. forbesi* is abundant in Suisun Marsh in July and August, but absent in October and November. Cyclopoid copepods are most abundant in October and *Daphnia* become present in October and then reach high

densities in November (Winzter, AP unpublished data). This is consistent with the proportion of these species found in the *C. caspia* gut contents each month, suggesting that *C. caspia* is feeding upon prey items as they are readily available in the system. Copepod nauplii also appear to be a particularly important resource. Durand and Kimmerer (2006) found high stage dependent mortality of copepod nauplii in Suisun Bay, possibly due to clam predation. If these same forces are acting on the copepod population in Suisun Marsh, it is likely that *C. caspia* presence in the system acts as a further stressor on an already highly impacted community. Further work is needed to investigate food preferences, prey availability, and seasonal and diel changes in feeding of *C. caspia*.

## Implications

Given *C. caspia's* capabilities for high growth and feeding rates, the population of *C. caspia* in the SFE has the potential to have a considerable impact on the zooplankton community. This impact may be detrimental to native fish populations which also depend on zooplankton as a food source. Additionally, our experiments found increasing growth rates with increasing temperature and we did not reach an upper limit temperature where growth rate decreased. Under projections of climate change, Suisun Marsh will experience between 2-6 C warming in the next decade (Brown, 2004). As the recorded average summer temperature between 1980-2008 in Suisun Marsh hasn't exceeded 23 C, it is possible that *C. caspia* will continue to thrive in the area, and may increase its range, spreading into new habitats as conditions change (O'Rear et al., 2009). In addition to *C. caspia,* there are three other Ponto-Caspian hydrozoans that have become established in the SFE (Rees, 1999). These species (*Maeotias marginata, Blackfordia virginica,* and *Moerisia* sp.) also have a polyp form and together this suite of hydroids may be putting substantial pressure on the food webs of SFE.

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ACKNOWLEDGEMENTS

We would like to thank Nicole Elen, Dr. Peter Moyle, Dr. Mike Dawson, Dr. Nadine Folino-Rorem, Suisun City Marina, and the Suisun Resource Conservation District for their assistance during this project. This research was supported by funding from CALFED Science Program Grant #1036 (P.I.s: B May and PB Moyle), NOAA Dr. Nancy Foster Scholarship (to MH Meek), UC Davis Biological Invasions IGERT NSF-DGE#0114432 (to AP Wintzer), UC Davis Jastro-Shields Research Scholarship (to MH Meek and AP Wintzer), UC Davis Block Grant (to AP Wintzer), and the Giles W. and Elise G. Mead Foundation (to PB Moyle).

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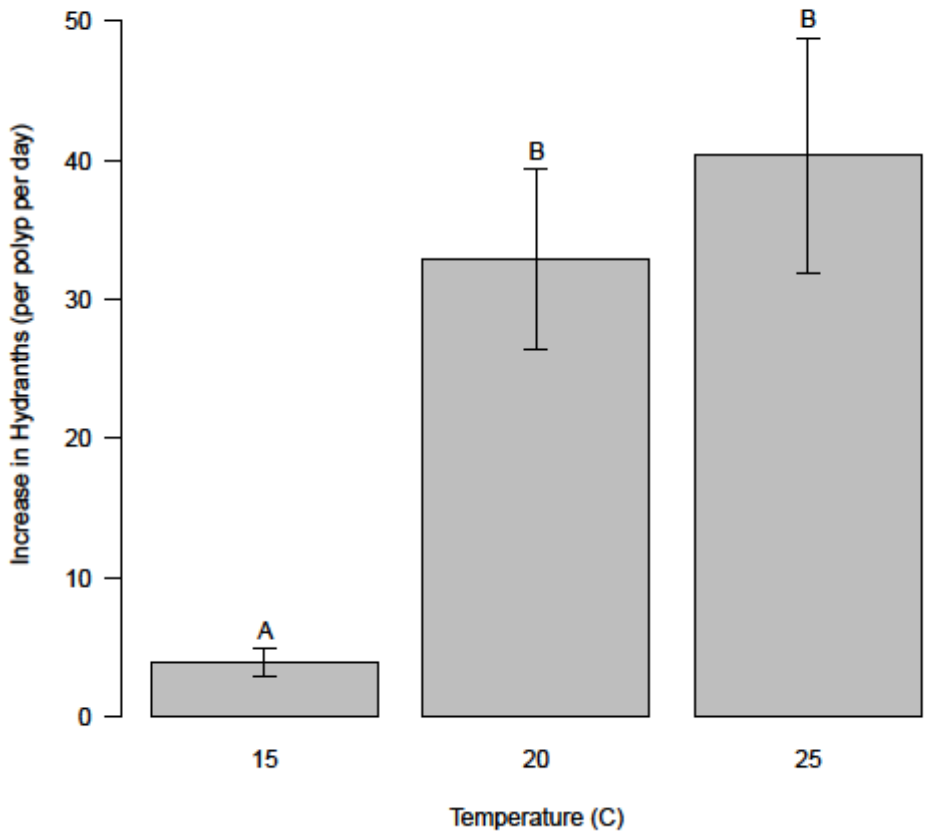


Figure 1: Mean increase in hydranths per polyp per day at each temperature treatment. Bars denote standard error and letters denote significant difference. Treatments with different letters are significantly different at the $p<0.05$ level.

Table 1: Population growth parameters of *C. caspia* at each temperature treatment (means ±standard error). All values at the 15 C treatment were significantly different from 20 and 25 C treatments at the $p<0.05$ level.

| Temperature (C) | Sample size | Intrinsic growth rate ($r_m$, per hr) | Finite rate of increase **(Λ, per hr)** | Doubling time ($D_t$, per hr) |
|---|---|---|---|---|
| 15 | 13 | 0.11±0.01 | 1.12±0.01 | 7.40±1.00 |
| 20 | 14 | 0.24±0.01 | 1.28±0.02 | 2.99±0.20 |
| 25 | 15 | 0.27±0.02 | 1.31±0.02 | 2.95±0.46 |

Table 2: Mean predation rate (I) per polyp per hour at each treatment level, using the average number of *Artemia* nauplii fed.

| | | Time (hr) | | | Temperature | | | Salinity (ppt) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | 1 | 2 | 4 | 15 | 20 | 25 | 2 | 6.5 | 11 |
| Mean ± S.E. | 4.06 ± 0.52 | 6.24 ± 1.05 | 2.92 ± 0.48 | 3.15 ± 0.51 | 5.48 ± 0.63 | 3.11 ± 1.13 | 4.05 ± 0.60 | 4.56 ± 0.67 | 3.44 ± 1.19 | 4.32 ± 0.55 |
| N | 109 | 35 | 37 | 38 | 28 | 42 | 40 | 29 | 41 | 40 |

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Table 3: Diet of *C. caspia* as described by percent of polyps with a particular prey item in their gut (% FO) and percent of total prey number in guts (% N). Calculated for all polyps sampled and by month in 2007.

| | *Total* | | *July* | | *August* | | *October* | | *November* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 22 polyps | 49 prey | 7 polyps | 10 prey | 7 polyps | 12 prey | 6 polyps | 22 prey | 2 polyps | 5 prey |
| Prey | % FO | % N | % FO | % N | % FO | % N | % FO | % N | % FO | % N |
| Copepod nauplii | 54.55 | 42.86 | 71.43 | 80.00 | 57.14 | 58.33 | 50.00 | 27.27 | 0.00 | 0.00 |
| *Pseudodiaptomous forbesi* | 22.73 | 12.24 | 28.57 | 20.00 | 42.86 | 33.33 | 0.00 | 0.00 | 0.00 | 0.00 |
| Nematode | 4.55 | 2.04 | 0.00 | 0.00 | 14.29 | 8.33 | 0.00 | 0.00 | 0.00 | 0.00 |
| Cyclopoid copepod | 18.18 | 32.65 | 0.00 | 0.00 | 0.00 | 0.00 | 66.67 | 72.73 | 0.00 | 0.00 |
| *Daphnia* sp. | 9.09 | 10.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 100.00 | 100.00 |

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Aquat Ecol
DOI 10.1007/s10452-010-9343-7

# Ecological insights into the polyp stage of non-native hydrozoans in the San Francisco Estuary

**Alpa P. Wintzer · Mariah H. Meek · Peter B. Moyle · Bernie May**

Received: 17 March 2010 / Accepted: 21 September 2010
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**Abstract** The populations of several invasive jellyfish appear to be increasing around the globe. While data on non-native hydromedusae in the San Francisco Estuary have been accumulating in recent years, little is known regarding their polyp phase. The goal of this study was to gather the first field-derived ecological data for polyp stages of *Blackfordia virginica*, *Moerisia* sp., and *Cordylophora caspia* in the estuary. Monthly fouling plates were deployed at five sites during 2007 and 2008. Settlement data indicate a seasonal presence of *B. virginica* and *Moerisia* sp., with both distribution and abundance correlated with a combination of water quality and physical parameters. *Cordylophora caspia* appeared to be present beyond the time period sampled and may be active in the system year-round. The ability of polyps to persist month to month was low, likely due to predation by other non-native species and competition for space.

**Keywords** *Moerisia* · *Blackfordia virginica* · *Cordylophora caspia* · San Francisco Estuary · Hydrozoan polyp ecology

Handling Editor: Jacco C Kromkamp.

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## Introduction

Many jellyfish (scyphozoan and hydrozoan medusae) populations appear to be increasing around the world, most likely in response to human-induced alterations of the oceanic environment, such as global warming, eutrophication, and over-harvesting of fish stocks (Mills 2001). However, information on jellyfish biology is limited, especially on the ecology of the polyp phase compared to that of their medusae counterparts (Mills 2001). This disparity is often related to the small size and cryptic nature of the benthic stage, making them difficult to detect in the wild. Yet, knowledge of the ecology of both life stages is crucial to understanding the causes of bloom events (Mills 2001), as well as to being able to predict and manage any resulting impacts.

Four hydrozoan jellyfish, *Maeotias marginata, Moerisia* sp. (species indet.), *Blackfordia virginica,* and *Cordylophora caspia* (polyp phase only), have become established within the low-salinity waters of the upper San Francisco Estuary (Mills and Rees

Published online: 08 October 2010

2000). Originally native to the Ponto-Caspian region, this quartet was likely introduced through ballast water release (Rees and Gershwin 2000). *Cordylophora caspia* was introduced as early as the 1920s (Folino-Rorem et al. 2009), while *B. virginica* has been noted since 1970 (Mills and Sommer 1995). *Moerisia* sp. was first collected in 1993 (Mills and Rees 2000). The cryptic nature of these species may have allowed them to be present, but undetected, in the system even longer. Gelatinous zooplankton are novel predators in this region and increasing trends in medusae abundance may be linked to sharp declines in four planktivorous fish species (Schroeter 2008).

Morphological descriptions of medusae (Mills and Sommer 1995; Rees and Gershwin 2000), diets (Mills and Sommer 1995; Schroeter 2008), and their seasonal trends in the estuary (Rees and Kitting 2002; Schroeter 2008) have been reported in recent years. However, information on polyp biology in the system is largely lacking. Rees and Gershwin (2000) were able to culture the earliest stages of a single *M. marginata* polyp in the laboratory, but it expired before it reached full development. *Moerisia* sp. polyps were successfully reared in the laboratory from a polyp specimen found in a sample bag at the Monterey Bay Aquarium (Mills and Sommer 1995) and also developed from ripe medusae (Rees and Gershwin 2000). Additionally, Mills and Rees (2000) found wild populations of both *Moerisia* sp. and *B. virginica* polyps in the Napa River. *Cordylophora caspia* is a global invader that is commonly studied in both laboratory and field (Folino 2000; Jankowski et al. 2008). Ecological information on this species in the San Francisco Estuary, however, is limited.

The goal of this study was to gather the first field-derived ecological data for the polyp stages of these hydrozoan species in the upper San Francisco Estuary. Specifically, we aimed to (1) document patterns of recruitment and distribution of polyps within the estuary, (2) identify the abiotic parameters associated with their distribution and abundance patterns, and (3) determine seasonal patterns of persistence of polyps in the estuary. This was part of larger study to develop a solid understanding of the ecology of both medusa and polyp phases of invasive hydrozoans in the San Francisco Estuary in order to determine their impact on the estuarine ecosystem.

## Materials and methods

### Field collections

Fouling arrays were hung at five brackish water locations within the estuary, with one in each of the Napa and Petaluma Rivers and one in each of three sloughs (Suisun, Montezuma, and Boynton) within Suisun Marsh (Fig. 1). Each array was constructed

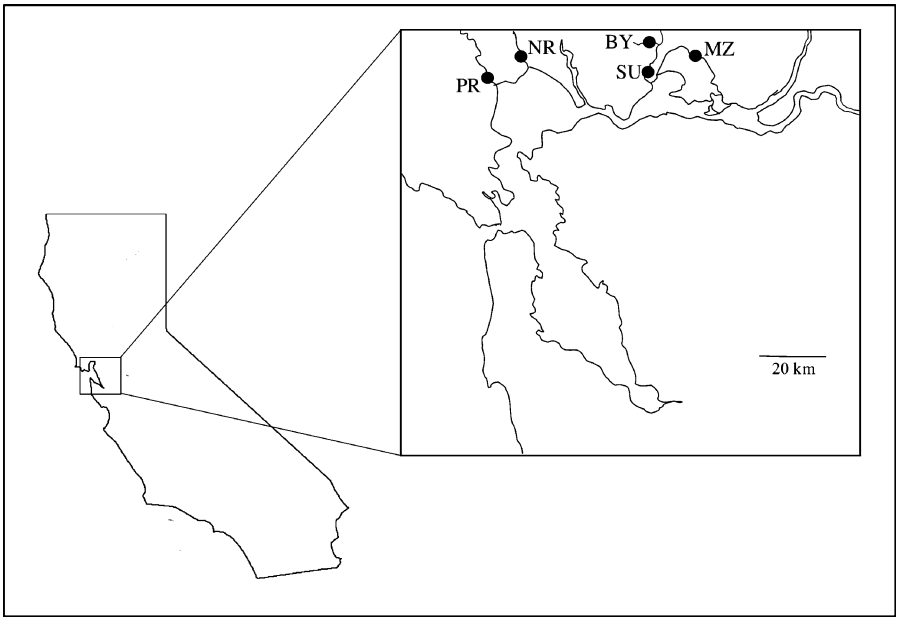


**Fig. 1** Map indicating sites of settlement array collections in the San Francisco Estuary, *PR* Petaluma River, *NR* Napa River, *BY* Boynton Slough, *SU* Suisun Slough, *MZ* Montezuma Slough

to hang at two levels within the water column, one at 0.5 m below the water's surface and the other 0.5 m above the bottom. Six 100 $cm^2$ sheet PVC plates, roughed with an orbital sander on both sides, were suspended from each level.

To assess monthly trends in polyp distribution and recruitment, including settlement of both planula larvae and asexually produced polyps, fouling arrays were deployed during the medusae bloom period from May–November 2007 and April–November 2008. Of the six PVC plates per array level, three were randomly oriented vertically and three were hung horizontally. This was meant to maximize the chance of capturing polyps, because we had no prior knowledge of their environmental preferences for settlement. All plates were replaced monthly, and the collected plates were preserved in 95% ethanol for laboratory work. Additionally, monthly water quality measurements of temperature, salinity, and dissolved oxygen were collected using a YSI meter; water transparency was determined with a Secchi disk.

To examine trends in polyp persistence, fouling arrays were deployed during April 2008, with all six plates per array level hung horizontally. Two randomly chosen plates per level were then collected from each site in June, August, and October 2008 and preserved in ethanol. The sample plates were replaced with new plates to maintain a constant array surface area, but these plates were not used in the analyses.

### Laboratory processing

In the laboratory, the polyps were initially identified to species with genetic sequencing using ITS1 primers from Dawson and Jacobs (2001). These regional sequences were then compared to the ITS1 regions, sequenced as noted earlier, of the easily identifiable medusae stages of *Maeotias marginata* ($n = 8$; Genbank accession # GU198208), *Moerisia* sp. ($n = 5$; Genbank accession # GU198209, GU198210), and *Blackfordia virginica* ($n = 5$; Genbank accession # GU198211–GU198213), as well as a positively identified *C. caspia* polyp specimen ($n = 7$; Genbank accession # GU198206, GU19820). No variation was found at the locus within each species, making this a reliable method of species identification. After genetic confirmation, polyp species were classified based on morphology alone and all hydranths were enumerated from the plates.

### Analyses

The monthly abundance numbers were standardized to number of days each plate was deployed to yield polyp recruitment rates. The rates were then scaled up to an area of one square meter. Means of these data were plotted by site and by month to allow for visual inspections of distribution and recruitment trends within the estuary.

A canonical correspondence analysis (CCA) was performed with CANOCO (CANOCO ver. 4.0) to examine relationships among monthly recruitment rates of polyps, four water quality variables, and the depth each plate was hung in the water column. The forward selection process was used to build a final model, which was then tested for significance with Monte Carlo simulations ($P < 0.05$, 499 permutations). Collection sites were added to gradients on the resultant ordination plot. The values of each of the model variables associated with the 5th and 95th percentiles of polyp recruitment were then identified to estimate an optimal range of abiotic parameters for each species. In addition, *t*-tests were performed to determine differences in recruitment on plates hung vertically versus horizontally and for top versus bottom sides of the horizontal plates.

To examine persistence of polyps over time, two-way ANOVAs, with month and treatment (monthly vs. persistence) effects, were performed on recruitment data for each polyp species found at each site (when present). These analyses included data for monthly and persistence plates from June, August, and October 2008. Tukey–Kramer HSD *post hoc* tests were then run to pinpoint the source of any variance. The loss of October plates in Boynton Slough precluded a full sample set. Thus, persistence analyses of this site were performed with a one-way ANOVA for each effect.

## Results

The fouling arrays were successful at sampling polyp recruitment for all hydrozoan species except *M. marginata*, with cumulative plate totals yielding more than 1 million individuals. Visual plots of mean monthly recruitment data indicate variability among sites, species, and years (Fig. 2). *Blackfordia virginica* was present in the estuary from June through October

and had the greatest recruitment rates at the Napa River site (Fig. 2a, b). Fewer *B. virginica* were collected from the Petaluma River, Boynton, and Montezuma sites, while they were not found in Suisun Slough (Fig. 2a). This species peaked in recruitment in June and July and had higher rates in 2007 than in 2008 (Fig. 2a, b). *Moerisia* sp. recruited to plates at all sites, except for the Napa River, and had the highest rates in Montezuma Slough in 2007 and Boynton Slough in 2008 (Fig. 2c). It was collected between June and November, peaking in August and September (Fig. 2d). *Cordylophora caspia* was found at all Suisun Marsh sites but not in the Napa and Petaluma Rivers (Fig. 2e). It was collected during all sampling months, although recruitment rates were highly variable among months (Fig. 2f). These rates were highest in Boynton Slough during both years (Fig. 2e).

All five environmental variables put through forward selection were retained to build the final CCA model. The first two CCA axes were associated with 54.9% (48.4 and 6.5%, respectively) of the variability in species recruitment (Table 1). Monte Carlo simulations showed statistical significance on both the first axis ($F = 108.062$, $P = 0.002$) and the whole model ($F = 27.971$, $P = 0.002$). The canonical correspondence ordination diagram (Fig. 3) plots the species scores in relation to gradients of the

**Table 1** Results of a canonical correspondence analysis relating recruitment of hydrozoan polyps onto settling plates to environmental variables in the San Francisco Estuary

| | Axis 1 | Axis 2 |
|---|---|---|
| Eigenvalue | 0.732 | 0.097 |
| Species–environment correlation | 0.889 | 0.408 |
| Cumulative percentage of variance explained | | |
| Species | 48.4 | 54.9 |
| Species–environment relation | 88.3 | 100.0 |

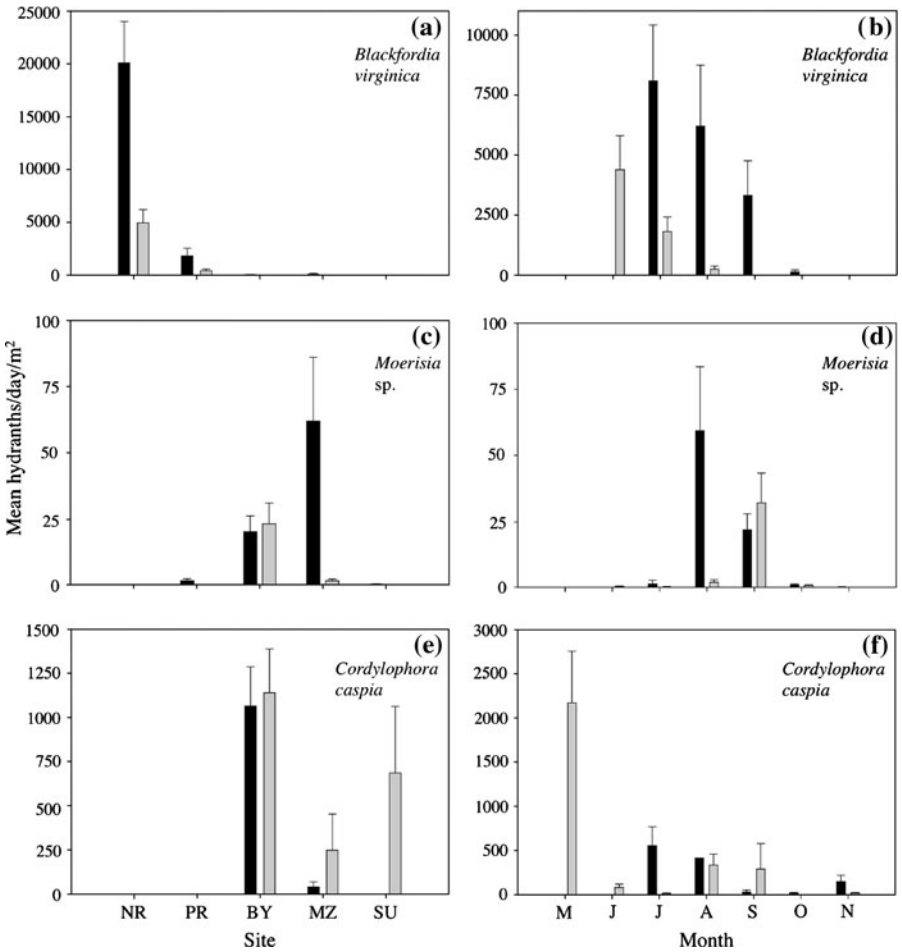


**Fig. 2** Mean recruitment rates of hydrozoans to settling arrays at five sites within the San Francisco Estuary; **a**, **c**, **e** by site and **b**, **d**, **f** by month; *NR* Napa River, *PR* Petaluma River, *BY* Boynton Slough, *MZ* Montezuma Slough, *SU* Suisun Slough; Months: May–November; *black bars* 2007, *gray bars* 2008; *error bars* reflect standard error

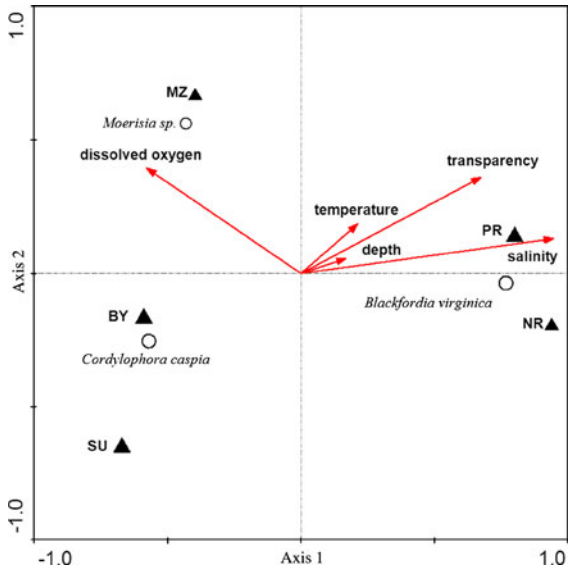


**Fig. 3** Canonical correspondence ordination diagram depicting relationships among polyps of three hydrozoan species (*circles*), sites (*triangles*), and habitat variables in the San Francisco Estuary; *NR* Napa River, *PR* Petaluma River, *BY* Boynton Slough, *MZ* Montezuma Slough, *SU* Suisun Slough

environmental model variables. The length of each environmental variable's vector represents its relative explanatory power (e.g., salinity explains a high degree of the variability in the species data), while direction of the vector indicates an increase (e.g., salinity is greatest toward the right side of the plot). Relative to other species, *B. virginica* polyps were associated with regions of high salinity, higher water transparency, low dissolved oxygen, high temperatures, and greater depths. *Moerisia* sp. polyps were linked to mid-levels of salinity, water transparency, temperature, and depth, as well as high dissolved oxygen. *Cordylophora caspia* was associated with low salinity, transparency, temperature, and depth, as well as mid-levels of dissolved oxygen. The three species differed in their correlations with environmental variables measured (Table 2), as indicated by the "optimal ranges" at which they were found, based on 5th and 95th percentiles of recruitment rate (Schroeter 2008).

Recruitment patterns, measured as total hydranths settled per day per $m^2$, differed among species in response to the physical orientation of the plate. *Blackfordia virginica* showed significantly higher recruitment for horizontal plates ($\bar{x}_{\text{horizontal}} = 331.5$, $\text{SD} = 487.3$) than vertical plates ($\bar{x}_{\text{vertical}} = 113.3$, $\text{SD} = 198.0$; $t = 3.433$, $P = 0.001$). *Moerisia* sp. showed a similar, though non-significant trend ($\bar{x}_{\text{horizontal}} = 2.2$, $\text{SD} = 4.3$; $\bar{x}_{\text{vertical}} = 0.8$, $\text{SD} = 1.7$; $t = 1.979$, $P = 0.053$). Neither orientation was related to recruitment patterns in *C. caspia* ($\bar{x}_{\text{horizontal}} = 43.6$, $\text{SD} = 78.1$; $\bar{x}_{\text{vertical}} = 28.9$, $\text{SD} = 66.7$; $t = 1.186$, $P = 0.238$). An examination of recruitment differences between the top-side of the horizontal plate versus the bottom side, revealed that both *B. virginica* ($\bar{x}_{\text{top}} = 32.0$, $\text{SD} = 64.1$; $\bar{x}_{\text{bottom}} = 310.2$, $\text{SD} = 454.9$; $t = 5.162$, $P = 0.000$) and *Moerisia* sp. polyps were more abundant on the bottom sides ($\bar{x}_{\text{top}} = 0.6$, $\text{SD} = 0.7$; $\bar{x}_{\text{bottom}} = 2.2$, $\text{SD} = 4.6$; $t = 2.160$, $P = 0.037$). *Cordylophora caspia* exhibited no difference in abundance on either side of the plates ($\bar{x}_{\text{top}} = 23.4$, $\text{SD} = 44.9$; $\bar{x}_{\text{bottom}} = 23.6$, $\text{SD} = 38.2$; $t = 0.023$, $P = 0.982$).

Persistence plates did not show the expected cumulative effect of polyps settling over a longer time period, for any species at any site. They did, however, often mirror the monthly trends in recruitment. Two-way ANOVAs of plates collected and replaced monthly showed generally low trends in polyp persistence over time, but the details were related to site and species (Fig. 4). The Napa River, which only had *B. virginica* present in 2008, had significant differences in treatment ($F = 7.3093$,

**Table 2** Optimal environmental variable ranges, represented by 5th and 95th percentile, for hydrozoan polyps recruitment onto settling plates in the San Francisco Estuary

| Variable | *Blackfordia virginica* | *Moerisia* sp. | *Cordylophora caspia* |
|---|---|---|---|
| Salinity (‰) | 14.9–22.2 | 4.6–21.8 | 1.4–8.9 |
| Temperature (°C) | 20.0–23.1 | 18.9–22.1 | 18.1–23.5 |
| Dissolved oxygen (mg/l) | 2.3–6.3 | 5.7–6.9 | 4.6–7.6 |
| Water transparency (cm) | 30.5–91.5 | 32.0–60.0 | 12.0–55.0 |
| Plate depth (m) | 0.3–2.7 | 0.3–1.5 | 0.3–1.2 |

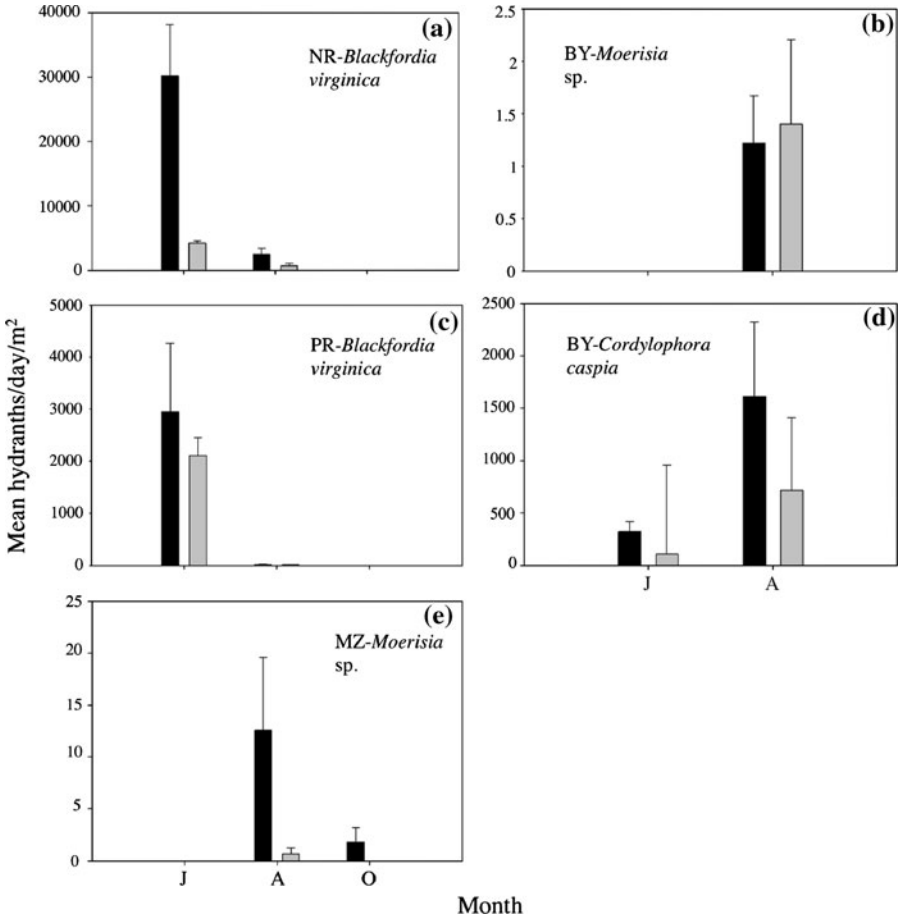


**Fig. 4** Mean recruitment rates of hydrozoans to monthly and persistence settling arrays at five sites within the San Francisco Estuary; *NR* Napa River, *PR* Petaluma River, *BY* Boynton Slough, *MZ* Montezuma Slough, *SU* Suisun Slough; Months: June, August, and October; *black bars* monthly plates, *gray bars* persistence plates; *error bars* reflect standard error

$P = 0.0124$), month ($F = 10.5657$, $P = 0.000$), and their interaction ($F = 6.2854$, $P = 0.006$). Tukey tests revealed that these results were due to extremely high polyp recruitment numbers on monthly plates collected in June. There were no differences among any other monthly or persistence plates. Plates from the Petaluma River that tested solely for *B. virginica* showed an effect of month only ($F = 9.0323$, $P = 0.001$). Multiple comparison tests identified that both monthly and persistence plates had high recruitment in June, compared to other months. Polyps in Boynton Slough, due to the loss of October persistence plates, were analyzed as one-way ANOVAs for the treatment effect for each of the *Moerisia* sp. and *C. caspia* and neither was significant. Montezuma Slough plates, analyzed for *Moerisia* sp. persistence, were also not significant. Persistence plates from Suisun Slough did not contain any polyps and therefore were not compared to monthly plates.

## Discussion

### Distribution and abundance

The polyp phases of *Moerisia* sp. and *B. virginica* follow the common recruitment pattern in benthic hydroids in temperate waters, showing a peak in productivity during spring and summer, which slows down in fall and winter (Coma et al. 2000). Polyps that appear the following season are likely to be either coming from an existing population of active individuals from an alternate location or re-generating after a period of dormancy. The re-vitalization of polyp colonies when optimal seasonal water quality parameters are reached may be a strong indication of the latter (Calder 1990). Dormancy is a common method of survival until optimal conditions that promote growth are restored and is seen in a variety of marine taxa (Boero et al. 2008). Thus, the closely

related *M. lyonsi* is known to form a durable cyst resting stage that gives rise to the next year's population (Purcell et al. 1999). There is currently no information available about a resting stage of *B. virginica*, but their rapid seasonal reappearance in the Napa and Petaluma Rivers strongly suggests it is likely to be present.

*Cordylophora caspia* appeared to follow a different recruitment pattern with little seasonal influence. Although our sampling was limited only to the period around medusae blooms, fish dietary studies from Suisun Marsh noted a year-round presence of the hydroid in this system (Matern and Brown 2005). Similarly, a study by Calder (1990) indicates that it also remains active throughout the year in warmer waters along the coast of South Carolina. *Cordylophora caspia* has successfully invaded a broad latitudinal range along the eastern coast of North America, extending from Quebec to the Caribbean Sea (Calder 1990). In extreme salinities (Kinne 1971) and temperatures (Fulton 1962), likely associated with the two ends of such a range, it stops growth and may form diapause stages, known as "menonts," by keeping dormant tissues within stems and stolons (Roos 1979). The year-round presence of *C. caspia* in the San Francisco Estuary indicate that temperature and salinity ranges found in this system remain within this species' tolerance levels.

The reason for lack of *M. margniata* polyps on the plates remains unknown and may be related to polyp size or substrate preference. Schroeter (2008) stated that the extremely rapid increase in abundance of medusae in relation to environmental factors indicates that polyps are present in the system, but information on them is limited. The single early stage polyp cultured by Rees and Gershwin (2000) grew on detritus, rather than the glass of its holding vessel. This may indicate that *M. marginata* polyps prefer soft substrate settlement or it may simply be a lack of preference for glass. Our inability to find this species may also be due to its small, cryptic nature. The single primary polyp reared in the laboratory was only ~0.1 mm in height and lacked tentacles (Rees and Gershwin 2000). This specimen expired before it could develop further. Thus, morphology of the fully mature stage is unknown. A similarity to the primary polyps of the freshwater jelly *Craspedacusta sowerbii* has been noted (Rees and Gershwin 2000) and *M. marginata* has been named as the extant sister group to freshwater jellies (Collins et al. 2006). Therefore, it is possible that the small, simple polyps of *C. sowerbii* may be similar to *M. marginata* polyps and were overlooked on the plates. Additionally, the ethanol preservative may have shrunk polyps down to an unrecognizable specimen. Future samples may require alternate plate materials and formalin preservation.

## Physical correlates

The CCA results demonstrate that water quality factors are strongly correlated with the majority of variation in our polyp data and thus explain a large portion of our seasonal observations. It is commonly believed that temperature is the main driver of seasonal hydroid patterns (Gili and Hughes 1995). In our system, while temperature was a factor of influence, it was salinity that had the most importance. The San Francisco Estuary has a seasonally dynamic salinity regime compared to the more stable marine systems involved in most hydroid research (Boero 1984). Similarly, work in tropical regions with rainy seasons and related salinity shifts has noted salinity as an important variable in seasonal hydroid abundance (reviewed in Gili and Hughes 1995). Salinity also was a major factor for *M. lyonsi* polyps in Chesapeake Bay (Ma and Purcell 2005a, b). Salinity did not appear to dictate a seasonal presence of *Cordylophora*, which may be due to this species' remarkable ability to physically remodel itself under changing saline conditions to maintain effective metabolic function (Kinne 1964).

Surprisingly, dissolved oxygen levels were highly correlated with higher recruitment rates of *Moerisia* sp. and *C. caspia* in this study. While many medusae are well known for their abilities to withstand low oxygen conditions, the limited information about tolerance in the polyp stage for some species suggests otherwise. Reduced abundances of hydromedusae in the Adriatic sea, for example, were hypothesized to result from an intolerance of polyps to depleted oxygen levels in the benthos (Benovic et al. 1987). Similarly, polyps of the scyphozoan *Chrysaora quinquecirrha* had reduced long-term survival and asexual reproduction rates in laboratory treatments with low dissolved oxygen levels compared to those with higher levels (Condon et al. 2001).

Water transparency is a known variable that influences hydroid distribution. *Blackfordia virginica* recruitment was greatest at high (for the estuary) levels of transparency. An increase in water transparency in this system is often related to reduced levels of suspended sediment and detrital matter. This in turn may reduce contact damage to their small, fragile polyps. Increased light levels may also result in an increase in prey abundance. *Cordylophora caspia* showed an opposing trend, thriving in less transparent sites, which could be a behavior associated with the avoidance of filamentous algae substrate competitors (Gili and Hughes 1995).

Some alternative biotic factors that may influence trends of hydrozoan polyps include food availability and strategic temporal staggering of seasonal cycles with those of substrate competitors (Bavestrello et al. 2006). Other species have been known to possess some form of internal clock, going through the motions of seasonality even when kept at constant laboratory conditions (Brinkmann 1964; Brock 1975). Further investigation would be required to test these alternatives.

The interplay of abiotic factors in relation to plate orientation may explain some of the trends observed in this study. For example, the fact that both *Moerisia* sp. and *B. virginica* polyps were at higher densities on plates hung horizontally versus vertically is likely related to water flow. The San Francisco Estuary is a tidally driven system. Many hydroid species will grow perpendicularly to the major direction of flow, with benefits of increased water exchange lending itself to rapid replenishment of oxygen and planktonic prey (summarized in Boero 1984) while also acting to effectively disperse recently budded medusae (Gili and Hughes 1995). The preference of *B. virginica* to settle on the bottom side of horizontal plates may be a strategy to avoid being covered with sediment, because this species is quite small, to reduce competition with filamentous algae, or to expel self-generated waste products from their proximity (C. Widmer, personal communication). The lack of position preference for *C. caspia* colonies may be due to their sets of stacked annular rings at branching points. Similar annuli in *Obelia longissima* were found to significantly reduce local soft tissue damage during flexion (Hunter 1987), such as that induced by water flow.

In terms of settlement substrate, it is clear from this study that *Moerisia* sp., *B. virginica*, and *C. caspia* will recruit to a wide variety of substrates including PVC. Calder (1990) collected *M. lyonsi* polyps from the Chesapeake Bay growing on shells of the mussel *Brachidontes recurvas* and on detrital matter. This species was also observed on oyster shells (Purcell et al. 1999). *Blackfordia virginca* was collected in the Napa River on both floating docks and shells of the barnacle *B. improvisus* (Mills and Rees 2000). *Cordylophora caspia* is well known for its ability to settle on a variety of surfaces, including intake screens (Folino-Rorem and Indelicato 2005), rocks, floating, and emergent vegetation (Roos 1979), and even the dorsal surface of a live cyprinid fish, the Sacramento splittail, *Pogonichthys macrolepidotus* (A. Wintzer, unpublished observation)!

## Persistence

Like most hydrozoans, the polyp phases of non-native jellyfish in the San Francisco Estuary are exceptional pioneers, settling early on the fresh surfaces of our array plates. Boero (1984) credited the taxa's early establishment success to its predisposition for rapid settlement and growth. High estimates (hydranths/day/m$^2$) for single settling plates in this study included 124,976 *B. virginica,* 1,107 *Moerisia* sp., and 22,998 *C. caspia*. Boero (1984) also noted that, as in this case, after initial settlement, most hydrozoans are then often out-competed for space by more robust species. In the persistence study, polyps were competing, unsuccessfully, for space with a number of other non-native fouling species, including the Australian tubeworm, *Ficopomatus enigmaticus*, the common sea grape, *Molgula manhattensis*, the bay barnacle, *Balanus improvisus*, and a particularly aggressive unidentified bryozoan species.

Polyp persistence may have also been limited by presence of predators commonly associated with the settling plates. Both the shimofuri (*Tridentiger bifasciatus*) and shokihaze (*T. barbatus*) gobies, native to Asia (Moyle 2002), were commonly seen resting on our plates. Gut content examinations of shimofuri gobies in the San Francisco Estuary have revealed that *C. caspia* accounted for 18–23% of diet volume and that it was a prey item consumed year-round (Matern and Brown 2005). Shokihaze gobies were also found to be *C. caspia* predators, but to a lesser degree, with it comprising up to 10% of total gut content weight depending on season (Slater 2005). Several other

species of Suisun Marsh fishes have been found to consume hydroids, but in very small amounts (<1% total weight). These include native Sacramento splittail and prickly sculpin (*Cottus asper*), as well as non-native striped bass (*Morone saxatilis*) and yellowfin goby (*Acanthogobius flavimanus*) (Feyrer 1999). The low percentages for these species, however, may reflect incidental ingestion while consuming other prey items and suggest that their cropping potential on hydroids is quite low. Finally, dozens of the miniature aeolid nudibranch, *Tenellia adspersa,* were found on our settling plates. Also from the Ponto-Caspian region, this species is known to voraciously feed on benthic hydroids (Mills and Sommer 1995; Chester 1996).

## Implications

There is a great need to understand ecological impacts of non-native hydrozoans in the San Francisco Estuary. Fish-monitoring studies have recently revealed sharp declines in populations of delta smelt (*Hypomesus transpacificus*), longfin smelt (*Spirinchus thaleichthys*), age-0 striped bass and threadfin shad (*Dorosoma petenense*) in the upper San Francisco Estuary (IEP 2005, 2006, 2008). A decrease in the abundance of calanoid copepods was also noted for this system (IEP 2008).

Studies are currently underway to examine the trophic implications, through both competition and larvae predation, of the medusae stage on these fishes. This, however, may only be a piece of the puzzle, because in some species the polyps are voracious predators themselves. Gili and Coma (1998), for example, noted that hydroids, which constituted less than 0.5% of biomass in a community, were able to consume 10% of the system's annual algal production. Additionally, polyps of both *Hydra canadensis* and *Craspedacusta sowerbii* can capture and consume larval fishes (Dendy 1978, Elliot et al. 1997). Further investigations will be necessary to understand the polyps' role in the trophic ecology of the estuary, and thus, total impact of these non-native jellyfish on the food web.

The reduced ecological health of the heavily modified San Francisco Estuary has resulted in plans for habitat restoration. As Rees (1999) remarked, knowledge of general habitat use of this suite of hydrozoans, as well as environmental factors that increase populations, will be advantageous to restoration efforts, in order to minimize creation of conditions beneficial to these unwanted species. An avoidance of the "optimal" abiotic conditions given in Table 2 will negatively influence polyp survival. However, for a more effective restoration strategy, these studies should be paired with field studies on medusae that give an understanding of population dynamics of these species.

Finally, aided by unregulated ballast water transport and global shifts in thermal and salinity regimes, many jellyfish species are increasing their ranges. Each of our study species has now been identified on several continents (Kramp 1961; Paranaguá 1963; Denayer 1973; Santhakumari et al. 1997; Folino 2000; Buecher et al. 2005; Genzano et al. 2006; Bardi and Marques 2009). While some findings from this study can be applied to these systems, a continued effort to study the poorly understood polyp stage is necessary to develop predictive tools for bloom formation, ecological impact, and management decisions, as well as to determine sites vulnerable to future invasions.

**Acknowledgments** We thank CALFED Science Program Grant #1036, National Science Foundation grant NSF-DGE #0114432 IGERT UC Davis, NOAA Dr. Nancy Foster Scholarship, UC Davis Jastro-Shields Research Scholarship, UC Davis Block Grant, and the Golden West Women Flyfishers for financial support. We are grateful to the Suisun Resource Conservation District, Dr. Nadine Folino-Rorem, Dr. Ted Grosholz, Dr. Sharon Lawler, the Napa Valley Marina, and the Petaluma Marina for assistance during this project.

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APPENDIX G

# Life history and population dynamics of *Moerisia* sp., a non-native hydrozoan, in the upper San Francisco Estuary (U.S.A.)

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**Abstract**

Some populations of gelatinous zooplankton appear to be increasing and causing ecological disruptions during large bloom events. The goal of this study was to investigate the life history and population dynamics of the small, invasive *Moerisia* sp. and its potential ecological impacts in the upper San Francisco Estuary, California. Medusae and polyps were collected from 8 and 2 sites, respectively, during 2007 and 2008. Polyps emerged from a resting stage during June. Asexual reproduction of medusae buds was positively correlated with temperature and negatively related to salinity. The production of polyp buds was positively correlated with dissolved oxygen and water transparency levels. Sexual reproduction, defined by the presence of eggs, was related to the size of medusae. Cessation of reproduction of both polyps and medusae occurred in October, when temperatures dropped below 17ºC. This life history is similar to other hydrozoans and allows *Moerisia* sp. to reach large numbers seasonally in the San Francisco Estuary, possibly contributing to the recent declines of pelagic fish and zooplankton.

*Keywords: Moerisia, life history, population dynamics, hydrozoa, sexual reproduction, asexual reproduction*

## 1. Introduction

Blooms (normal, seasonal cycles) of gelatinous zooplankton can have tremendous impacts on trophic interactions and nutrient cycling. Sudden pulses of medusae can significantly reduce numbers of zooplankton prey (Brodeur et al., 2002; Hansson et al., 2005; Smith and Alexander, 2008). Fish abundances may be reduced through competition for copepods and other zooplankton prey (Lynam et al., 2005) or direct predation on fish eggs and larvae (Purcell and Grover, 1990; Purcell et al., 1994). Blooms of gelatinous zooplankton may also alter carbon, nitrogen, and other elemental fluxes via large-scale prey consumption, excretion, and mass post-bloom decomposition (reviewed in Pitt et al., 2009). Because the scale and frequency of many blooms appear to be increasing (Mills, 2001), an understanding of each species' life history and population dynamics is needed to predict trends and consequences of these events.

Detailed ecological knowledge of hydromedusae is especially scant, due to their small size and highly seasonal nature (Boero et al., 2008). A yet-to-be-identified hydrozoan species of *Moerisia*, believed to be native to the Black Sea region, has recently become established in the low-salinity waters of the San Francisco Estuary (Mills and Rees, 2000). Because of its small size (typically < 5 mm), its effects in the San Francisco Estuary have been little studied. Rees and Gershwin (2000) cultured *Moerisia* sp. in the laboratory and found they exhibited a classic hydrozoan 2-stage reproduction cycle: 1) polyps asexually produce more polyps, and 2) polyps bud dioecious medusae that mate sexually, producing planula larvae, which settle to form more polyps. In the same study, both the medusae and polyp stages were found to be efficient predators, killing and eating *Artemia* nauplii at a rapid rate. A San Francisco Estuary jellyfish survey found the densities of medusae to be relatively low, $\geq 1/m^3$ (Rees and Kitting, 2002). More recent sampling in the upper estuary, however, yielded density estimates as great as

197/$m^3$, with this species comprising 88-71% of the total medusae numbers over a 2-year study (Schroeter, 2008).

The goal of our study was to identify trends in the population dynamics of *Moerisia* sp. in Suisun Marsh within the upper estuary, a region identified as key nursery area for many fish and invertebrate species (Meng and Matern, 2001; Schroeter, 2008). Specifically, we aimed to document density, size structure, and biomass of the medusae population over the seasonal bloom period, as well as the biotic and abiotic factors influencing egg production by medusae and asexual reproduction by polyps. Additionally, we considered these data in the context of previous studies.

## 2. Materials and methods

### *2.1 Field sampling*

Zooplankton samples, including hydrozoans and their planktonic prey, were collected from eight sites within Suisun Marsh, located in the northeast part of the San Francisco Estuary (Fig. 1) at monthly intervals during the 2007 and 2008 seasonal bloom period of June-November. Collections were made with a horizontally towed conical zooplankton net (230$\mu$m mesh, 0.5m diameter, 2m length) paired with a General Oceanics flowmeter to determine water volume sampled. Tows in sloughs less than 15m in depth were performed at a mid-water column depth. In sloughs greater than 15 m deep, tows were made in the top (approximately 1 m below the surface) and bottom (approximately 1 m above the bottom) halves of the water column. Samples were immediately preserved in 5% non-buffered formalin. A single additional zooplankton tow was performed during August 2009, and the collected medusae were transported fresh to the laboratory for biomass processing.

From April-November of 2008, fouling arrays were deployed at two locations within the

study area (Fig. 1) to collect the polyp stage of *Moerisia* sp. Each array hung at two levels within the water column, 0.5m below the surface and 0.5m above of the bottom. Six 100cm$^2$ roughed PVC plates were suspended from each level. All plates were replaced monthly and the sample plates were preserved in 95% ethanol.

Water quality data, including temperature, salinity (measured using the Practical Salinity Scale), and dissolved oxygen, were measured with a model 30 YSI meter in conjunction each sample collected. Additionally, measures of water transparency were collected using a secchi disk. Mean values of abiotic water quality from all 8 sites were plotted by sample month.

*2.2 Copepod densities*

In the laboratory, subsamples of formalin-preserved calanoid copepods, that contained at least 100 individuals of the most abundant species, were used to determine monthly concentrations. Calanoid copepods were targeted to represent the prey field in this study because they were the most numerous taxa found in the zooplankton samples and they are the most common prey item found in *Moerisia* sp. gut contents (Wintzer, 2010).

*2.3 Medusae densities and population structure*

Preserved *Moerisia* sp. medusae were also subsampled, so that individuals from 1/3 of each sample were counted. Additionally, medusae bell diameters were measured using a SPOT Idea camera and software. Subsamples of medusae and bell diameter values were scaled up using sample volume to estimate monthly totals. Site maps with medusae concentrations and size frequency histograms were created based on these estimates.

A bell diameter-dry weight relationship was developed for the fresh medusae specimens. Sixty fresh medusae were rinsed with fresh water, measured for bell diameter, and initially weighed. They were then left to dry at 25°C. Specimens were re-weighed every two weeks until

a constant value, the dry weight, was obtained. The resulting exponential equation was then combined with the size structure data to develop monthly mean medusa biomass estimates during the blooms.

*2.4 Medusae egg production*

The interradial surfaces of the stomach and lobes of medusae (n = 1,168 individuals) were inspected for the presence of eggs using a dissecting microscope. To examine the factors correlated with presence of eggs in medusae, a forward step-wise regression model was built using temperature, salinity, dissolved oxygen, water transparency, calanoid copepod prey density, hours of daylight, and medusae bell diameter as potential parameters. After selection, a nominal logistic model, utilizing a likelihood-ratio $\chi^2$ test, was run on the retained variables. We were unable to detect fertile males in our preserved samples.

*2.5 Polyp reproduction*

The presence or absence of medusae buds and polyp buds were noted on polyps (n = 198) from the monthly fouling plates. As described for medusae egg presence in section 2.3 above, regression models were built using the same parameter pool, with the exception of medusae bell diameter, to find factors related to the production of both medusae buds and polyp buds by polyps, and nominal logistic models were then run for each case.

## 3. Results

*3.1 Monthly water quality and copepod densities*

Water temperature data (Fig. 2a) showed typical summer and fall seasonal trends. Temperatures were highest in the summer months (June-August), approximately 21.0ºC during both years, and then sharply declined beginning in August, reaching a low of 11.3ºC in 2007 and 14.8ºC in 2008.

The trend for salinity was generally opposite to that of temperature (Fig. 2b). Salinity was lowest in June of both years: 3.0 in 2007 and 4.3 in 2008. Values increased throughout the summer to a maximum in the fall. In 2007, salinity was highest in November (7.2) and in 2008, salinity peaked in September (8.9) and then fell to 6.6, likely due to earlier rains and freshwater inflow. These trends are typical for this region, which receives little to no rain, and associated increased freshwater inflow, until the fall or winter.

Dissolved oxygen values (Fig. 2c) had a fair amount of variability, but showed an overall increasing trend from July (6.1mg/l) to September (7.1 mg/l) in 2007 and June (6.0 mg/l) to October (7.3 mg/l) in 2008, in response to the decrease in temperature. Dips in dissolved oxygen are common in Suisun Marsh during fall, as a result of multiple anthropogenic factors, including increased urban runoff and wetland drainage (Schroeter, 2008).

Water transparency generally tracked trends of salinity (Fig. 2d). The sampling period began in June with reduced water clarity (24 cm) in both 2007 and 2008, increasing to maximums of 32 cm and 40 cm in October of 2007 and 2008, respectively. The sharp decrease in transparency seen in November 2008 reflects turbidity associated with increased freshwater flows.

Density estimates for calanoid copepods showed an increasing trend from month to month during the two study years (Fig. 3). The samples for 2008, however, had much greater variability and higher average density values than those for 2007.

*3.2 Medusae densities and spatial patterns*

An estimated 80,000 *Moerisia* sp. medusae were collected during the study. This species exhibited a bloom pattern of low densities in June, with numbers gradually increasing throughout the summer and a rapid decrease by October (Fig. 4). The 2008 densities did appear to increase

faster than the previous year, and both sampling years showed extremely high densities of greater than 200 individuals/$m^3$ at some sites between July and September.

Spatial trends place the first medusae of the bloom in the southeastern-most site sampled. By July, *Moerisia* sp. could be found throughout the system. The highest concentrations of medusae were found in the northwestern region of the marsh, and the few individuals remaining in October were in the northern reaches (Fig. 4).

*3.3 Medusae size distribution and biomass*

Monthly bell diameter histograms revealed a discernable trend over the bloom period (Fig. 5). The few medusae found and measured in June of 2007 and 2008 fell into the smallest size classes, less than 2mm. Over the following three months, the medusae were found in nearly all of the classes, although individuals greater than 5 mm were not common. The continued representation of smallest size class indicates continuous production of new medusae through October of both years. At the completion of the bloom, the two medusae that were collected were fairly large, greater than 3 mm.

The relationship between bell diameter and dry weight was fit with an exponential curve ($R^2 = 0.97392$) (Fig. 6):

$$y = 0.00002\exp(0.5362x)$$

Total medusa biomass plots per month show overall trends of initial low values, rising to peaks in September 2007 and August 2008, and then decreasing again by October in both years (Fig. 7). Biomass values for July and August of 2008 are much larger than those for the same months in 2007.

*3.4 Correlates of egg production by medusae*

Medusae carrying eggs were found from July-September during both 2007 and 2008.

Only medusae bell diameter was retained as a variable after forward parameter selection. The nominal logistic fit whole model test showed a significant, positive correlation between bell diameter and the likelihood ratio that medusae were producing eggs, $\chi^2$ (1, N = 1,168) = 65.34, p < 0.001. The range of *Moerisia* sp. bell diameters for medusae that had eggs was 1.9-5.2 mm, while the mean size was 3.3 mm.

*3.5 Correlates of medusae bud and polyp bud production by polyps*

Polyps produced medusae buds from June to October. The variables temperature and salinity were retained by the stepwise regression and integrated into a significant nominal logistic model, $\chi^2$ (2, N = 198) = 39.85, p < 0.001. Temperatures that correlated to medusae bud production ranged from 16.9-25.6ºC, with an average of 22.1ºC. Salinities correlated with medusa bud production were between 1.5 and 7.1, with a mean of 5.2.

Polyp buds were also produced from June to October and were positively correlated with dissolved oxygen and water transparency. The logistic model created with these variables was significant, $\chi^2$ (2, N = 198) = 26.16, p < 0.001. The production of polyp buds occurred within a dissolved oxygen range of 3.7-7.4 mg/l, with an average of 7.0 mg/l. The water transparency range was broad, from 19 to 60 cm, with an average of 56 cm.

**4. Discussion**

The life history of *Moerisia* sp. in Suisun Marsh has four distinct stages, which correspond to the dynamics of the annual bloom: polyp regeneration, asexual reproduction, sexual reproduction and bloom cessation. These stages are similar to those documented for other cnidarians, especially *M. lyonsi,* the species that *Moerisia* sp. most closely resembles (Rees and Gershwin, 2000). The blooms observed in Suisun Marsh are the result of a sequence of environmental conditions that seasonably favor the different life history stages.

*4.1 Polyp regeneration*

In our study area, *Moerisia* sp. polyps appear during June (Wintzer et al., 2011). Temperature appears to be the principal trigger that initiates regeneration from resting stages in temperate estuarine and freshwater hydrozoans (Dunham, 1941; Calder, 1990; Folino, 2000). While no encysted specimens were recovered, the rapid appearance of medusae implies that local populations of polyps are re-vitalized from a state of dormancy rather than from the immigration of medusae from other parts of the estuary. This is further supported by the lack of medusae collected in the regions surrounding the marsh prior to polyp appearance in this study. The polyps of *M. lyonsi* are also believed to utilize a dormant resting stage, because they were able to repeatedly colonize mesocosm set-ups even after enduring desiccated conditions (Purcell et al., 1999).

*4.2 Polyps and asexual reproduction*

We found temperature to be positively related to production of medusae buds in *Moerisia* sp., while salinity had a negative correlation. Schroeter (2008) noted that the *Moerisia* sp. medusae blooms appeared at an average temperature of 21.2°C, and an average salinity of 5.8. These values fall within the ranges of salinity and temperature correlated with bud production, but the early season values from the current study suggest this event may occur at conditions of 25.6°C and 1.5. The discrepancy may be attributed to a larger collection mesh size (500 $\mu$m) of the net used by Schroeter (2008), which would not have captured the earliest, small medusae.

We also found that the production of polyp buds was related to high dissolved oxygen levels. This is in agreement with Wintzer et al.'s (2011) study, correlating water quality variables to polyp distribution and abundance in Suisun Marsh, which found recruitment rate of *Moerisia* sp. polyps is most strongly influenced by dissolved oxygen. Given this, and the lack of

a relationship between dissolved oxygen and sexual reproduction in medusae, it is likely that asexual reproduction is the most utilized mode of population increase for the benthic stage in this system. A similar trend has been demonstrated for stolonation by *Chrysaora quinquecirrha* polyps (Condon et al., 2001).

Interestingly, high values of water transparency were also related to polyp bud production. Because water transparency values may be considered to be a crude proxy for light intensity, this trend is the opposite of that found in *Aurelia aurita*, in which polyps are produced at lower light intensities and medusae buds are formed as this value increases (Liu et al., 2009). The polyps of *A. aurita* settle on the undersides of substrate, which have low light intensities. *Moerisia* sp. polyps, however, recruit to both vertical and horizontal surfaces without significant difference (Wintzer et al., 2011). These variations in settlement behavior may reflect behavioral differences between these species, which can explain the alternate trends regarding light and polyp production.

It may be that prey availability also impacts rates of asexual reproduction by *Moerisia* sp. polyps. Purcell et al. (1999) found that the creation of *M. lyonsi* polyp buds was related to high food levels, based on treatments of 1 to 8 copepods/day. The polyp stage of *Moerisia* sp. in Suisun Marsh consumes a variety of small zooplankton and benthic crustaceans (Wintzer, unpublished data), which were not collected during this study.

Once re-established in the estuary, *Moerisia* sp. polyps were collected between June and November, with peaks in August and September (Wintzer et al., 2011). The "optimal conditions" for this life stage (based on the 5$^{th}$ and 95$^{th}$ percentile values of density data) include temperatures between 18.9-22.1ºC, a broad salinity range of 4.6-21.8, dissolved oxygen levels of 5.7-6.9mg/l, and a water transparency range of 32.0-60.0cm (Wintzer et al., 2011). Recruitment

within the system seems to infer high rates of asexual reproduction, with a single settlement plate estimate of 1,107 *Moerisia* sp. hydranths/day/$m^2$ (Wintzer et al., 2011). Purcell et al. (1999) noted that the rapid rate of asexual reproduction in *M. lyonsi* is also striking in relation to the few published data for other cnidarians. As with most hydrozoan polyps, they are well adapted to early colonization of surfaces, but are soon out-competed for space (Boero, 1984). Persistence of polyps was generally poor from month to month, as non-native substrate competitors, including the bay barnacle, *Balanus improvisus*, and predators, such as shimofuri (*Tridentiger bifasciatus*) and shokihaze (*T. barbatus*) gobies reduced their numbers (Wintzer et al., 2011).

*4.3 Medusae and sexual reproduction*

Fast growth rates have been observed in *Moerisia* medusae post-release from polyps. Under laboratory conditions, Rees and Gershwin (2000) found 10-day-old *Moerisia* sp. medusae ranged in size from 1.5-2.5 mm bell diameter and reached 3.5-4.2 mm by day 24. This rate, of approximately 13% growth/day, was slower than the 33% growth/day recorded for laboratory-raised *M. lyonsi* (Purcell et al., 1999), but on par with other species, including field measured *A. aurita* (Hansson, 1997).

The development of eggs in medusae of *Moerisia* sp. was correlated only with bell diameter. The fast growth rate of this species, coupled with its small final size and a high level of available prey may explain these findings. Hamner and Jenssen (1974) found that maturation in *A. aurita* was also dependent upon size of medusae. Mature *Moerisia* sp. females were found during several months of both sampling years, over a period in which water quality varied considerably. During the course of the seasonal bloom, medusae occurrence was greatest under the apparently optimal conditions of 19.5-22.7 ºC temperature, 2.8-9.9 salinity, and 4.6-7.8 mg/l dissolved oxygen in 2005 and 15.7-22.0 ºC temperature, 3.1-9.3 salinity, and 4.6-7.8 mg/l

dissolved oxygen in 2006 (Schroeter, 2008).

Combinations of physical forces (*e.g.* circulation patterns, thermoclines, etc.) and behaviors commonly influence the distributions of hydromedusae (Arai, 1992). The rapid, wide-spread appearance of medusae in Suisun Marsh during the early months of the bloom is likely a due to polyp re-generation and asexual reproduction, in addition to the movement of the weak-swimming *Moerisia* sp. via tidal motion (Rees and Gershwin, 2000).

*4.4 Bloom cessation*

Large-scale declines of *Moerisia* sp. polyps were observed during October. A decrease in temperature, and its associated physiological and ecological impacts, is the commonly accepted trigger for dormancy in polyps during the winter (Coma et al., 2000). The reduction of *Moerisia* sp. polyps occurred with temperatures of 16.9-17.1ºC, which may be the thermal limit for the benthic stage of this species before it enters a diapause stage (Wintzer et al., 2011).

Mortality in medusae may be linked to physiologically intolerable environmental conditions. A minimum temperature of 19ºC was estimated for survival of the medusae stage, explaining their typical reductions in early October (Schroeter, 2008). *Moerisia* sp. medusae were collected at a minimum temperature of 17ºC in this study, suggesting a slightly lower thermal tolerance for this stage. Some mortality in medusae may also be due to natural senescence, which is common after sexual reproduction (Boero et al., 2008). There are no known medusae predators in the upper San Francisco Estuary (Rees and Gershwin, 2000) that would cause a reduction in their population. Additionally, prey limitation is an unlikely contributor to the declines, as there were numerous calanoid copepods present at the end of the bloom.

*4.5 Future investigations of Moerisia* sp.

To date, most data on *Moerisia* sp. come from field studies, and detailed laboratory experiments are required to identify fine-scale patterns of life history and population dynamics. For example, studies on the polyp phase may allow us to identify the exact conditions required for the promotion of polyp re-generation and regression to a resting stage. Additionally, we may gain insight into how temperature, salinity, dissolved oxygen, and water transparency interact to influence the asexual production of medusae and polyp buds. Laboratory experiments with medusae can investigate the factors that promote the maturation of male medusae, as well as the conditions at the physiological threshold of survival for medusae nearing the culmination of the bloom.

*4.6 Implications of Moerisia sp. blooms*

The flexible and rapid reproductive capacities, coupled with the ability to form resting stage "seed banks" during unsuitable conditions, may allow *Moerisia* sp. to have a very large seasonal impact on other pelagic species in the upper San Francisco Estuary, especially via trophic interactions. Perhaps the greatest concern relates to recent precipitous declines in the local populations of four planktivorous fish species, delta smelt (*Hypomesus transpacifus*), longfin smelt (*Spirinchus thaleichthys*), age-0 striped bass (*Morone saxatilis*), and threadfin shad (*Dorosoma petenense*) (Sommer et al., 2007). Large reductions in the number of calanoid copepods, a major prey source for these fishes, have also been documented in the system. *Moerisia* sp. consumes a wide variety of zooplankton, especially calanoid copepods (Wintzer, 2010). Although individuals of this species are small, diet data demonstrate that it rarely has an empty gut and is capable of digesting multiple items at once (Wintzer, 2010). Additionally, laboratory culture has revealed that *Moerisia* sp. leaves many prey items dead, but uneaten (Rees and Gershwin 2000), likely increasing the level of competition with fishes for food resources.

A second potential trophic impact of *Moerisia* sp. is their alteration of nutrient cycling through the marsh foodweb. Because there are no known predators of the medusae in the estuary, the nutrients obtained from the pelagic realm are likely not significantly released back into the system until the medusae die off in late fall. This event shunts the energy from the pelagic food web directly to the detrital loop (Schroeter, 2008). Another contribution to the benthos may be from *Moerisia* sp.'s tendency to kill more zooplankton than it consumes (Rees and Gershwin 2000), allowing this energy source to sink to the bottom of the system. It is unknown how this alteration may impact the trophic relationships of the estuary.

**Acknowledgements**

We would like to thank Dr. May, B., Dr. Schroeter, R.E., Dr. Grosholz, E.D., Dr. Lawler, S.P., Durand, J.R., Purdy, S.E., and O'Rear, T.A. for their assistance. We are grateful to an anonymous reviewer whose comments greatly improved this manuscript. This research was supported by funding from the CALFED Science Program, UC Davis Biological Invasions IGERT NSF-DGE#0114432, Dr. Nancy Foster Scholarship, Marin Rod and Gun Club Scholarship, Stockton Sportsmen's Scholarship, and the Giles W. and Elise G. Mead Foundation.

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**Figure captions**

Figure 1. Sampling stations in Suisun Marsh, California. Black circles = medusae collection sites. Grey triangles = polyp collection sites.

Figure 2. Monthly temperature, salinity, dissolved oxygen, and water transparency in Suisun Marsh, California during the 2007 and 2008 *Moerisia* sp. seasonal bloom periods. Error bars = standard error.

Figure 3. Monthly density of calanoid copepods in Suisun Marsh, California during the 2007 and 2008 *Moerisia* sp. seasonal bloom periods. Error bars = standard error.

Figure 4. Monthly medusae densities at sampling stations in Suisun Marsh, California during the 2007 and 2008 *Moerisia* sp. seasonal bloom periods.

Figure 5. Percentage frequency histograms of *Moerisia* sp. medusae in Suisun Marsh, California during the 2007 and 2008 *Moerisia* sp. seasonal bloom periods. Vertical dashed line = mean size. n = number of medusae examined.

Figure 6. Relationship between bell diameter and biomass of *Moerisia* sp. medusae in Suisun Marsh, California.

Figure 7. Monthly biomass densities of medusae in Suisun Marsh, California during the 2007 and 2008 *Moerisia* sp. seasonal bloom periods. Error bars = standard error.

Figure 1

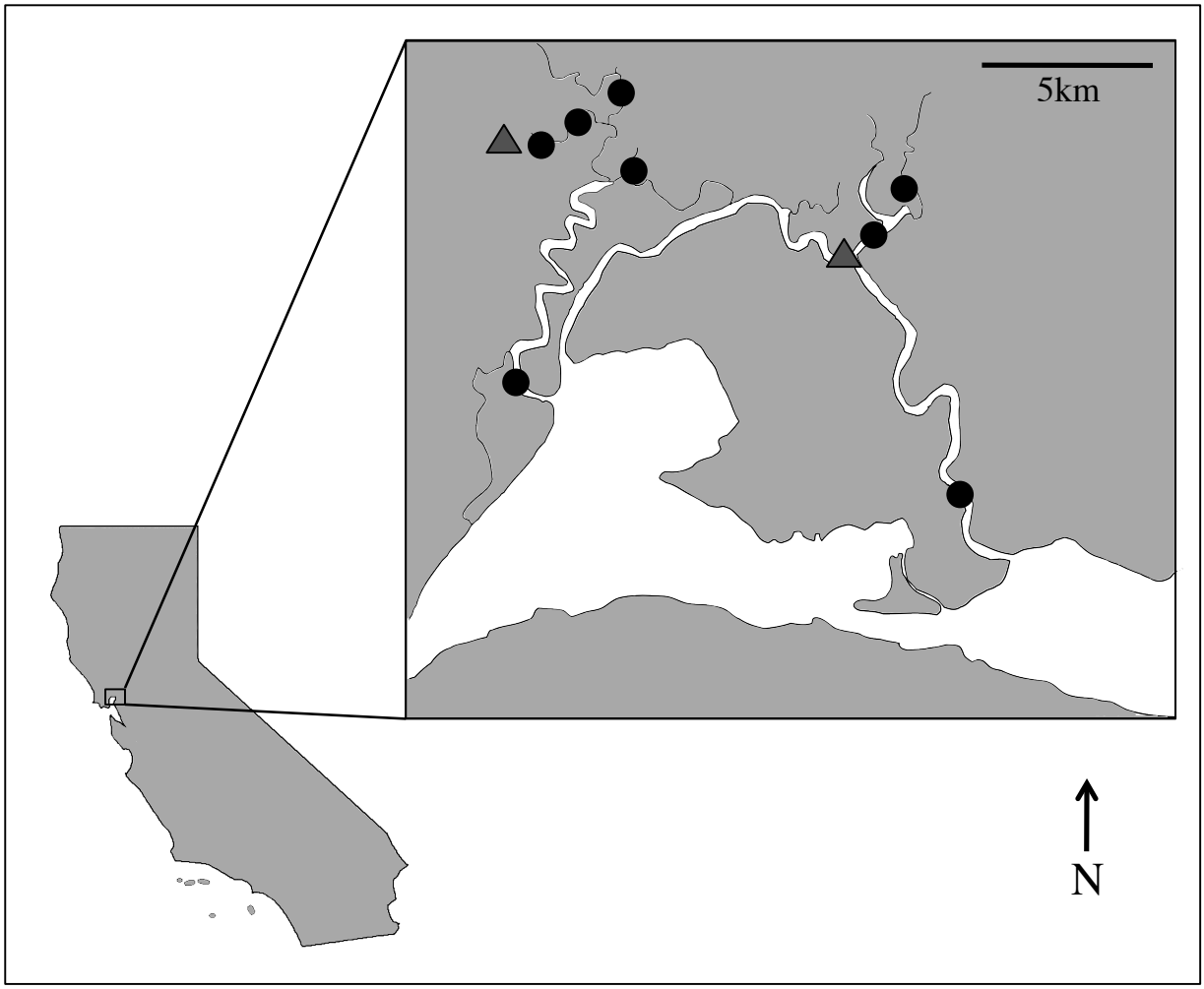


Figure 2

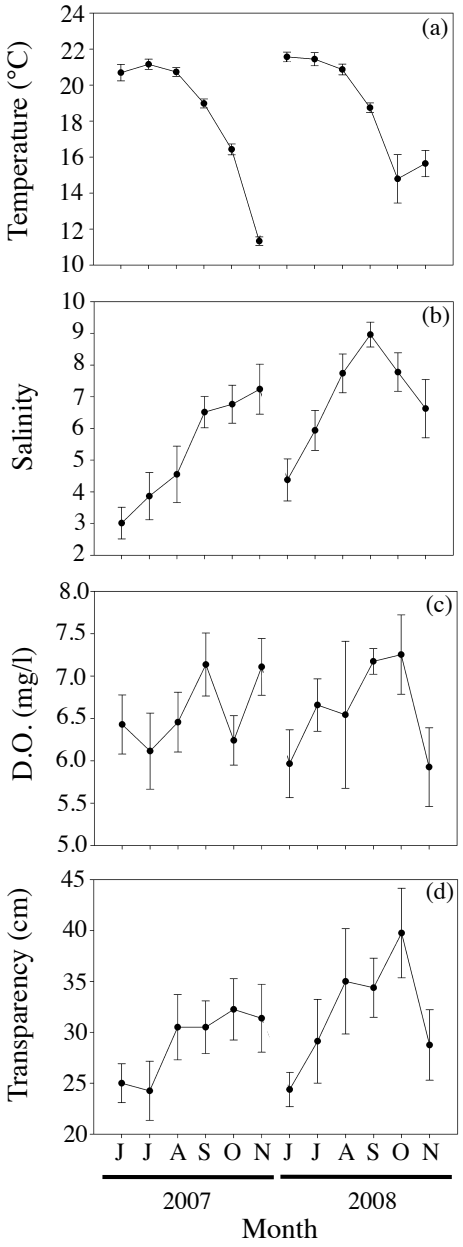

(a)
Temperature (°C)
24
22
20
18
16
14
12
10
(b)
Salinity
10
9
8
7
6
5
4
3
2
(c)
D.O. (mg/l)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
(d)
Transparency (cm)
45
40
35
30
25
20
J J A S O N J J A S O N
2007
2008
Month

Figure 3

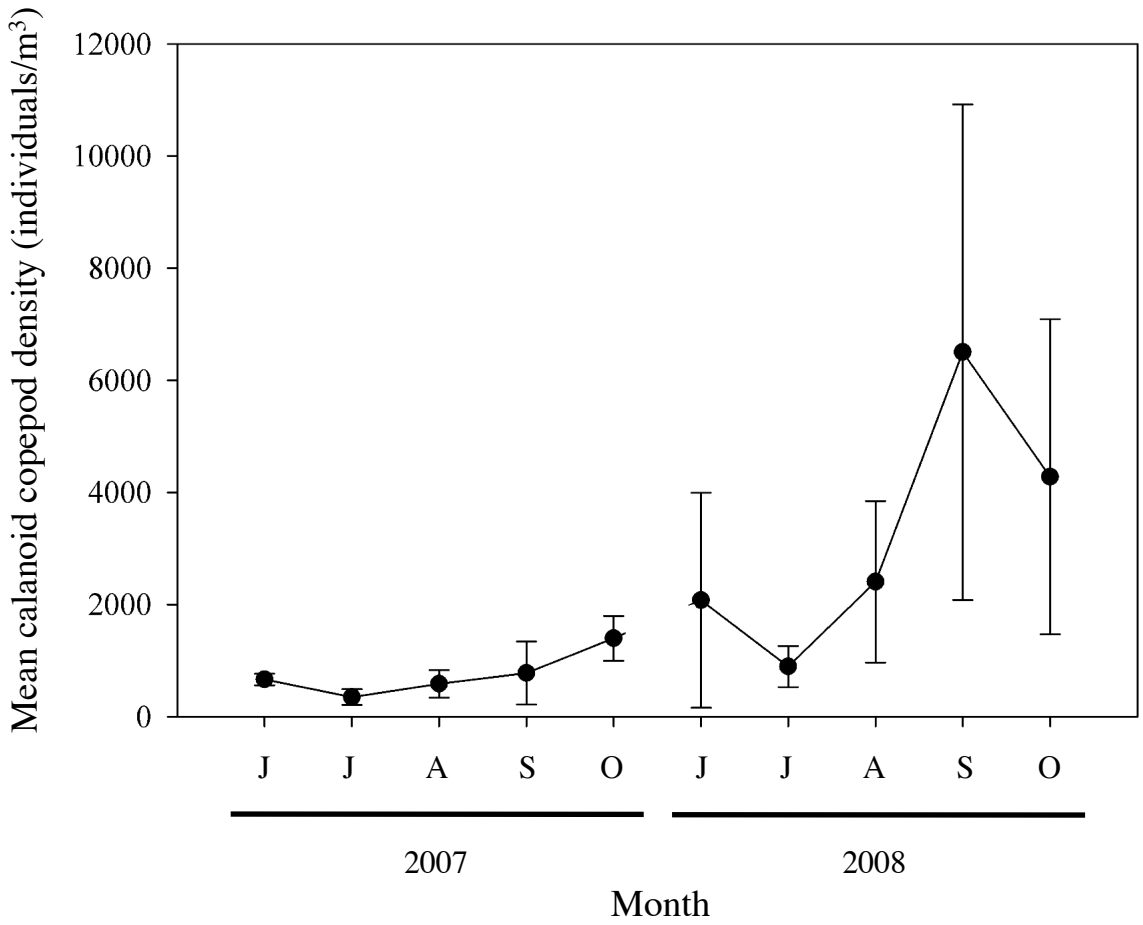

Mean calanoid copepod density (individuals/m3)
12000
10000
8000
6000
4000
2000
0
J
J
A
S
O
J
J
A
S
O
2007
2008
Month

Figure 4

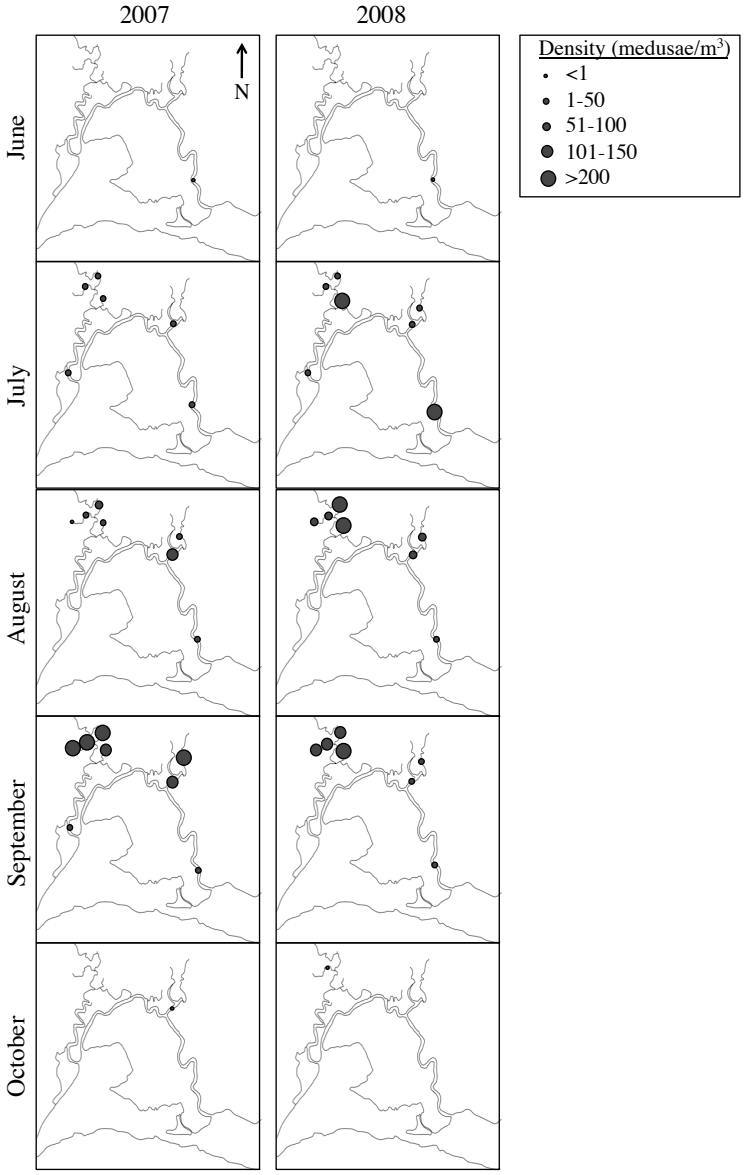

2007
2008
June
July
August
September
October
N
Density (medusae/m3)
<1
1-50
51-100
101-150
>200

Figure 5

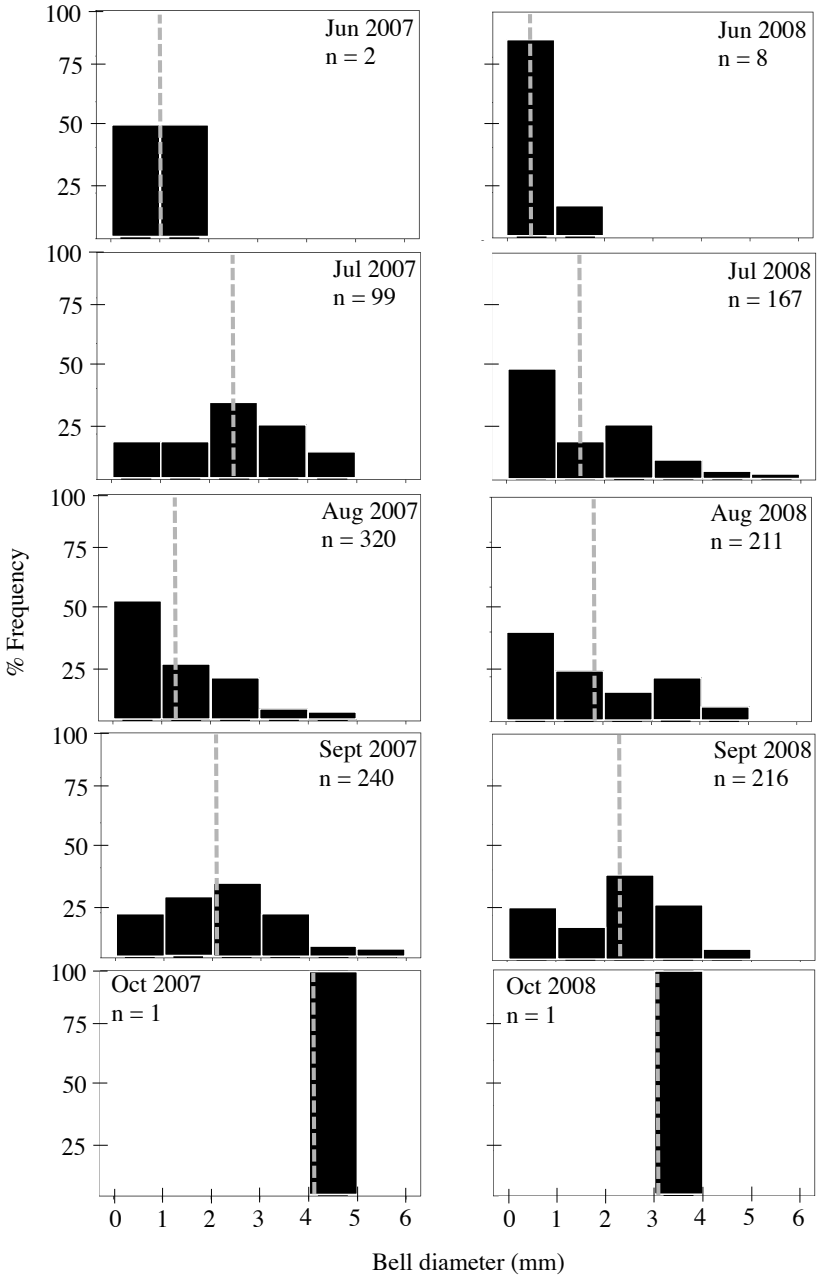

Jun 2007
n = 2
Jun 2008
n = 8
Jul 2007
n = 99
Jul 2008
n = 167
Aug 2007
n = 320
Aug 2008
n = 211
Sept 2007
n = 240
Sept 2008
n = 216
Oct 2007
n = 1
Oct 2008
n = 1
% Frequency
Bell diameter (mm)

Figure 6

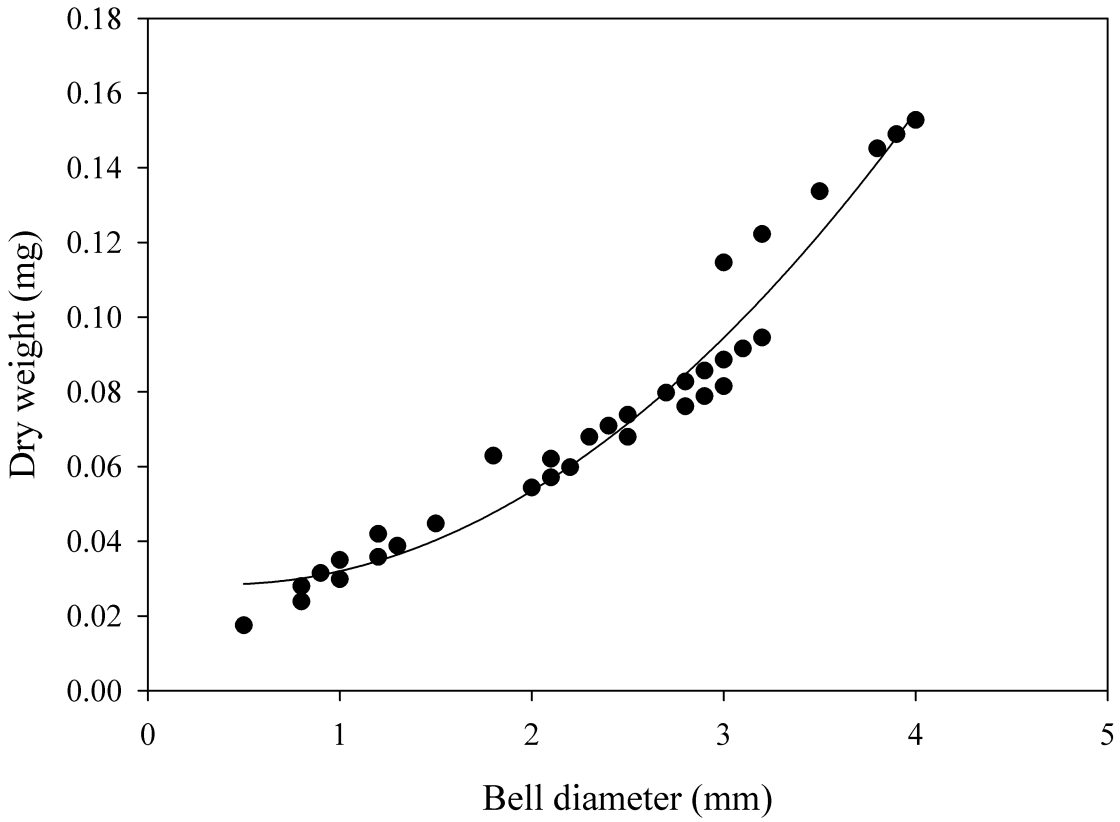
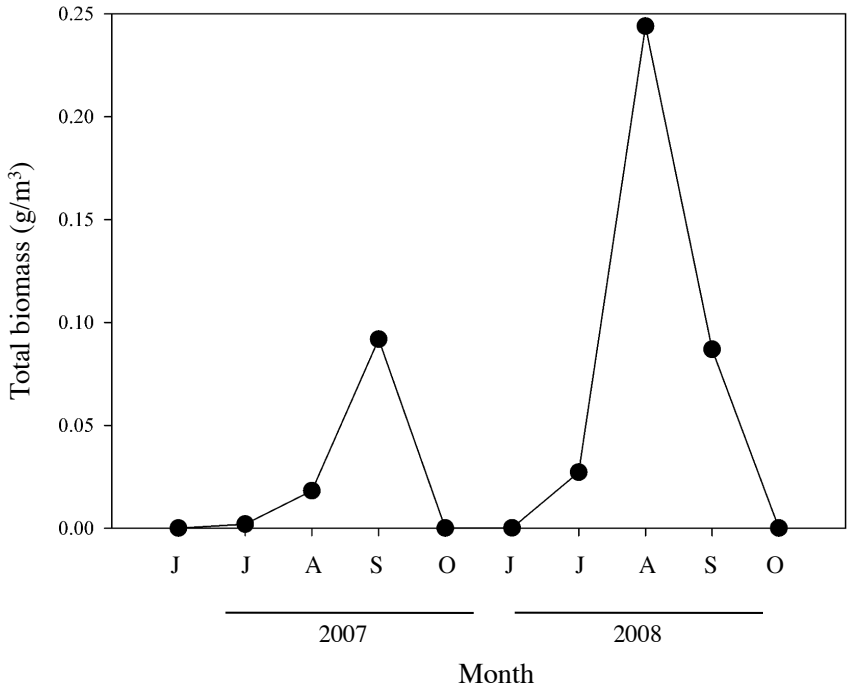

0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Dry weight (mg)
0
1
2
3
4
5
Bell diameter (mm)

Figure 7


0.25
0.20
0.15
0.10
0.05
0.00
Total biomass (g/m3)
J
J
A
S
O
J
J
A
S
O
2007
2008
Month

APPENDIX H

# Trophic ecology of two non-native hydrozoan medusae in the upper San Francisco Estuary

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**Abstract**

Blooms of some gelatinous zooplankton are increasing worldwide, often disrupting foodwebs. Invasions of non-native jellyfish are a growing problem in many estuaries, including the San Francisco Estuary, where at least two species of Ponto-Caspian hydrozoans, *Maeotias marginata* and *Moerisia* sp., are abundant. This study investigates their trophic ecology, testing the hypotheses: 1) diets over the bloom and at the diel scale are comprised of a variety of prey items; 2) hydrozoans are generalist feeders; 3) hydrozoans feed on the larvae of declining fish species; and 4) the potential for prey competition exists between the hydrozoans and two declining planktivorous fishes, striped bass (*Morone saxatilis*) and threadfin shad (*Dorosoma petenense*). Both hydrozoans ate a variety of crustaceans, most notably calanoid copepods, which were found in greater proportion in the guts than the environment. The only fish larvae consumed were gobies. Density of *Moerisia* sp. was negatively correlated to gut fullness for both fishes, and diet overlap was high between shad and hydrozoans, but low for bass. Because of strong spatial and temporal overlap between hydrozoans and shad, competition for zooplankton may be occurring. These hydrozoans have invaded other systems, and should be monitored to assess potential ecological interactions in these locations.

**Additional keywords:** *Maeotias marginata, Moerisia*, *Morone saxatilis*, *Dorosoma petenense*, intra-bloom, diel, diet

**Running head:** Trophic ecology of hydrozoans in the San Francisco Estuary

## Introduction

Gelatinous zooplankton have proven to be important, yet understudied, predators in marine and estuarine systems. Increases in bloom size and frequency of several jellyfish species, coupled with their ability to consume copious amounts of prey, can dramatically alter pelagic foodwebs, including the initiation of trophic cascades (Greve 1994; Jankowski *et al*. 2005; Pitt *et al*. 2007). Fish populations may be effected through strong competition for zooplankton prey (Purcell 2003; Hansson *et al*. 2005; Lynam *et al*. 2006) or through direct predation on eggs and larvae (Purcell and Arai 2001; Shoji *et al*. 2005).

Several pelagic, plankton-feeding fishes have undergone long-term declines, in the upper San Francisco Estuary with step-declines since 2002, resulting in record low numbers of delta smelt (*Hypomesus transpacificus*), young-of-year striped bass (*Morone saxatilis*), threadfin shad (*Dorosoma petenense*), and longfin smelt (*Spirinchus thaleichthys*) (Feyrer *et al*. 2007; Rosenfield and Baxter 2007; Sommer *et al*. 2007). These trends, combined with declines in zooplankton species, are considered to be part of a general pelagic organism decline in the estuary (IEP 2005).

Three small hydrozoans, *Maeotias marginata* (bell width < 40 mm), *Moerisia* sp. (< 8 mm), and *Blackfordia virginica* (< 18 mm), all native to the Ponto-Caspian system (Calder and Burrell 1969), have become established in the upper San Francisco Estuary. Hydrozoans are novel predators in this region, resulting in an especially high likelihood of foodweb alteration (Moyle and Light 1996). Schroeter (2008) analyzed otter trawl data collected for *M. marginata* from 1981-2005 within Suisun Marsh in the upper San Francisco Estuary, finding significant increases in the annual percent occurrence per site across the system. Of the nearly 29,000 *M. marginata* collected during these years, more than 90% were captured

after 1992 and more than 80% after 1998. Long-term data are not currently available for the other two species in the estuary, however if their populations are following growth trends such as those found for *M. marginata,* their impact on the system the may be substantial.

Preliminary diet studies on *M. marginata* and *B. virginica* demonstrate that they feed on a wide variety of planktonic organisms, including larval fishes (Mills and Sommer 1995; Schroeter 2008). Studies on the diet of *Moerisia* sp. have never been published. The limited, but compelling, data on these invasive jellyfish indicates that more information is needed to properly assess their potential role in the declines of zooplankton and pelagic fishes in the upper San Francisco Estuary.

This study begins to address these data needs by examining the trophic ecology of *M. marginata* and *Moerisia* sp., the two most common hydrozoans in the estuary, to better understand how they might interact with two declining species of fish, striped bass and threadfin shad. Specifically, the hypotheses under investigation include 1) the diets of the hydrozoans are comprised of a variety of prey items, both over the bloom period and at the diel scale; 2) the hydrozoans are generalist feeders, consuming prey in relation to their availability in the environment; 3) the hydrozoans feed on the larvae of declining fish species; and 4) the potential for competition for prey exists between the hydrozoans and the two pelagic fishes.

## Methods

### *Study area*

This study was conducted in Suisun Marsh (38.1707°N 121.9736°W) in the upper San Francisco Estuary. Suisun Marsh is a brackish water system covering 34,000ha. Approximately one third of this area is formed by an array of tidally influenced sloughs,

while the remainder is a combination of diked seasonal pools and upland grasslands (DWR 1999; Meng and Matern 2001). The system receives the majority of its freshwater input from the Sacramento River, via the Sacramento-San Joaquin Delta to the east, with smaller contributions made by a waste treatment facility outfall, and a series of small inflowing creeks. Saline water enters the marsh by tidal action from three southerly-located bays (Meng and Matern 2001). Water quality (*e.g.* salinity, temperature, dissolved oxygen) varies both spatially and seasonally, making this a very dynamic system (Schroeter 2008).

*Intra-bloom diets*

To investigate the hypotheses that the hydrozoans feed on a wide variety of prey items during the seasonal bloom, prey are consumed in relation to their availability over the bloom period, and that they consume larvae of declining fish species, monthly field sampling during the medusae bloom (July-October) was undertaken in 2007 and 2008. Four sites were sampled for *Moerisia* sp., small-sized *Maeotias marginata*, and their planktonic prey using a zooplankton net (230$\mu$m mesh, 0.5m diameter, 2m length) coupled with a General Oceanics (Miami, Florida, USA) flowmeter to estimate the towed volume. This mesh size was chosen because it was able to capture both the smallest sizes of medusae as well as the majority of their potential prey items. Samples collected from sloughs less than 15 m in depth were performed at a mid-water column depth, while tows in sloughs greater than 15 m deep were performed in each of the top (approximately 1 m below the surface) and bottom (approximately 1 m above the bottom) halves of the water column. All tows were 5 minutes in length. Upon retrieval of the zooplankton net, the samples were immediately preserved in 5% formalin to minimize net feeding. Some prey consumption is inevitable for this type of sampling and may have slightly skewed the diet data.

In order to collect the larger, more mobile sizes of *M. marginata*, an otter trawl (1.5m x 4.3m mouth, 5.3m length, 35mm stretch graded to 4mm stretch mesh at the cod end) was towed at all sites. In the deep sloughs, hydrofoils were fitted to the otter boards (Trent 1967), and additional trawls were performed to sample the upper half of the water column. All trawls were paired with a flowmeter. The larger mesh size of the trawl precluded net feeding. Medusae were preserved in 5% formalin.

In the laboratory, the bell diameters of up to 20 individuals from each species in each sample were measured using a Leica Z16 APO dissection microscope (Wetzlar, Hesse, Germany) and attached SPOT Idea camera and software (Sterling Heights, Michigan, USA). The medusae gut contents were removed and identified to the lowest possible taxonomic level. In addition, subsamples from the zooplankton samples, commensurate with the total sample density, were identified and enumerated. Zooplankton assemblages and densities from samples taken in the upper and lower portions of a site's water column were found to be similar and were combined for all subsequent analyses.

A Spearman's ρ rank correlation was performed to identify possible relationships between medusae bell width and the number of prey items consumed. Non-parametric tests were utilized because data did not meet normality assumptions. Percent occurrence values over the 4 sample months per year were also calculated for each prey item found in the gut contents.

Pearre's selectivity index (Pearre 1982) was used on pooled site data to assess dietary patterns for each species by comparing the proportion of prey items found in medusae guts with the proportion of these prey items available in the zooplankton samples:

$$C = \pm(((n((\,|\,a_d b_e - a_e b_d\,|\,)-(n/2))^2)/abde)/a+b)^{.5}$$

where, $a_d$ is the number of a specific prey type ingested, $a_e$ is the number of that prey in the environment, a is the total number of that prey type (ingested + environment), $b_d$ is the number of all other prey ingested, $b_e$ is the number of all other prey in the environment, b is the sum of all other prey ingested and in the environment, d is the total number of prey ingested, e is the total number of prey in the environment, and n is the sum of d and e.

A positive value of C indicates a high occurrence of that prey type in the diet as compared to the amount in the environment, a value of 0 represents no difference, and a negative value indicates a disproportionately low occurrence in the diet. All calculated indices were tested for significance using a modified $\chi^2$ statistic (Pearre 1982):

$$\chi^2 = n*C^2$$

where, n and C are calculated as described for Pearre's selectivity index above. A Bonferroni correction was also applied to the $\chi^2$ statistic to reduce the risk of type 1 error.

*Diel diets*

Diel sampling occurred on August 11-12, 2008 in a single slough to explore the hypotheses that dietary patterns over a 24-hour period involve a broad prey base that the hydrozoans are generalist feeders. Laboratory studies have shown that the digestion times for several zooplankton species were 1 hour or less for both hydrozoan species (Wintzer, unpublished data). Therefore, in order to collect discrete time series diet data, samples were taken approximately every 3 hours, during slack, ebb, and flood portions of the tidal cycle. The zooplankton net and flowmeter were used during collection. In the laboratory, medusae were measured and their gut contents identified. A subsample of the ambient zooplankton were identified and counted, as described above. Pearre's selectivity index calculations were performed for each collection period.

*Trophic interactions with fishes*

The hypothesis that the potential for prey competition exists between the hydrozoans and planktivorous fishes was assessed using a variety of methods, including gut fullness calculations before, during, and after the bloom period and in relation to medusae density. Comparisons of diet overlap between fishes and jellyfish were also undertaken.

Juvenile young-of-the-year striped bass and, both juvenile and mature, threadfin shad were chosen for this portion of the study because both species were still relatively abundant. Up to 20 of each species captured in each of the otter and midwater trawls were preserved in 10% formalin throughout the 2007 and 2008 sampling seasons. Beach seines were also performed at two sites to augment fish numbers. Additionally, monthly fish and plankton surveys were conducted at all four sample sites before (April and May) and after (December and January) the seasonal bloom in 2008 to investigate the possibility of changes in fish feeding during the bloom.

In the lab, the wet weights of the fishes and their stomach contents were measured to the nearest 0.00000g using a Mettler Toledo balance (Columbus, Ohio, USA). All gut contents were identified to the lowest possible taxon and enumerated.

An estimate of gut fullness (Smyly 1952):

$$F = (\text{stomach content weight}/\text{fish weight}) \times 100$$

was calculated for each specimen. Kruskal-Wallis tests were performed for each species to identify differences in gut fullness between the pre-bloom, bloom, and post-bloom time periods. Additionally, during the bloom period, possible correlations between gut fullness and medusae density were examined with Spearman's $\rho$ tests. Because large and small sized

*M. marginata* were collected with different sampling gear, only densities for the large *M. marginata* were used to test for correlations.

The level of monthly dietary overlap at the sample locations between Suisun Marsh fishes and hydromedusae was evaluated with Pianka's symmetric niche coefficient (Pianka 1974).

$$\phi_{ij} = (\Sigma P_{ij} P_{ik})/(\sqrt{(\Sigma P_{ij}^2 \Sigma P_{ik}^2)})$$

where, $P_{ij}$ is the proportion of prey type i found in the diet of species j and $P_{ik}$ is the proportion of prey type i in the diet of species k. A resultant value of 0 indicates no overlap, while a value of 1 shows total dietary overlap (Huey and Pianka 1977). The impact of month and year on dietary overlap between striped bass and hydromedusae was investigated with non-parametric Kruskal-Wallis tests. Due to low numbers of threadfin shad, collections of this fish and medusae at the same collection period were somewhat rare, making it difficult to statistically analyze these data by month and year. Instead, ranges and means of overlap values were examined for these species combinations.

**Results**

*Intra-bloom diets*

226 *M. marginata* and 182 *Moerisia* sp. from 2007 and 200 *M. marginata* and 201 *Moerisia* sp. from 2008 were dissected for gut contents. The nonparametric Spearman's ρ correlations indicated that the number of prey items per individual medusa had a significant positive relationship to bell width for both *M. marginata* ($\rho=0.620$, d.f.=422, $p<0.000$) and *Moerisia* sp. ($\rho=0.190$, d.f.=381, $p<0.000$) (Fig 1).

*Maeotias marginata* were found to feed on a large number of taxa, consuming 13 different prey types over their bloom period. Their diet in 2007 consisted mainly of calanoid

copepods (65% ±8.92), along with barnacle nauplii (14% ±8.20), cyclopoid copepods (14% ±6.53), corophiid amphipods (4% ±.60), and calanoid egg sacs (no longer attached to the copepod) (1% ±.70). Mysids, gammarid amphipods, crab zoae, and cumaceans were also consumed, but contributed less than 1% to the gut contents each. In 2008, *M. marginata* fed upon calanoid copepods (80% ±3.74), barnacle nauplii (4% ±1.23), corophiid amphipods (4% ±.88), crab zoea (4% ±1.56), mysids (3% ±1.56), calanoid egg sacs (3% ±.74), and gammarid amphipods (1% ±.32), with lesser contributions by fish larvae, cumaceans, cyclopoid copepods, *Moerisia* sp. medusae, ostracods, and hydranths of *Cordylophora caspia*, another Ponto-Caspian hydrozoan.

A monthly assessment of gut contents over the bloom (Fig 2*a*) reveals that *M. marginata* consumed mainly calanoid copepods each period. Barnacle nauplii and cyclopoid copepods comprised a large percentage of the diets during September and August, respectively, in 2007.

The percent occurrence of prey items in *M. marginata* guts was highest for calanoid copepods (100% both years) and mysids (100% both years), followed by cyclopoid copepods (75%, 100%), and corophiid amphipods (75%, 100%). Crab zoea (75% both years), barnacle nauplii (75% both years), egg sacs (75%, 50%), gammarid amphipods (75%, 50%), and cumaceans (25%, 50%) showed moderate occurrence over the study, while fish larvae (0%, 50%), hydranths (0%, 25%) and medusae (0%, 25%) were low.

Monthly selectivity calculations for *M. marginata* indicate that not all prey items in the guts were consistent with the amount available in the system, refuting hypothesis 2. A larger proportion of corophiid amphipods were detected in the diets as compared to the environments, while the opposite was found for larval fishes, *Moerisia* sp. medusae, and

ostracods. Both positive and negative values were found for the remaining prey items, except for cumaceans, harpacticoid copepods, and *C. caspia* hydranths, which were never significant (Table 1).

*Moerisia* sp. also consumed a wide variety of items, feeding upon 8 taxa over the study period. In 2007, they consumed mostly calanoid copepods (87% $\pm$3.92), barnacle nauplii (8% $\pm$3.22), cyclopoid copepods (3% $\pm$1.42), and calanoid egg sacs (1% $\pm$.57), while cumaceans were eaten in small quantities. In 2008, gut contents included calanoid copepods (94% $\pm$2.73), mysids (2% $\pm$.61), and barnacle nauplii (2% $\pm$2.10). Zoea and fish larvae each contributed less than 1%.

*Moerisia* sp. gut contents during each month of the bloom period were predominantly comprised of calanoid copepods over both study years (Fig 2*b*). The percent occurrence of calanoid copepods in *Moerisia* sp. diets, based on monthly collections, was 100% for both sample years. The remaining prey items had variable occurrence during 2007 and 2008, including barnacle nauplii (50%, 25%), mysids (0%, 75%), zoea (0%, 50%), calanoid eggs (25%, 0%), cyclopoid copepods (25%, 0%), cumaceans (25%, 0%), and fish larvae (0%, 25%).

A larger proportion of calanoid copepods was found in *Moerisia* sp. guts as compared to the environment. Significant and opposite trends were found for all other important taxa (Table 1). These results refute the second hypothesis.

Of the calanoid copepods consumed by both medusae species, *Acartiella sinensis* made up the greatest percentage, followed by *Pseudodiaptomus forbesi*. *Tortanus dextrilobatus* and *Eurytemora affinis* were found in smaller numbers (Fig 3). In terms of monthly percent occurrence of calanoid copepods, *A. sinensis*, and *T. dextrilobatus*, were

present 100% of the time in monthly *M. marginata* gut contents for each year, while *P. forbesi* (100%, 75%) and *E. affinis* (75%, 75%) were more variable. *Acartiella sinensis* had 100% occurrence in the monthly diets of *Moerisia* sp. during 2007 and 2008, with *T. dextrilobatus* (75%, 75%), *P. forbesi* (75%, 75%) and *E. affinis* (50%, 25%) present less consistently.

*Diel diets*

During a 24-hour period, *M. marginata* and *Moerisia* sp. consumed fewer prey taxa, 6 and 3, respectively, compared to the monthly data. Pearre's selectivity index values for the diel study were less variable than those of the monthly surveys (Table 2), with the ratios of some prey items not corresponding with that seen in the water. *Maeotias marginata* displayed a larger proportion of calanoid copepods and corophiid amphipods in their diets compared to the environment. The opposite trend was found for all other prey items, except *C. caspia* hydranths, which were not significantly consumed, and gammarid amphipods and barnacle nauplii, which had both positive and negative significant index values. *Moerisia* sp. gut contents had a higher proportion of calanoid copepods compared to the environment over the entire study period. The remaining items showed an opposite trend, with the exception of *C. caspia* hydranths and fish larvae, which were not significantly consumed.

*Trophic interactions with fishes*

Disproving the third hypothesis, *M. marginata* and *Moerisia* sp. never consumed the larvae of declining pelagic fishes. They did, however, feed on larvae of the non-native shimofuri goby, *Tridentiger bifasciatus,* at two sites during 2008. The overall contribution of fish larvae to medusae diets was low, comprising just 4% (2 fish) and 1% (2 fish) of the

number of individual prey items for *M. marginata* and *Moerisia* sp., respectively during July. August gut contents contained 1% fish larvae (5 fish) for *M. marginata*.

There was no difference in gut fullness for striped bass over pre-bloom, bloom, and post-bloom periods ($\chi^2$=1.141, d.f.=2, p=0.565; mean values: pre-bloom 1.293, bloom 1.320, post-bloom 1.281). Similarly, no trend was found for threadfin shad ($\chi^2$=0.216, d.f.=2, p=0.898; mean values: pre-bloom 0.586, bloom 0.500, post-bloom 0.630). These results do not lend support the idea of competition for prey between fishes and medusae during the bloom. Overall, the mean index of fullness was greater for striped bass (1.318) than for threadfin shad (0.595). Gut contents of shad, however, contained sand on several occasions during August and September, which may have compromised their fullness index values.

Within the bloom period, there was a slight positive correlation between striped bass gut fullness and density of large *M. marginata* ($\rho$=0.130, d.f.=230, p<0.044), and a negative trend with density of *Moerisia* sp. ($\rho$=-0.160, d.f.=230, p<0.013) (Fig 4*a* and *b*). There was no relationship with threadfin shad gut fullness and large *M. marginata* density ($\rho$=-0.180, d.f.=39, p<0.267) and a negative correlation with *Moerisia* sp. density ($\rho$=-0.490, d.f.=39, p<0.001) (Fig 4*c* and *d*). These findings do not rule out the possibility that *Moerisia* sp. may compete with pelagic fishes for prey.

The levels of diet overlap, as calculated by Pianka's symmetric niche coefficient for striped bass and *M. marginata* were typically low (0.01-0.27, average 0.10, n=16 comparisons). However, there were some high-level overlap periods with *Moerisia* sp. (0.00-0.76, average 0.10, n=14). In general, the level of dietary overlap was greatest between threadfin shad and both *M. marginata* (0.06-0.99, average 0.70, n=4) and *Moerisia* sp. (0.67-0.92, average 0.79, n=3). The overlap between striped bass and *M. marginata* during blooms

was not affected by month ($\chi^2$=6.311, d.f.=7, p=0.971) or year ($\chi^2$=1.751, d.f.=1, p=0.186). Overlap levels between striped bass and *Moerisia* sp. showed similar Kruskal-Wallis results, with no significant effect of month ($\chi^2$=0.815, d.f.=7, p=0.846) or year ($\chi^2$=0.436, d.f.=1, p=0.509). These results support the hypothesis for competition between both hydrozoan species and threadfin shad.

## Discussion

### *Intra-bloom and diel feeding ecology*

Over the course of this work, *M. marginata* did consume a wide variety of prey, which was consistent with results of smaller-scale studies. Mills and Sommer (1995) examined medusae from the Petaluma River, California during June 1993, with copepods being the second greatest prey item in numerical abundance, behind barnacle nauplii. Interestingly, in addition to many of the prey found in the current study, the Petaluma River medusae also consumed items such as seeds, fecal pellets, terrestrial insects, and filamentous algae in small amounts. Schroeter (2008) examined gut contents of *M. marginata* collected from Suisun Marsh in the summer of 2004 and fall of 2005. Calanoid copepods were numerically dominant in both samples and the majority of the remaining prey items were similar to those found in this study. Some exceptions include cladocerans during the summer and copepod nauplii during the fall, both in small quantities.

This is the first study to document the trophic ecology of *Moerisia* sp.. Purcell *et al.* (1999), however, performed laboratory feeding experiments using six prey taxa with *Moerisia lyonsi* from the Chesapeake Bay, which is considered to be a close relative of *Moerisia* sp. (Rees and Gershwin 2000). This species fed exclusively on larval and adult stages of the calanoid copepod, *Acartia tonsa*, and harpacticoids. Comparatively, this study

shows Suisun Marsh *Moerisia* sp. possess a more diverse prey base than its relative. This may simply be related to fewer available prey taxa in the Chesapeake system; however, barnacle nauplii were used in their feeding study, and while captured on tentacles, they were never consumed by *M. lyonsi* (Purcell *et al*. 1999). An additional difference is that copepod nauplii were never found in *Moerisia* sp. guts, but were consumed by *M. lyonsi*.

Pearre's selectivity index indicated that both *M. marginata* and *Moerisia* sp. do not simply consume prey in relation to their proportional availability, but may be consuming specific prey types. It should be noted that this metric does not take into account important factors that influence true selectivity (*e.g*. capture efficiency, handling time (Eggers 1977), digestion rate of prey items) and, therefore, the results must be applied with some caution. It does, however, offer preliminary insight into the feeding strategies of these hydrozoan species.

Several morphological and behavioral mechanisms allow *M. marginata* and *Moerisia* sp. to be unique and effective predators on crustacean prey, such as calanoid copepods, in the San Francisco Estuary. First, these medusae possess numerous, closely-spaced tentacles, appropriate for capturing small prey items (Purcell and Arai 2001). *Maeotias marginata*, in particular, can have up to 600 densely packed tentacles around the bell (Mills and Sommer 1995), while the much smaller *Moerisia* sp. can possess up to 32 (Rees and Gershwin 2000).

Second, both have nematocysts used for the capture of crustacean prey. Stenoteles are found in tightly packed rings along the tentacles and clustered along the manubrial lips of *M. marginata* (Mills and Sommer 1995). This form of nematocyst is effective at penetrating hard surfaces, such as chitinous exoskeletons (Purcell and Mills 1988). *Moerisia* sp. tentacles, in addition to containing stenoteles, also have desmonemes (Rees and Gershwin

2000). Desmonemes have been found to wrap around small structures, such as copepod setae, to secure prey (Östman *et al*. 1991).

Finally, swimming behavior and extension of the tentacles can increase the chance of prey encounter (Madin 1988). *Maeotias marginata* appears to employ a two-stage feeding strategy. This species swims to the surface, flips over, and sinks slowly to the bottom with its tentacles outstretched. Planktonic organisms are captured during the descent. Once it contacts the substrate, *M. marginata* remains exumbrella downward while twitching the ends of its tentacles, likely as a luring behavior (Mills and Sommer 1995). This may allow for the capture of mostly benthic prey, such as corophiid amphipods (Schroeter 2008), and could explain the positive Pearre's selectivity index values for this diet item.

*Moerisia* sp., which possesses prominent ocelli at the base of each tentacle bulb, is strongly phototactic (Rees and Gershwin 2000). Although weak swimmers, they can be observed pulsing towards the water's surface during calm periods. Upon reaching the top of the water column, *Moerisia* sp. drift towards the bottom, without flipping over, their tentacles outstretched (Wintzer, personal observation). It is unclear how much, if any, time is spent resting on the bottom, but lack of benthic prey in their diets suggests that feeding takes place only in the water column. A similar feeding strategy description is given for *M. lyonsi*, which does not spend time on the bottom (Purcell *et al*. 1999).

Specializations of the prey species may also play a large role in determining hydromedusae diets. Physical characteristics of prey species can also also impact feeding efficiency of hydromedusae (Madin 1988). Suchman and Sullivan (1998) found that size of the copepod *Acartia hudsonica* impacted its selectivity by the scyphozoans *Aurelia aurita* and *Cyanea* sp., with small copepodites experiencing reduced predation. Small prey items

367 may evade capture due to the smaller surface area of their bodies (Suchman and Sullivan
368 1998), the ability to pass between tentacles without contact (Spadinger and Maier 1999), or
369 by tentacle collision that is not powerful enough to cause nematocyst discharge (Larson
370 1987). This may explain why only one individual of *Limnoithona tetraspina*, a small
371 cyclopoid copepod that has recently become very abundant in the upper San Francisco
372 Estuary, was found in the gut contents of *M. marginata*, and why copepod nauplii were not
373 consumed in this study.

374 Prey behavior can also influence capture. Predator avoidance behavior may include
375 the speed that zooplankton swim (Mills 1981), their ability to detect hydrodynamic
376 disturbances by predators (Suchman 2000), and the directionality and strength of their escape
377 response (Suchman and Sullivan 1998). These behaviors may vary with the stage of
378 particular taxa (Suchman 2000) and certainly differ among species.

379 Finally, the density of a prey species can impact its rate of capture by predators.
380 Madin (1988) indicates that because medusae are non-visual predators, they must rely on
381 random entanglement with their prey. At least one species, *A. aurita*, however, shows
382 chemosensory ability and is attracted to water conditioned by its prey (Arai 1991). In both
383 scenarios, prey capture would be increased when prey are at high densities. In this study,
384 both hydrozoans consumed some prey species over the bloom period that were present in
385 high abundance.

386 *Hydrozoan predation on larval fishes*

387 While some cnidarians are soft prey specialists, feeding exclusively on fish larvae,
388 this taxa only constitutes a small portion of most gelatinous zooplankton diets (Purcell 1985).
389 Medusae that prey primarily on crustaceans will commonly feed upon fish eggs and larvae

390 when their densities are high compared to other potential prey items (Purcell and Arai 2001).
391 The capture of the youngest fish larval stages, which have limited escape responses, may be
392 particularly vulnerable prey items for medusae (Purcell *et al*. 1987).

393 *Maeotias marginata* has been previously shown to consume fishes. Mills and
394 Sommer (1995) maintained medusae in the laboratory, occasionally feeding them small fish.
395 Additionally, Schroeter (2008) found 6 goby (*Tridentiger* sp.) larvae in the guts of 40 wild-
396 caught *M. marginata*. There was, however, no indication that the much smaller *Moerisia* sp.
397 could catch and consume fish larvae. In this study, medusae as small as 3.05mm bell width
398 were quite capable of feeding on larvae of shimofuri goby (*T. bifasciatus*). The trend of
399 proportionally more larvae in the environments as compared to gut contents of *Moerisia* sp.
400 may be a factor of size limitation, as many of the *T. bifascfiatus* were larger than this species
401 of medusae. Large *Maeotias marginata*, however, were able to consume the largest sizes of
402 *T. bifasciatus* (20mm) available.

403 While both *M. marginata* and *Moerisia* sp. consumed larval fish, none of the
404 declining pelagic fish species were found in their guts. Striped bass and the imperiled delta
405 and longfin smelts spawn early in the year so their larval stages may have limited temporal
406 and spatial overlap with the medusae bloom (Schroeter 2008). Larval threadfin shad do co-
407 occur with the early portion of the bloom, typically July (Schroeter 2008), but were never
408 consumed. These results indicate no direct predation impact on larvae of declining pelagic
409 fishes in Suisun Marsh by non-native hydrozoans.

410 *Prey competition between hydrozoans and pelagic fishes*

411 Competition is a difficult interaction to prove (Purcell and Arai 2001). However,
412 substantial dietary overlap has been documented between scyphozoans, a ctenophore, a

hydrozoan and four juvenile fishes in Alaska (Purcell and Sturdevant 2001). High levels of prey resource competition were deemed possible in that study based on temporal and spatial co-occurrence of potential competitors (Purcell and Sturdevant 2001).

In Suisun Marsh, the plankton feeding stages of the four declining pelagic fishes have strong spatial and temporal overlap with *M. marginata* (Schroeter 2008) and *Moerisia* sp. (Wintzer, personal observation). Striped bass did not show a great degree of dietary overlap with either of the hydrozoans, however, threadfin shad did, especially with *Moerisia* sp.

While our study did not perform gut content analyses on the threatened smelt species, some level of dietary overlap may still be inferred. In an analysis of dietary patterns of Suisun Marsh fishes, for example, Feyrer *et al*. (2003) found that delta smelt and threadfin shad had high levels of pelagic copepods in their diets, along similar levels of gut fullness and diet composition. Additionally, longfin smelt and juvenile striped bass were similar due to the high mysid content of their diets. Given these results, it is likely that delta smelt and longfin smelt follow the dietary overlap trends of threadfin shad and juvenile striped bass, respectively.

The lack of high dietary overlap, in addition to similar trends in gut fullness during pre-bloom, bloom and post-bloom periods, refutes the hypothesis that juvenile striped bass are competing with non-native hydrozoans for prey at any significant level. Longfin smelt, although not directly studied here, are likely similar. Conversely, threadfin shad have high dietary overlap with medusae. The sand found in their stomachs makes their gut fullness data difficult to interpret, but Ingram and Ziebell (1983) reported that shad feed on benthic prey when their preferred planktonic prey is limited. Additionally, shad gut fullness calculations were negatively correlated with *Moerisia* sp. densities. For these reasons, it is

possible that this fish species, and perhaps the trophically similar delta smelt, will compete for prey resources with *M. marginata* and especially *Moerisia* sp. during jellyfish blooms.

*Conclusions*

*Maeotias marginata* and *Moerisia* sp. are predators on a variety of small crustaceans in the San Francisco Estuary. Evidence suggests that they may employ some selectivity during feeding, but this requires further study to confirm. While their ability to consume the larvae of imperiled fishes is reduced by limited spatial and temporal overlap, the medusae and some planktivorous fishes do consume a similar prey base. Threadfin shad and delta smelt populations, in particular, may be affected by both hydrozoans due to their dietary overlap of copepods with these fish species. These findings are troubling, especially in conjunction with evidence of increasing medusae populations (Schroeter 2008), declining zooplankton populations, and the presence of additional well-established invasive zooplanktivores, such as the overbite clam (*Corbula amurensis*).

As mentioned above, true competition is a difficult phenomenon to confirm. The next step for research within the San Francisco Estuary will involve determining the cropping potential of *M. marginata* and *Moerisia* sp. in relation to the production rate of calanoid copepods. These data should provide an estimate of the potential level of prey depletion by medusae, as well as the level of prey availability for planktivorous fishes, which can inform our hypothesis of competition between hydrozoans and both threadfin shad and delta smelt for prey resources.

In a global context, there are reports of *M. marginata* in Holland (Hummelinck 1941), the Sea of Azov (Ostroumoff 1896), the Loire Estuary in France (Denayer 1973), Chesapeake Bay (Calder and Burrell 1969), and the Baltic Sea (Vainola and Oulasvirta

2001). Similarly, *M. lyonsi* (a close relative of *Moerisia* sp.) has invaded several systems in the eastern United States (Calder and Burrell 1969; Porrier and Mulion 1977; Purcell *et al*. 1999) and the Baltic Sea (Väinölä and Oulasvirta 2001). The findings from this study add to the limited understanding of how jellyfish blooms may impact the trophic ecology of an invaded system and may be applicable to other locations. It is urged that studies on negative interactions between these hydrozoans and planktivorous fishes continue in order to determine any potential ecological impacts to specific systems and to establish necessary management options.

**Acknowledgements**

The authors would like to thank B. May, R. Schroeter, E. Grosholz, S. Lawler, J. Durand, S. Purdy, T. O'rear, and C. Alemdar for their assistance, and two anonymous reviewers whose comments greatly improved this manuscript. This research was supported by funding from the CALFED Science Program, UC Davis Biological Invasions IGERT NSF-DGE#0114432, Dr. Nancy Foster Scholarship, Cen Cal Marine Science Scholarship, UC Davis Block Grant, James Micheletti Research Fellowship, Bob Wisecarver DVFF Scholarship, and the Giles W. and Elise G. Mead Foundation. California Depatment of Fish and Game scientific collection permits #001928 and #007681 and University of California, Davis Institutional Animal Care and Use protocol #12338 were used for this study.

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**Figure Legends**

Fig. 1. Relationship between medusae bell diameter and number of prey items found in the guts of (*a*) *Maeotias marginata* and (*b*) *Moerisia* sp.

Fig. 2. Monthly percent of prey items found in the guts of (*a*) *Maeotias marginata* and (*b*) *Moerisia* sp. during the 2007 and 2008 sample periods. Sample size is indicated above each bar

Fig. 3. Percent calanoid copepods found in the gut contents of *Maeotias marginata* and *Moerisia* sp. during the 2007 and 2008 sample periods. Sample size is indicated above each bar

Fig. 4. Comparison of medusae density and the gut fullness of pelagic fishes for (*a*) large *Maeotias marginata* and striped bass, (*b*) *Moerisia* sp. and striped bass, (*c*) large *M. marginata* and threadfin shad, and (*d*) *Moerisia* sp. and threadfin shad

**Table Captions**

Table 1. Monthly selectivity calculations for prey by *Maeotias marginata* and *Moerisia* sp. in Suisun Marsh using Pearre's selectivity index. 0.00 values indicate no difference between relative abundance of plankton in the guts and in the water. Bold values=significance ($\alpha$=0.05); *=significance with Bonferroni correction ($\alpha$=0.004)

Table 2. Diel selectivity calculations for prey by *Maeotias marginata* and *Moerisia* sp. in Suisun Marsh using Pearre's selectivity index. 0.00 values indicate no difference between

618 relative abundance of plankton in the guts and in the water. Bold values=significance

619 ($\alpha$=0.05); *=significance with Bonferroni correction ($\alpha$=0.004)

620

Table 1

| n | Month | Barnacle nauplii | Calanoid copepods | Calanoid egg sacs | Cyclopoid copepods | Corophiid amphipods | Cumaceans | Fish larvae | Gammarid amphipods | Harpacticoid copepods | Hydranths | Medusae | Mysids | Ostracods | Zoea |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2007 *Maeotias marginata* | | | | | | | | | | | | | | | |
| 45 | July | -0.01 | **0.12*** | **0.12*** | 0.00 | **0.15*** | -- | **-0.14*** | **-0.17*** | -- | -- | **-0.07*** | 0.00 | **-0.05** | 0.00 |
| 61 | August | **0.04*** | **0.05*** | **-0.05*** | -0.01 | **0.12*** | -0.02 | **-0.03** | **0.02** | -- | 0.00 | **-0.09*** | **0.05*** | -0.02 | **-0.03** |
| 67 | September | **-0.21*** | **0.24*** | -- | **-0.06*** | **0.19*** | 0.00 | **-0.07*** | 0.00 | -- | -- | **-0.05*** | -0.02 | -- | -0.02 |
| 51 | October | -0.01 | **-0.05*** | **0.04*** | **0.03** | **0.16*** | -- | -- | -- | -- | -0.01 | -- | **-0.02** | -- | -- |
| 2007 *Moerisia* sp. | | | | | | | | | | | | | | | |
| 41 | July | **-0.06** | **0.08** | **0.12*** | -0.04 | -- | -- | -0.04 | **-0.07** | -- | -- | -0.01 | 0.00 | 0.00 | 0.00 |
| 60 | August | 0.03 | 0.03 | **-0.04** | -0.01 | 0.00 | -0.03 | -0.01 | **-0.05** | -- | **-0.04** | -0.03 | -0.01 | 0.00 | -0.01 |
| 80 | September | **-0.21*** | **0.24*** | -- | 0.01 | -0.02 | 0.01 | **-0.19*** | -0.01 | -- | -- | -0.01 | -0.01 | -- | 0.00 |
| 1 | October | **-0.32*** | 0.01 | **-0.20*** | -0.03 | -- | -- | -- | -- | -- | -- | -- | **-0.13*** | -- | -- |
| 2008 *Maeotias marginata* | | | | | | | | | | | | | | | |
| 59 | July | 0.00 | **-0.08*** | **0.18*** | 0.00 | **0.12*** | 0.00 | **-0.05*** | -0.01 | -- | -- | **-0.04*** | **0.04*** | **-0.03** | **0.03** |
| 60 | August | **-0.05*** | **-0.10*** | **0.06*** | -0.01 | **0.20*** | 0.00 | 0.01 | **0.08*** | 0.01 | 0.00 | **-0.02** | 0.01 | 0.00 | **0.05*** |
| 49 | September | **0.06*** | **-0.04*** | 0.00 | **0.09*** | **0.12*** | -0.01 | -0.01 | 0.00 | 0.00 | -- | **-0.03*** | **0.03*** | -0.02 | 0.01 |
| 31 | October | 0.02 | **0.03** | -0.02 | 0.00 | -- | -0.01 | -- | -0.01 | -0.01 | -- | -- | 0.00 | 0.00 | -- |
| 2008 *Moerisia* sp. | | | | | | | | | | | | | | | |
| 60 | July | **-0.11*** | **0.03** | -0.01 | -0.01 | -- | -0.02 | 0.00 | -0.01 | -- | -- | -0.01 | -0.02 | 0.00 | -0.01 |
| 80 | August | 0.00 | **0.04*** | 0.00 | **-0.03** | -- | 0.00 | -0.01 | 0.00 | -0.01 | -0.01 | -0.01 | 0.00 | -0.01 | **-0.04*** |
| 60 | September | -0.01 | 0.02 | -0.01 | -- | -- | 0.00 | -0.01 | **-0.03** | -0.02 | -- | -0.01 | 0.00 | 0.00 | 0.00 |
| 1 | October | -- | 0.06 | -- | **-0.06** | -- | -- | -- | -- | -- | -- | -- | -- | **-0.19*** | -- |

Table 2

| n | Time | Barnacle nauplii | Calanoid copepods | Calanoid egg sacs | Cyclopoid copepods | Corophiid amphipods | Cumaceans | Fish larvae | Gammarid amphipods | Harpacticoid copepods | Hydranths | Medusae | Mysids | Ostracods | Zoea |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Maeotias marginata* | | | | | | | | | | | | | | | |
| 1 | 10:29 | 0.00 | 0.01 | -- | **-0.03*** | -- | -0.01 | 0.00 | **-0.02** | **-0.02** | -- | -0.01 | 0.00 | -0.01 | -0.01 |
| 8 | 13:00 | 0.00 | 0.00 | -- | -- | -- | **-0.03*** | 0.00 | **0.03*** | -- | -- | **-0.02*** | 0.00 | -0.01 | -0.01 |
| 3 | 15:22 | **0.03*** | **0.02** | **-0.05*** | -- | **0.07*** | -0.01 | -0.01 | -0.01 | -- | 0.00 | **-0.03*** | **-0.02** | -0.01 | **-0.02** |
| 2 | 17:17 | -0.01 | 0.00 | **-0.14*** | **-0.22*** | -- | **-0.04*** | **-0.02** | **-0.02*** | -- | -- | **-0.18*** | 0.00 | **-0.04*** | 0.00 |
| 18 | 20:30 | **-0.03*** | **0.07*** | **-0.03*** | -0.01 | **0.07*** | 0.00 | **-0.01** | -0.01 | -0.01 | -- | **-0.03*** | **-0.01** | 0.00 | **-0.04*** |
| 9 | 23:08 | **0.07*** | 0.01 | **-0.05*** | -- | **0.20*** | 0.00 | **-0.02** | -0.01 | -0.01 | -- | -0.01 | -0.01 | 0.00 | **-0.02** |
| 6 | 3:05 | -0.01 | **0.01** | **-0.04*** | **-0.12*** | -- | -0.01 | 0.00 | 0.00 | **-0.12*** | -- | **-0.06*** | 0.00 | -0.01 | -0.01 |
| *Moerisia* sp. | | | | | | | | | | | | | | | |
| 20 | 10:29 | -0.01 | **0.03*** | -- | **-0.03*** | -- | -0.01 | 0.00 | -0.01 | -0.01 | -- | 0.00 | 0.00 | -0.01 | **-0.02** |
| 20 | 13:00 | 0.00 | **0.02*** | -- | -- | -- | **-0.03*** | 0.00 | 0.00 | -- | -- | **-0.02*** | 0.00 | -0.01 | **-0.02** |
| 20 | 15:22 | 0.01 | **0.02*** | **-0.06*** | -- | -- | -0.01 | -0.01 | **-0.02** | -- | -0.01 | **-0.04*** | -0.01 | **-0.01** | **-0.02** |
| 20 | 17:17 | 0.00 | **0.04*** | **-0.04*** | **-0.07*** | -- | -0.01 | 0.00 | 0.00 | -- | -- | **-0.06*** | **-0.03*** | -0.01 | **-0.01** |
| 20 | 20:30 | **-0.01** | **0.04*** | **-0.08*** | **-0.03*** | -- | **-0.01** | 0.00 | 0.00 | **-0.03*** | -- | **-0.08*** | -0.01 | -0.01 | **-0.02** |
| 20 | 23:08 | 0.00 | **0.03*** | **-0.09*** | -- | -- | 0.00 | -0.01 | -0.01 | **-0.02** | -- | **-0.03*** | -0.01 | -0.01 | **-0.02** |
| 20 | 3:05 | -0.01 | **0.02*** | **-0.02*** | **-0.07*** | -- | 0.00 | 0.00 | 0.00 | **-0.07*** | -- | **-0.03*** | 0.00 | 0.00 | **-0.01** |
| 20 | 7:45 | -0.01 | **0.08*** | **-0.02** | -0.01 | -- | -0.01 | 0.00 | -0.01 | -- | -- | **-0.04*** | **-0.03*** | 0.00 | **-0.03*** |

Figure 1

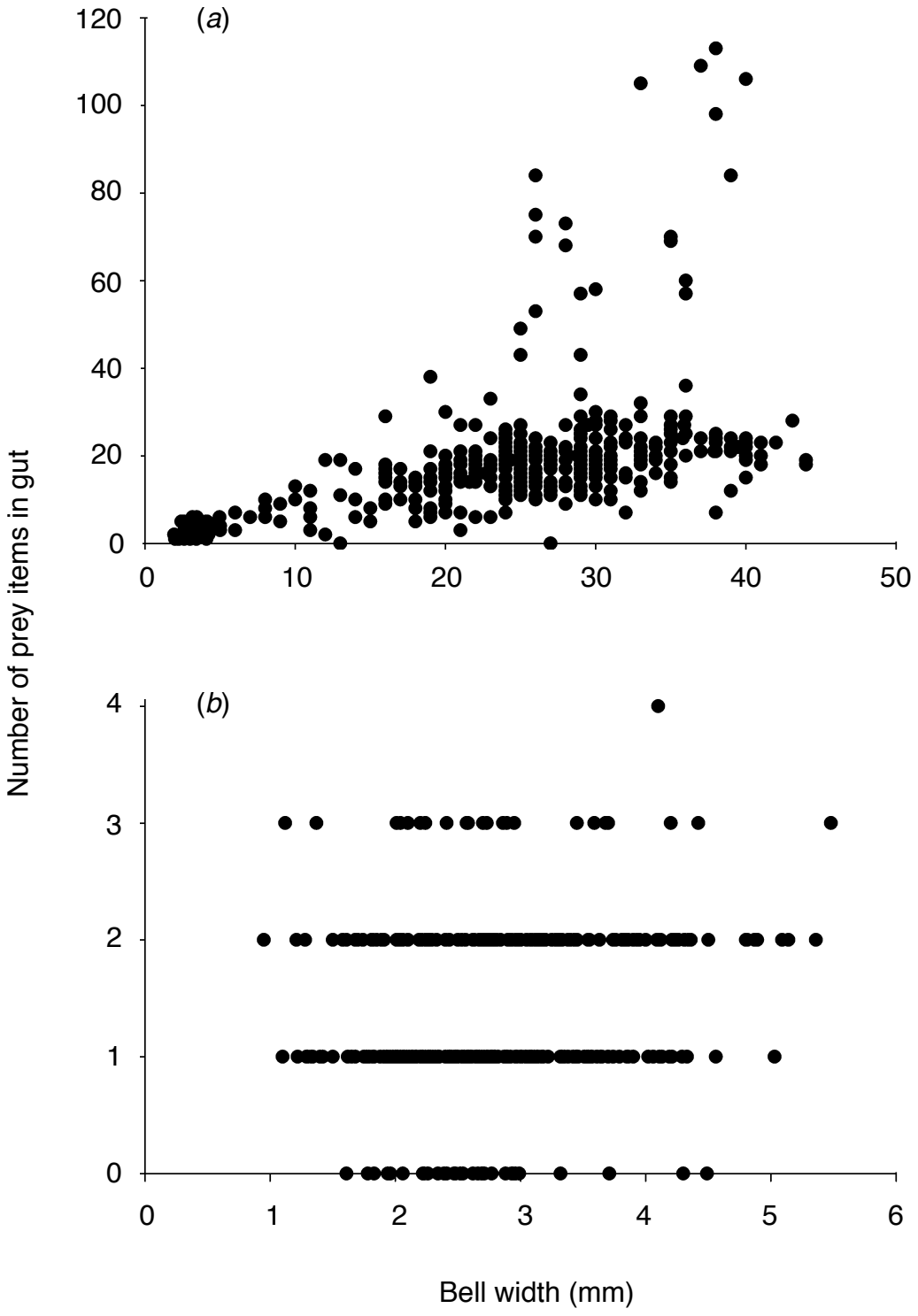

(a)
(b)
Number of prey items in gut
Bell width (mm)
0
20
40
60
80
100
120
0
10
20
30
40
50
0
1
2
3
4
0
1
2
3
4
5
6

Figure 2

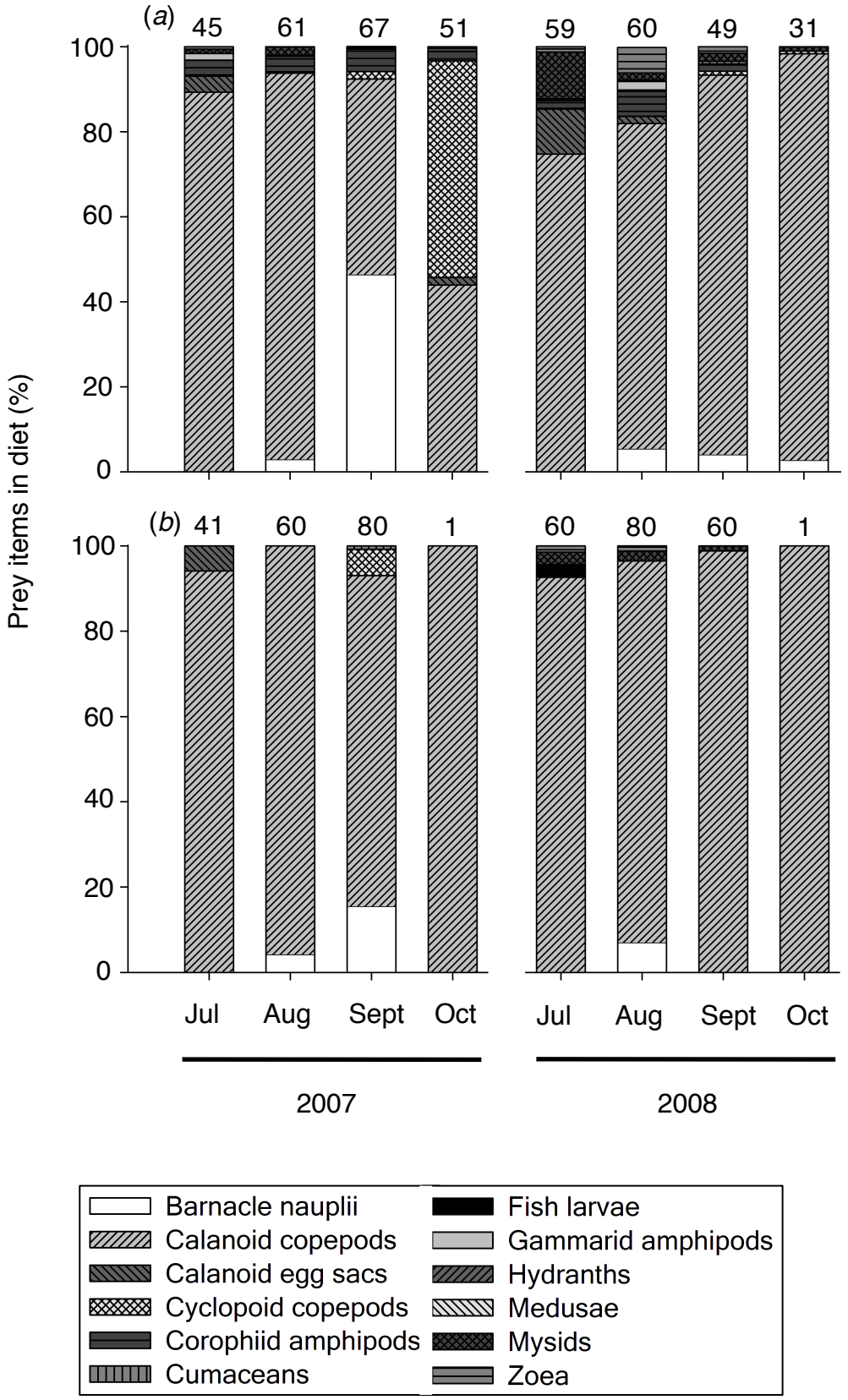

(a)
45
61
67
51
59
60
49
31
(b)
41
60
80
1
60
80
60
1
Prey items in diet (%)
100
80
60
40
20
0
Jul
Aug
Sept
Oct
2007
2008
Barnacle nauplii
Calanoid copepods
Calanoid egg sacs
Cyclopoid copepods
Corophiid amphipods
Cumaceans
Fish larvae
Gammarid amphipods
Hydranths
Medusae
Mysids
Zoea

Figure 3

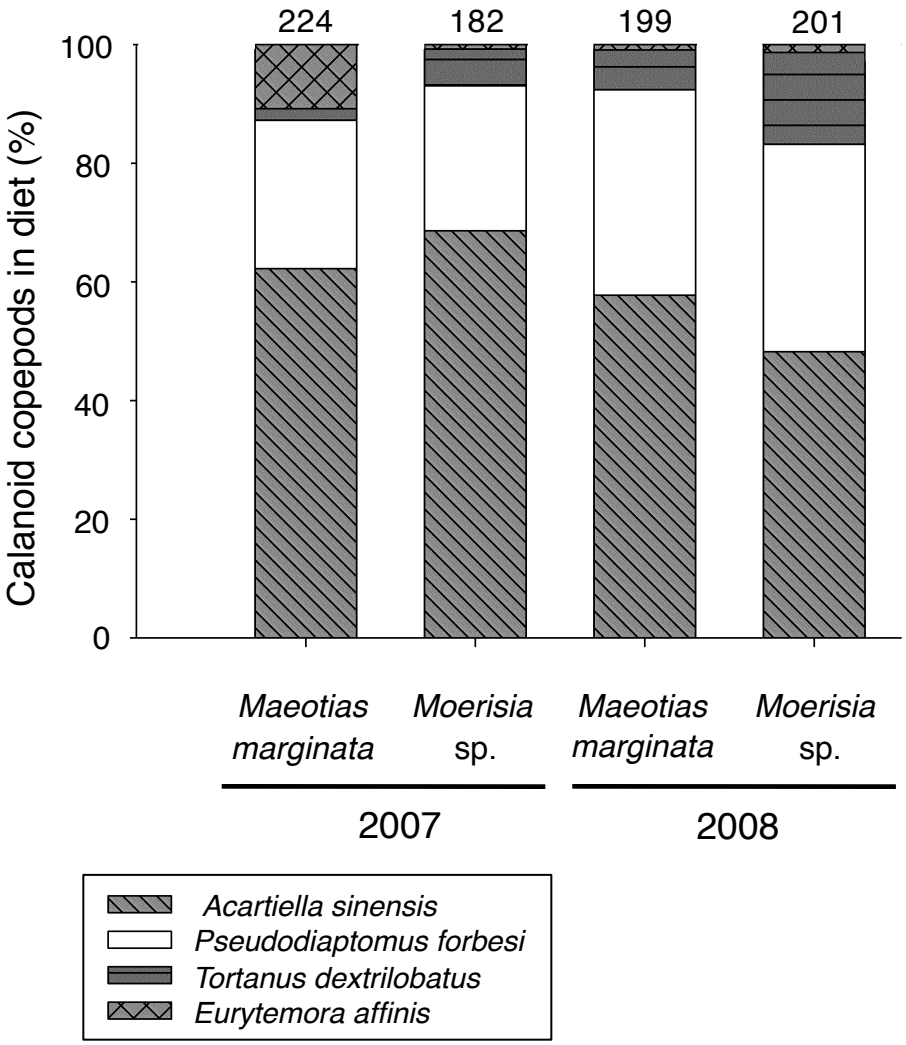
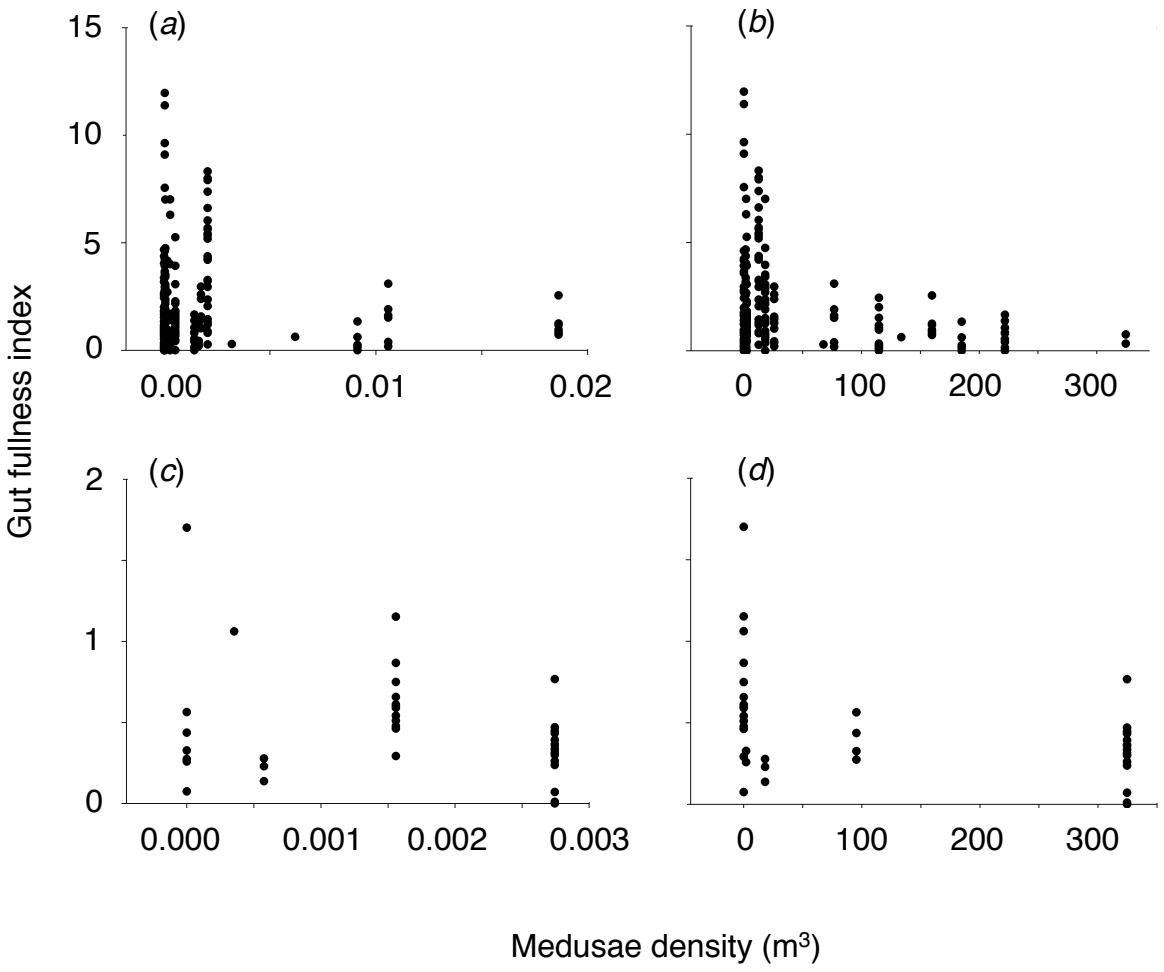

224
182
199
201
100
80
60
40
20
0
Calanoid copepods in diet (%)
Maeotias marginata
Moerisia sp.
Maeotias marginata
Moerisia sp.
2007
2008
Acartiella sinensis
Pseudodiaptomus forbesi
Tortanus dextrilobatus
Eurytemora affinis

Figure 4


(a)
(b)
(c)
(d)
Gut fullness index
Medusae density (m3)
15
10
5
0
0.00
0.01
0.02
0
100
200
300
2
1
0
0.000
0.001
0.002
0.003
0
100
200
300