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REPRODUCTIVE TIMING OF FRESHWATER MUSSELS AND POTENTIAL IMPACTS OF PULSED FLOWS ON REPRODUCTIVE SUCCESS

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Preface

The Public Interest Energy Research (PIER) Program supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

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Reproductive Timing of Freshwater Mussels and the Potential Impacts of Pulsed Flows on Reproductive Success is the final report for the Management of Ecological Evaluation of Hydropower Pulsed and Manufactured Flow Releases on California's Stream Systems Project (contract number 500-01-044) conducted by Spring Rivers Ecological Sciences, LLC, subcontracting for the Hydropower Pulsed Flow Program. The information from this project contributes to PIER's Energy-Related Environmental Research Program.

For more information about the PIER Program, please visit the Energy Commission's website at www.energy.ca.gov/pier or contact the Energy Commission at 916-654-5164.

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Abstract

Reproductive timing of native freshwater mussel species in the Pit River drainage in northeastern California was investigated from April 2004 through early May 2006 to assess the potential impacts of pulsed flow releases from hydroelectric facilities on mussel reproduction. Studied species included *Anodonta californiensis*, *A. nutalliana* (*wahlamatensis*), *Gonidea angulata*, and *Margaritifera falcata*.

Anodonta were gravid throughout most of the field seasons except from late July to mid-October, and their glochidia were observed on fish during all months except September and October. *Gonidea angulata* were gravid from late March to mid-July, and their glochidia were observed on fish from late March to late July/early August. Reproductive timing in these species was similar (within two weeks) in unregulated and regulated river reaches with different flow regimes. Gravid *Margaritifera falcata* were found in one Pit River reach in April and June 2004 and April 2005, in another reach in June and July 2005, and in a spring-fed tributary in July 2005.

In the Pit River, seasonal pulsed flows for channel maintenance/recreation should be scheduled during or after September to minimize interference with mussel reproduction and settlement of newly excysted juvenile mussels.

Keywords: *Anodonta californiensis*, *nuttalliana*, *wahlamatensis*, *Gonidea angulata, Margaritifera falcata,* freshwater mussels, mussel reproduction, glochidia, host fish, hydropower pulsed flows, Pit River, Fall River

Executive Summary

Introduction

Native freshwater mussel populations in North America are declining at a catastrophic rate. The construction and operation of dams for hydroelectric generation, flood control, and navigation have contributed substantially to the drastic declines in mussel populations. Downstream of dams, unnatural pulses in stream discharge (pulsed flows) have the potential to reduce mussel reproductive success if such events occur during the critical periods in the mussel reproductive cycle. Knowledge of mussel reproductive timing in California river systems is scant, and this information is necessary for assessing the potential impacts of seasonal pulsed flows (such as flushing/whitewater flows for channel maintenance or recreation) on mussel reproductive success and for scheduling these events to minimize reproductive interference.

Purpose

This study pinpointed time periods when mussel species in the Pit River drainage in northeastern California (Shasta County) would be most vulnerable to pulsed flows. Studied species included *Anodonta californiensis*, *A. nutalliana* (*wahlamatensis*), *Gonidea angulata*, and *Margaritifera falcata*. *Anodonta* were not identified to species because they were often difficult to distinguish in the field, and recent studies have emphasized the need for genetic analyses in identifying these species. To broaden the applicability of collected data, investigations were conducted in rivers and river reaches with different flow and/or temperature regimes.

Project Objectives

The primary objective was to collect mussel reproductive data from three Pit River reaches that experience different manufactured flows and from two spring-dominated Pit River tributaries that have relatively stable flow regimes. These reaches included the Pit 4 Bypass (receives reduced flow via stable releases from Pit 4 Reservoir except during spills); Pit River downstream of Pit 1 Powerhouse (receives full flow, with daily pulsed flows from hydroelectric peaking); Pit 1 Bypass (receives reduced flow, with a base flow release that varies seasonally: daily pulsed flows during the spring and summer from a hydroelectric project in the upper watershed, and pulsed flows for channel maintenance/recreation during three summer weekends); Hat Creek downstream of Hat 2 Powerhouse (run-of-river reach with stable, spring-dominated flow regime); and lower Fall River (unregulated reach with stable, spring-dominated flow regime).

Field investigations were conducted from April through November 2004 and from February 2005 through early May 2006 and focused on the following critical reproductive events:

- Spawning (when males expel sperm into the water column and females move eggs into portions of their gills modified to form brood pouches called marsupia).
- Glochidial release (the end of the gravid period when mature mussel larvae called glochidia are expelled from the marsupia in order to attach to suitable host fish).

- Juvenile excystment from host fish (when glochidia that have successfully transformed into juvenile mussels drop off of host fish).

To determine the timing of spawning and glochidial release, mussels were periodically collected and examined for gravidity (for incubating eggs or embryos), and samples of eggs/embryos were collected from the marsupia of gravid mussels to determine stages of development. In 2004, samples were also collected from the gonads of non-gravid mussels to determine their sex. In 2005–06, stream drift was sampled downstream of undisturbed mussel assemblages to determine seasonal peaks in glochidial release.

To determine periods of juvenile mussel encystment (attachment) on host fish and estimate periods of juvenile excystment (detachment), fish were collected concurrently with mussels and examined for signs of glochidial infection. A portion of the infected fish collected during late March through September 2005 was transported to the laboratory and held in freshwater aquaria to determine the timing of juvenile excystment and the species of fish that acted as hosts.

Results

Anodonta in all reaches spawned and released glochidia throughout most of the 2004 and 2005–06 field seasons, except from roughly late July to mid-October, and their glochidia were observed on fish during all months except September and October. Excysted juvenile *Anodonta* were collected from aquarium tanks containing native hardhead, Sacramento pikeminnow, tule perch, Pit sculpin, and non-native green sunfish during the months of April through July 2005.

Gonidea angulata in the Pit River reaches and Fall River (this species was not found in Hat Creek) spawned and released glochidia from late March/early April to mid-July in 2004 and 2005, and beginning in late April 2006. In 2005, *G. angulata* glochidia were more abundant in stream drift samples collected during June in the Pit 4 Bypass, Pit River downstream of Pit 1 Powerhouse, and Fall River. In the Pit 1 Bypass, stream drift was not sampled in June, but glochidia were more abundant in samples collected in early May than in mid-April or mid-July. Glochidia were observed on fish from late March to late July/early August. Juvenile *G. angulata* were collected from aquarium tanks containing native hardhead, tule perch, and Pit sculpin during June and July.

Spring/summer gravid periods for *Anodonta* and *G. angulata* were similar (within two weeks) in all reaches and ended as (or soon after) the hydrograph reached base flow and water temperatures peaked around mid-July in 2004 and 2005. *Anodonta* resumed spawning in mid-to-late October in both years, when flows began to increase and water temperatures dropped below 13°C (55.4°F). *Gonidea angulata* resumed spawning the following spring during variable flow conditions and after water temperatures exceeded 10°–12°C (50°–53.6°F).

Few gravid *Margaritifera falcata* were found during this study, and the timing of gravidity differed between river reaches and years. This species was not found in Fall River and was not sampled in the Pit 1 Bypass because it was rare. In the Pit 4 Bypass, spawning females were found in early April and mid-June in 2004, when flow regime was stable (200 cubic feet per second) and water temperatures ranged from 10°C to 16°C (50°–60.8°F); and in early and late

April 2005, when flows ranged from 200–2000 cfs and water temperatures averaged 12°C (53.6°F). In the Pit River downstream of Pit 1 Powerhouse, gravid females were not found in 2004, but were found from early June through early July in 2005. Those collected in early June had just spawned, and spawning occurred after a 5000-cfs drop in average daily river flow, when water temperature averaged 18°C (64.4°F). The single gravid female collected in early July incubated glochidia. In Hat Creek, one *M. falcata* spawned in early July 2005, but no other gravid *M. falcata* were found in this reach. The glochidia of *M. falcata* were not observed in samples of stream drift or on fish collected in 2005–06.

Conclusions

In the Pit River reaches, most adult mussels spawned and released glochidia during the months of April through July, and most juvenile mussels dropped off of their fish hosts to settle on the stream bottom during June, July, and early August. Seasonal pulsed flows or maintenance outages that occur during these critical time periods would have a greater impact on mussel recruitment than those that occur after August. The shorter reproductive periods observed for *G. angulata* and *M. falcata* may make these species more vulnerable to pulsed flows than *Anodonta*, especially during peak periods of glochidial release and juvenile excystment/ settlement. These peaks occurred for *G. angulata* during June and July in most locations.

Reproductive timing in *Anodonta* and *G. angulata* was similar in unregulated and regulated river reaches with different flow regimes. Reproductive timing in *M. falcata* varied between reaches, indicating that this species may be more sensitive to fluctuations in river flow and/or water temperature. Consequently, the annual timing and duration of *M. falcata* reproduction may be more difficult to predict in the Pit River and other California river systems.

Recommendations

Seasonal pulsed flows in the Pit 1 Bypass, which have been in effect since 2003, and maintenance outages in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse should be planned before and/or after peak periods of glochidial release and juvenile excystment/settlement for *G. angulata* and *M. falcata*. Although reproductive timing may vary somewhat annually, these critical events may occur for both species during June, July, and August. In the Pit 1 Bypass, license-prescribed pulsed flows for channel maintenance/recreation occurred in May/June, mid-July, and late August in 2004 and 2005. These high-flow events could have adversely impacted recruitment in *G. angulata* and the few *M. falcata* present in the reach by reducing contact between glochidia and host fish during glochidial release and/or preventing settlement of juveniles after excystment. Newly excysted juveniles may be especially vulnerable to scouring flows, and scheduling the summer pulsed flows after August, or September if possible, would allow juveniles time to grow to a less-vulnerable size.

Continued monitoring and assessment of specific impacts of pulsed flows on freshwater mussels, especially in the Pit 1 Bypass, is recommended. Because the timing of mussel reproduction observed in the Pit River may differ in other California drainages, reproductive data from rivers throughout the state should be gathered and compared to determine if site-specific, regional, or statewide flow recommendations would be appropriate.

Benefits to California

The data in this report increase the knowledge base of native freshwater mussel biology and ecology. This research provides the groundwork for future monitoring and assessment of pulsed flows in the Pit River drainage and for research of mussel reproductive timing in other regulated and unregulated rivers within California. Data from this and future studies on mussel reproductive timing will help managers of hydroelectric facilities, industry regulators, and policy makers assess the potential impacts of seasonal pulsed-flow releases on the reproduction of California mussel populations and plan these flow events to minimize adverse effects on mussel reproduction.

1.0 Introduction

1.1. Background and Overview

Freshwater mussels (Superfamily Unionoidea) are large, burrowing filter feeders that play an important role in healthy aquatic ecosystems. Mussels excrete nutrients that contribute to the aquatic food web (Vaughn et al. 2004; Howard and Cuffey 2006), improve water quality by filtering suspended solids and contaminants (McMahon 1991), and are prey for a number of aquatic and terrestrial mammals (Fuller 1974). Unfortunately, mussel populations are declining precipitously worldwide, and in North America more than 70% of the recognized mussel species are considered endangered, threatened, or of special concern (Williams et al. 1993).

In western North America, eight species of native freshwater mussels are recognized in the genera *Anodonta*, *Gonidea*, and *Margaritifera* (Turgeon et al. 1998), although uncertainties regarding the taxonomy of western *Anodonta* remain (see Mock et al. 2004; Nedeau et al. 2005). In California, these species include *Anodonta californiensis*, *Anodonta oregonensis*, *Anodonta nutalliana* (a.k.a. *Anodonta wahlamatensis*), *Gonidea angulata*, and *Margaritifera falcata* (Taylor 1981), all of which are found in the Pit River drainage in northeastern California (Figures 1 and 2). *Anodonta californiensis*, the California floater mussel, is a United States Forest Service (USFS) sensitive species. The conservation status of the other four native mussel species within California remains undetermined, because their historic and current distributions need to be evaluated (Williams et al. 1993; NatureServe 2005). The National Strategy for the Conservation of Native Freshwater Mussels (National Native Mussel Conservation Committee 1998) stated that the basic life history, reproductive biology, ecology, and habitat requirements of most mussels were unknown and that this information was critical for the successful conservation of freshwater mussels.

Freshwater mussels have a unique reproductive strategy that includes a brief period of parasitism on fish, or in rare cases, amphibians (Watters and O'Dee 1998). This strategy allows the relatively sedentary mussels to disperse upstream and to colonize new habitats. Their benthic (river bottom) existence and reliance on host fish during reproduction make mussels exceedingly vulnerable to habitat disturbance (Neves et al. 1985; McMahon 1991; Vaughn and Taylor 1999).

Alterations in riverine habitat resulting from the construction and operation of dams for hydroelectric generation, flood control, and navigation have contributed greatly to the drastic declines in mussel populations (Bogan 1993; Williams et al. 1993; Hughes and Parmalee 1999; Watters 2000). These losses have been attributed to inundation of river reaches by impoundments, changes in water quality (including water temperature regimes and sediment transport) downstream of impoundments, elimination of fish hosts by passage barriers, and alterations in daily and seasonal discharge regimes (Layzer and Madison 1995; Hardison and Layzer 2001). In river reaches affected by hydroelectric projects, like those on the Pit River, planned pulses in stream discharge (pulsed flows) or spills resulting from unscheduled powerhouse outages may also pose a threat to freshwater mussels if such events occur during critical periods in their reproductive cycle. Knowledge of mussel reproductive timing in

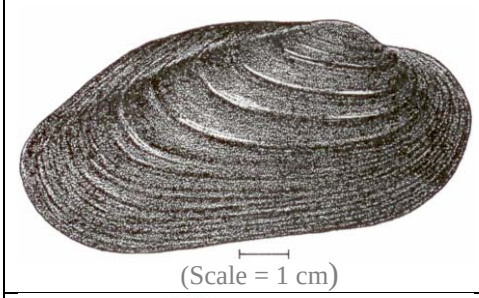
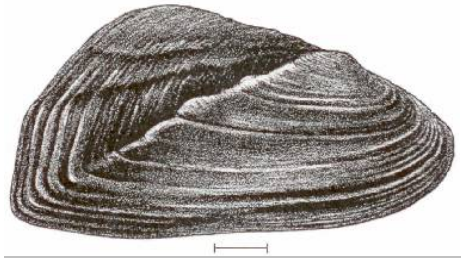
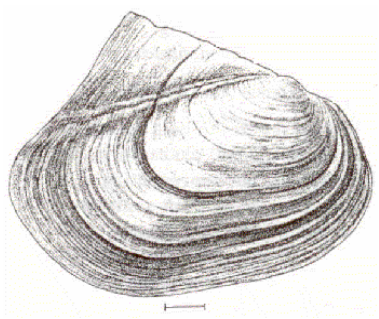
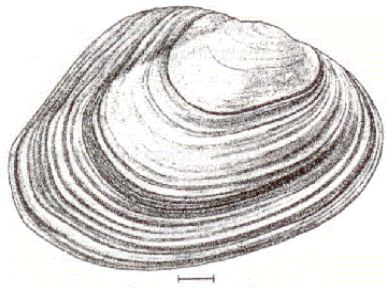
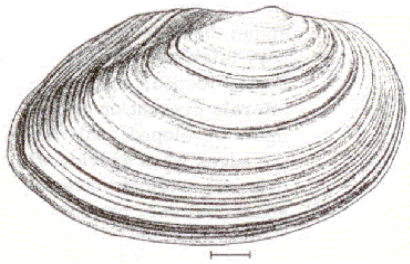
	*Margaritifera falcata,* western pearlshell mussel Thick, black or brown outer shell (periostracum), pearly interior (nacre), and well-developed hinge (pseudocardinal) teeth.
	*Gonidea angulata,* western ridged mussel Thick, black or brown outer shell, pearly interior, and well-developed hinge teeth. Distinct ridge running from beak to posterior ventral surface.
	*Anodonta nutalliana* (*wahlamatensis*), winged floater mussel Thin, fragile outer shell, pearly interior, and no hinge teeth. Shell with conspicuous winged hinge.
	*Anodonta californiensis,* California floater mussel Thin, fragile outer shell, pearly interior, and no hinge teeth. Shell with wing of moderate height.
	*Anodonta oregonensis,* Oregon floater mussel Thin, fragile outer shell, pearly interior, and no hinge teeth. Shell inflated primarily over median portion, with inconspicuous wing.

Figure 1. Native mussel species in the Pit River drainage (adapted from Burch 1975)

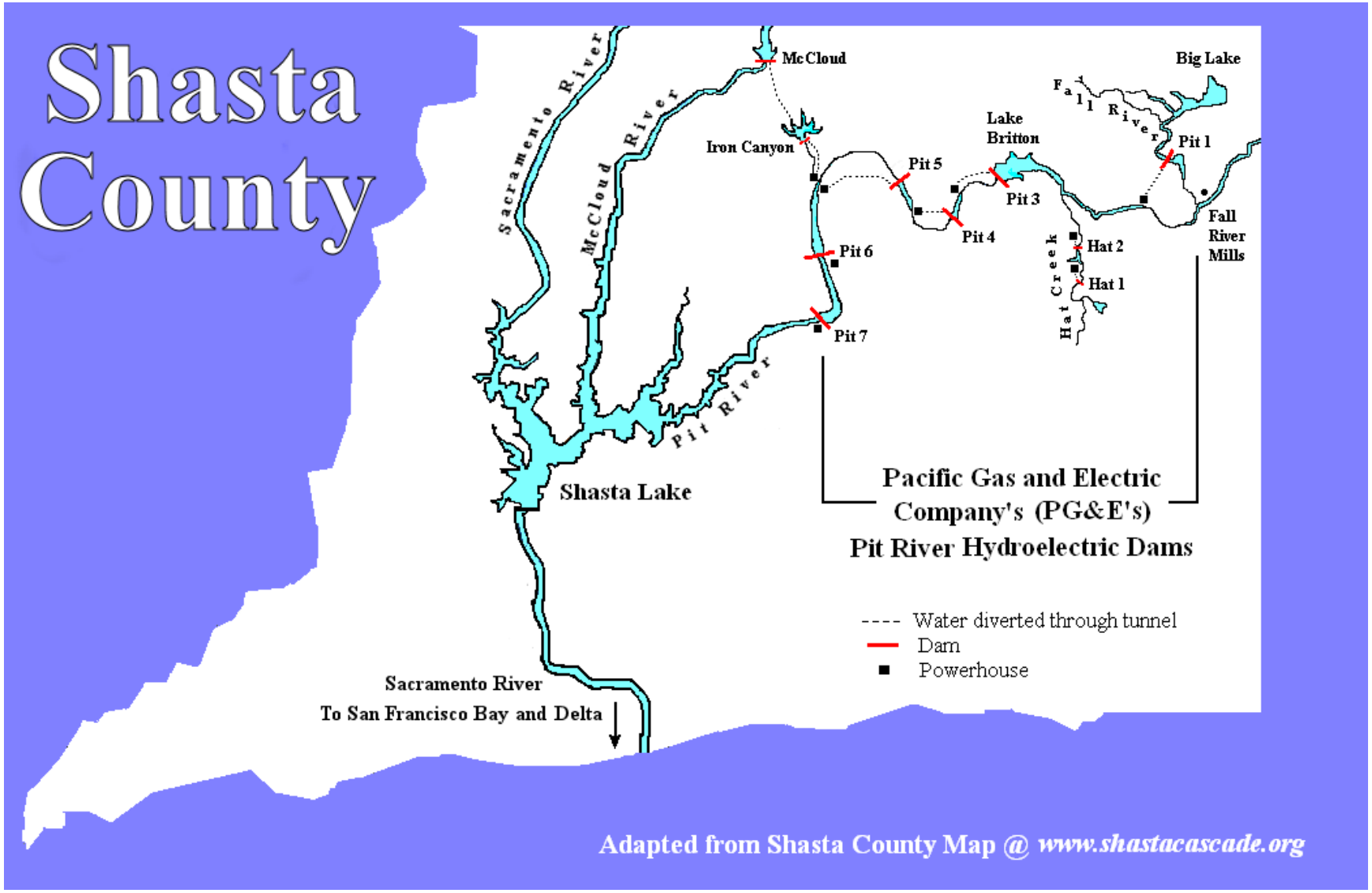


Figure 2. Lower Pit River drainage showing major hydroelectric dams and powerhouses operated by PG&E

California river systems is scant, however, and this information is necessary for assessing the potential impacts of seasonal pulsed flows (e.g., flushing flows for channel maintenance or whitewater flows for recreation) on mussel reproductive success and for scheduling these pulsed flow events to minimize reproductive interference.

To understand the potential for pulsed flows to interfere with freshwater mussel reproduction, it is important to understand their general reproductive strategy. During spawning, males expel sperm through their exhalent siphon into the water column and females move unfertilized eggs from their gonad into their gills, where one or more gill demibranchs are modified to form brood chambers called marsupia (McMahon 1991). All four of the gill demibranchs (there are two on each side of the body) function as marsupia in *G. angulata* and *M. falcata*, and the two outer demibranchs function as marsupia in *Anodonta* species. Eggs are fertilized within the marsupia of females when sperm are drawn into the gills along with the respiratory currents of water. Embryos are brooded in the marsupia through early development until the mature larvae, called glochidia, are released into the water column to complete development as ectoparasites on the fins, skin, or gills of suitable hosts. Glochidia cannot survive long in the water column, so attachment to hosts must occur within hours or days, depending on mussel species and water temperature (Surber 1912; D'Eliscu 1972; Fisher and Dimock 2000). When metamorphosis is complete, juvenile mussels fall from the host (excyst) and attach to substrate on the stream bottom. Juveniles can smother if they land in shifting sands or mud, so their survival prior to and immediately after excystment is entirely dependent on the currents and the migration patterns of the host (Isley 1911; Murphy 1942; Fuller 1974; Neves et al. 1985).

There are two primary reproductive strategies that have been described for unionoidean mussels. Some species exhibit prolonged incubation periods (long-term or bradytictic brooders) and are gravid throughout most of the year, while others exhibit short incubation periods (tachytictic or short-term brooders), with spawning and glochidial release occurring in the same season (Ortmann 1911, 1912; Heard and Guckert 1970; Heard 1998). Most *Anodonta* species have been described as long-term brooders (Lefevre and Curtis 1912; Heard 1975) and *Margaritifera falcata* typically exhibit short-term brooding strategies (Murphy 1942; Meyers and Millemann 1977; Fustish and Millemann 1978; Karna and Millemann 1978). Reproductive patterns have not been described for *Gonidea angulata*, which is a unique species assigned its own genus and occurring only in western North America. These general reproductive patterns can be affected by habitat variables such as water temperature and flow (Watters and O'Dee 2000; Hastie and Young 2003) and are thought to have evolved to coincide with seasonal fish migration and/or nesting periods (Zale and Neves 1982; Kat 1984). Consequently, the seasonal timing of reproduction within mussel species may differ both regionally and locally.

In California, over 260 hydroelectric power projects are distributed in many major rivers and streams. Many of these projects produce seasonal pulsed flows for recreation or channel maintenance that may interfere with mussel spawning, glochidial release, and/or juvenile excystment from host fish. Out-of-season high flows that occur during mussel spawning could reduce fertilization potential by diluting the concentration of sperm available for respiring females; those that occur during glochidial release could reduce encystment potential by flushing glochidia downstream and/or altering fish behavior; and those that occur during and

after periods of juvenile excystment from host fish could reduce juvenile survival by preventing attachment and settlement of juveniles on the stream bottom. The increased turbidity typically associated with pulsed flows could also reduce reproductive success by limiting available nutrients and energy available for reproduction (Aldridge et al. 1987). The possibility of such adverse impacts warrants research of mussel reproductive timing in all river systems affected by hydroelectric projects. Equipped with this information, managers of hydroelectric facilities can more accurately assess the potential impacts of seasonal pulsed flows on mussel reproduction and schedule these flow events to minimize reproductive interference.

1.2. Project Objectives

The goal of this research was to determine the seasonal timing of reproduction for native freshwater mussels in the lower Pit River drainage in northeastern California (Shasta County) (Figures 1 and 2). To broaden the applicability of the collected data, investigations were conducted in rivers and river reaches with differing flow and/or temperature regimes.

In 2004, the objective was to collect reproductive data from three reaches of the mainstem Pit River that are subject to different types of manufactured (pulsed) flows. These reaches included the Pit 4 Bypass (a reservoir-controlled reach not subject to pulsed flows), Pit River downstream of the Pit 1 Powerhouse (subject to daily pulsed flows from hydroelectric peaking), and the Pit 1 Bypass (subject to daily pulsed flows during the spring and summer and pulsed flows for channel maintenance/recreation during three summer weekends). In 2005–06, the objectives were to continue data collection in the three Pit River reaches and expand the study area to include the lower reaches of Hat Creek and Fall River, two spring-dominated tributaries to the mainstem Pit River that have relatively stable flow regimes.

Field investigations were conducted from April through November 2004 and from February 2005 through early May 2006 and focused on the following critical reproductive events:

- Spawning (i.e., when males expel sperm into the water column and females move eggs into portions of their gills modified to form brood pouches called marsupia).
- glochidial release (i.e., the end of the gravid period when mature mussel larvae called glochidia are expelled from the marsupia in order to attach to suitable host fish).
- Juvenile excystment from host fish (i.e., when glochidia that have successfully transformed into juvenile mussels drop off of host fish).

Although three species of *Anodonta* have been reported in the Pit River drainage based on features of shell morphology such as wing height (Figure 1), *A. californiensis* and *A. nutalliana* could not always be distinguished in the field, and new studies have emphasized the need for genetic analyses in identifying these species (Mock et al. 2004). The species *A. oregonensis* was not collected during this study, because it is rare in the Pit River and lower Fall River (Ellis 1996). Consequently, this study describes reproductive timing for the genus *Anodonta*, which includes *A. californiensis* and/or *A. nutalliana*.

1.3. Report Organization

Section 2 of this report describes the methods for determining reproductive timing in the lower Pit River drainage. Section 3 presents the detailed results from investigations conducted in the lower Pit River drainage during two field seasons (April through November 2004 and February 2005 through early May 2006). Results are described for each mussel species in each study reach (i.e., Pit River reaches, Hat Creek, and Fall River). Section 4 summarizes and discusses the results, includes a brief evaluation of the study methods, presents recommendations based on the results of the study, and outlines the benefits of this research to California. References cited in this report are included in Section 5 and a glossary of biological and technical terms is provided in Section 6.

2.0 Methods

2.1. Investigative Strategy

Periods of spawning and glochidial release were investigated by collecting mussels at regular intervals from study reaches in the Pit River drainage. Collected mussels were examined for gravidity (i.e., incubating eggs or embryos), and samples were collected from the marsupia of gravid mussels to determine the developmental stages of incubating embryos (e.g., uncleaved eggs, gastrula stage, glochidia). During the 2005–06 field season, stream drift was also sampled upstream of mussel collection sites to determine the timing of glochidial release without collecting mussels. Stream drift was sampled upstream of mussel collection sites to ensure that glochidia were collected from undisturbed mussels.

To determine periods of glochidial encystment on host fish (i.e., parasitic periods) and estimate periods of juvenile mussel excystment, fish were collected concurrently with mussels throughout the field seasons and examined for signs of glochidial infection. Mussel glochidia may not complete development on all species of fish that they infect (see Watters and O'Dee 1996), therefore a subsample of the naturally infected fishes collected during the 2005–06 field season was transported to the laboratory and held in freshwater aquaria to monitor the production of juvenile mussels.

2.2. Project Location and Study Reaches

The Pit River is the largest river in northeastern California, originating from forks draining Goose Lake, historically, and the Warner Mountains. The Pit River flows through portions of Modoc, Lassen, and Shasta counties to its historic confluence with the Sacramento River, now flooded beneath Shasta Lake. The lower Pit River begins at its confluence with the clear, spring-dominated Fall River, near the town of Fall River Mills (Figure 2). Upstream of Fall River, the mainstem Pit River is largely channelized and its discharge is dominated by slow moving, warm, and turbid agricultural return flows. As the Pit River passes Fall River Mills, it enters a more confined canyon reach, picks up accretion flows from the Fall River and other spring-dominated tributaries, and becomes a rapidly flowing, high volume, and somewhat cooler river (Ellis and Hesseldenz 1993). The lower Pit River has been extensively modified by PG&E (Pacific Gas and Electric Company) for power generation, and this study investigated mussel reproductive timing in reaches affected by the Pit 1 (FERC No. 2687); Pit 3, 4, 5 (FERC No. 233-081); and Hat Creek (FERC No. 2661) Hydroelectric Projects. The Pit River upstream of the PG&E projects is also influenced by the Muck Valley Hydroelectric Project, which has a small powerhouse on the Pit River several kilometers upstream of Fall River in Lassen County.

Field investigations were conducted in three reaches of the mainstem Pit River and in the lower reaches of its major tributaries, Hat Creek and Fall River. Study reaches in the Pit River included the Pit 4 Bypass (i.e., the reach from which water is diverted, or bypassed, to supply the Pit 4 Powerhouse), the Pit River downstream of the Pit 1 Powerhouse, and the Pit 1 Bypass (i.e., the reach from which water is diverted to supply the Pit 1 Powerhouse) (Figures 3 and 4).

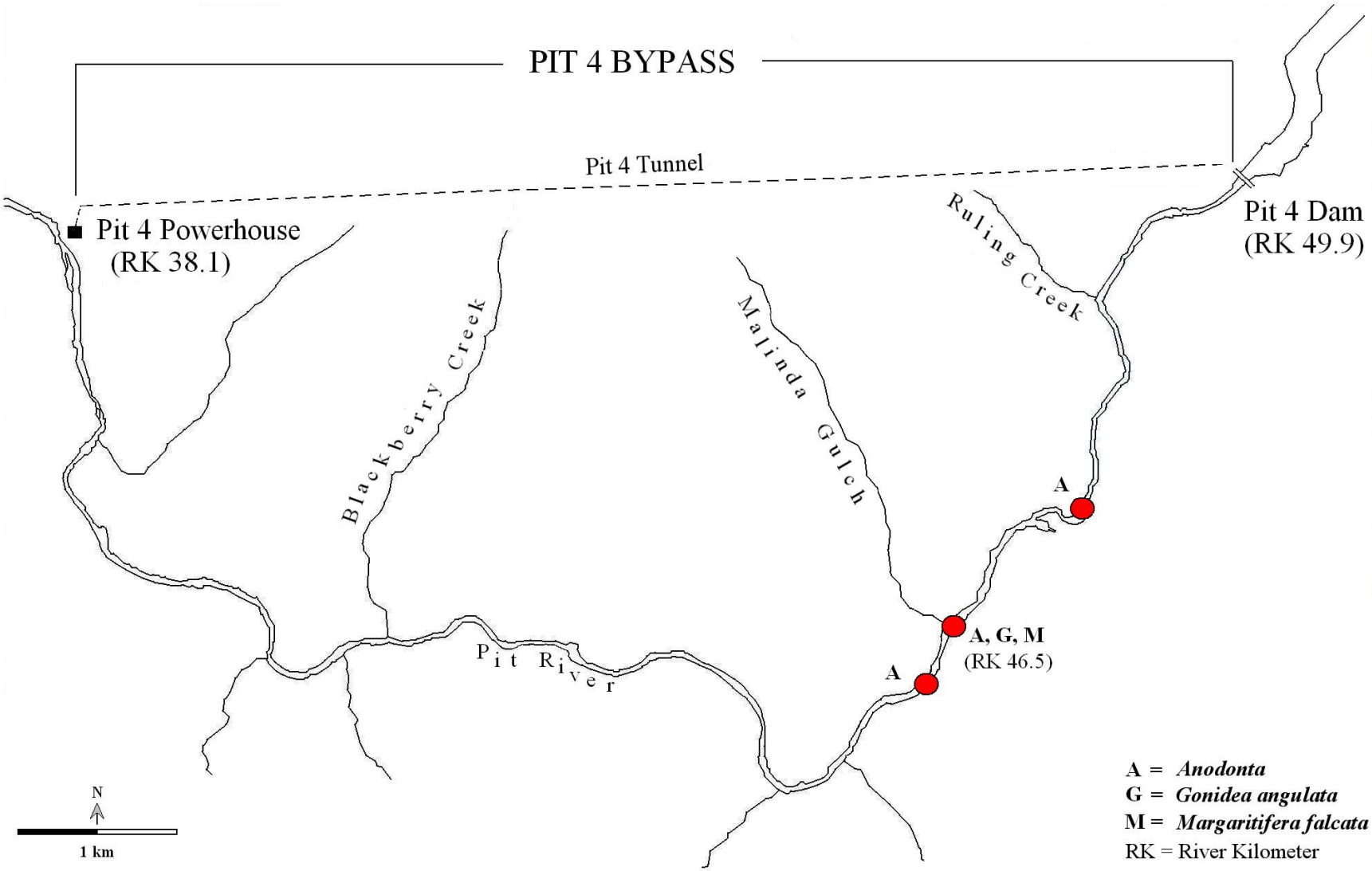


Figure 3. Study sites and mussel species in the Pit 4 Bypass reach of the Pit River

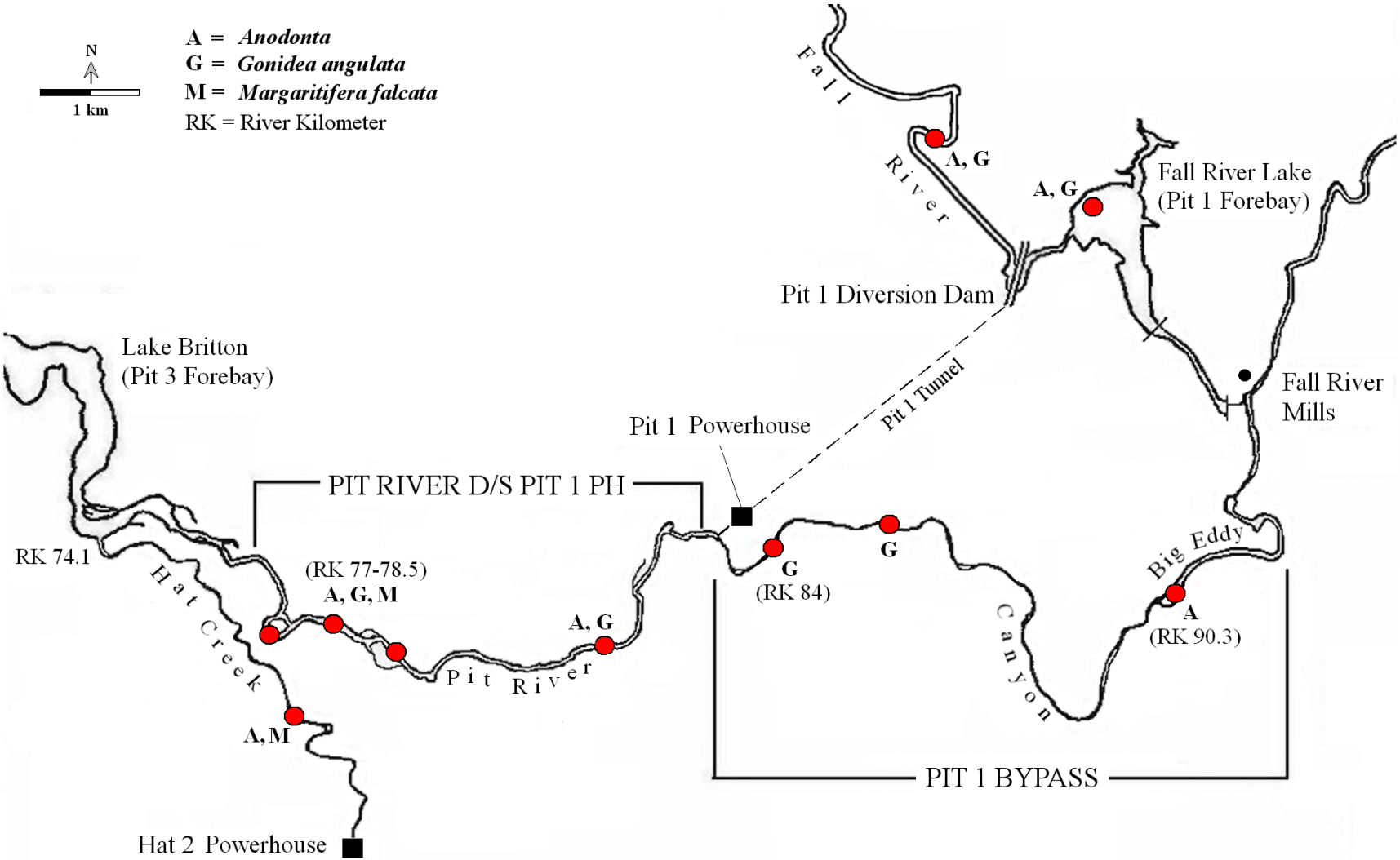


Figure 4. Study sites and mussel species in all reaches upstream of Lake Britton

In the tributary rivers, study reaches included the Hat Creek Wild Trout Area located downstream of the Hat 2 Powerhouse and Fall River upstream of the Pit 1 Diversion Dam. Some supplementary sampling was also conducted in Fall River Lake, which is the forebay for the Pit 1 Powerhouse. Mussels, fish, and/or stream drift were collected from one or more primary study sites within each river reach, and if reproductive data did not differ between sites, these data were pooled and compared between reaches. During the 2004 field season, time constraints limited field reconnaissance, so mussels and fish were collected from Pit River sites with known mussel assemblages. During the 2005–06 field season, new mussel assemblages were located in the Pit River and mussels, fish, and stream drift were collected from these sites as described in Sections 2.3 through 2.5.

Descriptions of the study reaches are as follows:

- **The Pit 4 Bypass** is located between the Pit 4 Dam and the Pit 4 Powerhouse (Figure 3). Water stored in the Pit 4 Reservoir is diverted via an underground tunnel into the Pit 4 Powerhouse, and a minimum of 150 cubic feet per second (cfs) is released to the Pit 4 Bypass. After implementation of the new license, the issue of which is anticipated in spring 2007, base flow in this reach will more than double as stipulated in the FERC license (USFS 2003). The flow regime in this reservoir-controlled reach is stable except during reservoir spills. The primary study site in this reach was located at Malinda Gulch, 3.4 river kilometers downstream of the Pit 4 Dam.
- **The Pit River downstream of Pit 1 Powerhouse** is located between the Pit 1 Powerhouse and Lake Britton, which is the Pit 3 Forebay (Figure 4). No water is diverted from this reach, which receives flow from the upper Pit River via the Pit 1 Bypass (natural channel) and from Fall River via the Pit 1 Hydroelectric Project. The Pit River downstream of Pit 1 Powerhouse has a minimum flow requirement of 700 cfs and experiences fluctuations in hourly average discharge from hydroelectric peaking. The primary study sites in this reach were located between 4 and 6 river kilometers downstream of the Pit 1 Powerhouse tailrace.
- **The Pit 1 Bypass** is located upstream of the Pit 1 Powerhouse and downstream of the confluence with Fall River (Figure 4). The majority of Fall River is diverted through the Pit 1 Hydroelectric Project, so flow in the bypassed channel is reduced. As of 2003, the Pit 1 Bypass has a summer base flow requirement from Fall River of 150 cfs with license-prescribed flushing/whitewater flows (1250 cfs or the natural flow, whichever is less) during three late spring/summer weekends. Flow in this reach is also influenced by the Muck Valley Hydroelectric Project, which has a small powerhouse on the Pit River several kilometers upstream in Lassen County. The primary study sites for *Gonidea angulata* were located between 1 and 2 river kilometers upstream of the Pit 1 Powerhouse tailrace, and the primary study site for *Anodonta* was located 7.5 river kilometers upstream of the Pit 1 Powerhouse tailrace. *Margaritifera falcata* were not studied in this reach, because only five scattered individuals were found at the downstream end of the Pit River Canyon.

- **Hat Creek** is a spring-dominated tributary to the Pit River, which flows through two run-of-river PG&E powerhouses before entering the upstream end of Lake Britton (Figures 2 and 4). Mussels were collected in the reach upstream of Lake Britton and downstream of the Hat 2 Powerhouse. Flow in this reach is not regulated by the run-of-river powerhouses, and the spring-dominated hydrograph is generally stable, although it is affected seasonally by upper Hat Creek's snow-melt driven hydrograph and by agricultural diversion in the upper watershed.
- **Fall River** is spring-dominated and the largest Pit River tributary. It rises from numerous springs in the upper drainage and flows through flat, mostly agricultural land before reaching the impoundment that diverts the majority of its flow through the Pit 1 Hydroelectric Project (Figure 4). Mussels were collected in lower Fall River, about 3 river kilometers upstream of the Pit 1 Diversion Dam, with some supplementary sampling in **Fall River Lake**, which is the Pit 1 Forebay.

All of the Pit River reaches are subject to naturally occurring seasonal peak runoff events, including the two bypass reaches, which typically have reduced flow. In past years, river discharge in the Pit 4 Bypass peaked at more than 30,000 cfs and discharge in the Pit 1 Bypass peaked at over 20,000 cfs.

2.3. Mussel Sampling

2.3.1. Collecting and Examining Mussels for Gravidity

Live mussels were collected in the Pit 4 Bypass, Pit River downstream of Pit 1 Powerhouse, and Pit 1 Bypass from April through November 2004, and in these three Pit River reaches, Hat Creek, and Fall River from February 2005 through April 2006 (Figures 3 and 4). All mussel species were collected in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse, *Anodonta* were collected from the Big Eddy pool in the upper Pit 1 Bypass, *G. angulata* were collected toward the downstream end of the Pit River canyon in the lower Pit 1 Bypass, *Anodonta* and *G. angulata* were collected in Fall River and Fall River Lake, and *Anodonta* and *M. falcata* were collected in Hat Creek. When possible, collections were every two weeks during the spring, summer, and fall and every four weeks during the winter.

Due to low abundance of mussels at most of the pre-selected study sites in 2004, an average of 10 and no more than 20 individuals of each genus present were collected and examined in each river reach on each date. More mussels were collected where abundance was high and when water conditions were suitable for collection. Based on the 2004 data and reports that as many as 30%–40% of *Margaritifera margaritifera* females may not reproduce in a given year (Young and Williams 1984; Bauer 1987a; Ross 1992; Hastie and Young 2003), numbers of *M. falcata* collected and examined were increased during the 2005–06 field season to improve detection of gravidity. No less than 20 *M. falcata* were collected at a given site on each date and more were collected in locations where abundance of this species was sufficiently high. Estimates of surface abundance were made at all of the primary study sites in February 2005 to determine the maximum number of *M. falcata* that could be collected at each site on each date throughout the 2005–06 field season without replacement (i.e., sample size=total adult abundance/expected number of

sampling dates). Surface abundance of adults was estimated using stratified random sampling designs (Strayer and Smith 2003). Similar abundance estimates were made for *Anodonta* and *G. angulata* during the 2005–06 field season, but collections of 10–20 individuals of these species per date were found to be sufficient for detection of gravidity.

Mussels were collected by hand using snorkeling or SCUBA gear and then transferred to five-gallon buckets filled with river water. Gravidity was determined by gently prying apart the shell valves using a stainless steel nasal speculum, inserting a thin piece of cork to prevent valve closure, and carefully examining the marsupia. Gravid mussels were distinguished from non-gravid mussels by their distended marsupia, which contained visible patches or striations of eggs or embryos.

2.3.2. Sampling Gravid and Non-gravid Mussels

In 2004, gravid mussels were sampled by extracting 0.05 ml of fluid from their marsupia using a 1.0-cc syringe with an 18-gauge, 1.5-inch needle (Milam et al. 2000; Shiver 2002). The same amount of fluid was also collected from the gonads of non-gravid mussels to determine their sex (Bauer 1987b; Grande et al. 2001). Although *Anodonta* and *M. falcata* may be functionally hermaphroditic (i.e., capable of producing both male and female gametes) (Heard 1975; Grande et al. 2001), the goal was to determine the total number of functional females sampled and the number of non-gravid females within that sample. Therefore, mussels with developing or mature eggs in the gonadal fluid were considered females and those with developing or mature sperm were considered males. Non-gravid mussels with both male and female gametes in the gonadal fluid were considered hermaphrodites. All mussels were sampled to determine sex prior to late July, but many collected during and after late July were checked for gravidity, but were not sampled due to time and funding constraints. After sampling, mussels were measured for length, width, and height using dial calipers (0.1 mm accuracy), marked using a battery-powered rotary tool, and returned to their point of capture. The shell metrics data are not presented in this report, but may be useful for future research and monitoring.

All samples were placed in an insulated cooler immediately after collection and were transported to the laboratory for microscopic analysis. Within eight hours of collection, samples were examined and digitally photographed under 4- or 10-power magnification using a compound microscope. The developmental stages of eggs/embryos in marsupium samples and the presence of eggs and/or sperm in gonad samples were categorized and recorded as described by Heard (1975) and Jones et al. (1986). In the marsupium samples, the presence of uncleaved eggs (i.e., eggs not yet fertilized or fertilized eggs not yet divided) indicated recent spawning activity, and the presence of glochidia (i.e., possessing fully developed adductor muscles and larval shells) indicated impending glochidial release. *Anodonta* can be an exception, because these long-term brooders may retain glochidia in their marsupia long after they reach maturity (Coker et al. 1921; Kat 1984). Mussels with mostly clear fluid and some degraded glochidia in their swollen marsupia were considered to have recently released glochidia (Murphy 1942; Jones et al. 1986).

During the 2005–06 field season, marsupium samples were collected from gravid mussels, but non-gravid mussels were not sampled. Marsupium samples were collected as previously described, but needle diameter was reduced to 20-gauge to minimize the potential for injury. Gravid *Anodonta* were only sampled from February through April in 2005. Thereafter, stages of embryonic development were estimated by noting the color of eggs/embryos within examined marsupia, which changed predictably during development (Avelar and de Mendonca 1998; Lima et al. 2006). Uncleaved eggs, which were white or cream-colored, were deposited in vertical striations within portions of each marsupium during the onset of egg deposition (i.e., spawning in females) (Figure 5). Developing embryos were a darker, beige color, but the specific stages of development after egg cleavage and before shell development could not be accurately estimated by coloration. Early and mature glochidia, which were a dark orange or brown color, were easily distinguished from uncleaved eggs and developing embryos. The marsupia of *Anodonta* that were actively releasing glochidia usually contained clear fluid in the posterior (i.e., near the siphons) marsupia and mature glochidia in the anterior (i.e., near the foot) marsupia.

2.3.3. Monitoring Marked Anodonta

In all study reaches, some marked *Anodonta* individuals (i.e., previously sampled mussels with numbers etched into their shells) were periodically re-collected and examined to monitor changes in gravidity status over time. *Anodonta* and other long-term brooders may be more tolerant of frequent disturbances than short-term brooders like *G. angulata* and *M. falcata* that are likely to abort their glochidial contents after examination (Yeager and Neves 1986; Howells 2000; Haag and Warren 2003). Some marked *G. angulata* were reexamined in 2004, but marked *M. falcata* were not reexamined in 2004 or 2005–06. When and where water conditions would allow, marked *Anodonta* were examined for gravidity underwater at the site of collection. In the

Anodonta gravid with uncleaved eggs | *Anodonta* gravid with glochidia | *Gonidea angulata* gravid with developing embryos

Figure 5. Appearance of marsupia during gravidity inspection

Pit 1 Bypass, where low visibility and submerged vegetation limited the efficacy of mussel collection, five female and six male *Anodonta* collected in March 2005 were kept in wire cages near shore and were examined for gravidity at regular intervals throughout the remainder of the 2005–06 field season (Woody and Holland-Bartels 1993).

2.4. Stream Drift Sampling for Glochidia

During the 2005–06 field season, stream drift samples were collected concurrently with mussels in all study reaches during the months of April through August 2005 and March through April 2006 by positioning two 25-cm diameter, 120-cm long plankton nets downstream of undisturbed mussel assemblages, which were immediately upstream or less than 1 river kilometer upstream of mussel collection sites. The nets and collection-bucket slots were constructed of 0.053-mm mesh to enable collection of *M. falcata* glochidia (Hastie and Young 2003), which are 0.05–0.06 mm in length (Murphy 1942) and ¼ to ½ the size of the other mussel species (D'Eliscu 1972). On each collection date, the nets were left in place for 30–60 minutes, depending on water turbidity, and the collection buckets were emptied and rinsed into one or more labeled sample jars, which represented a single drift sample (i.e., one sample/site/date). Samples were stored at 5°C (41°F) for no more than five days and were examined for glochidia by pouring 12-ml aliquots into gridded petri dishes and sorting through the contents under a dissecting microscope (Zale and Neves 1982). Cross-polarized light was used to illuminate glochidial shells and enhance detection of glochidia (Johnson 1995), which were identified to genus based on size and shell morphology. The number of glochidia in each aliquot and the total number of aliquots examined were recorded for each drift sample. Samples collected during periods of high turbidity required more aliquots than those collected during periods of low turbidity, so results are reported as number of glochidia per liter of sample examined.

2.5. Fish Sampling

In 2004, fish sampling was conducted in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse every two weeks from April through August using a 20-ft long beach seine. Fish sampling via beach seine was not feasible in the Pit 1 Bypass due to depth and stream bank configuration. Beginning in March 2005, a backpack electroshocker was used in the three Pit River reaches, and minnow traps and throw nets were used in Hat Creek and Fall River to increase the numbers and diversity of fish collected. Electroshocking was not permitted in Hat Creek or Fall River due to the presence of rough sculpin (*Cottus asperrimus*), which is a threatened species that is fully protected by the state of California. Fish were collected every two weeks during the spring, summer, and fall months and every four weeks, or when possible, during the winter months.

Upon collection, fish were held in 5-gallon buckets filled with river water and sorted by species and size. During the 2004 field season, fish under 25-mm fork length were immediately returned to the river, but all larger fish were sedated using sodium bicarbonate (i.e., Alka-Seltzer® or other effervescent antacid tablets) (Post 1979), and their fins and gills were examined for encysted glochidia. *Anodonta* have relatively large (0.2–0.4 mm) glochidia with ventral shell

hooks that enable them to attach to the fins and gills of host fish (D'Eliscu 1972; Heard 1975; Kat 1984), whereas *G. angulata* and *M. falcata* have smaller (0.05–0.1 mm), unhooked glochidia that usually encyst on the delicate gill filaments of the host (Murphy 1942; Kat 1984). To improve detection of encysted *G. angulata* and/or *M. falcata* glochidia, a maximum of 10 fish per species was sacrificed on each sampling date to examine their gills under magnification. Pit roach (*Lavinia symmetricus mitrulus*) and hardhead (*Mylopharodon conocephalus*), which are California species of special concern, were not sacrificed.

During the 2005–06 field season, collected fishes were examined for encysted glochidia without the use of anesthesia by placing them in a small glass dish that limited movement and provided magnification. Numbers and locations of encysted glochidia on each fish were recorded and no more than 20 fish per species were sacrificed on each sampling date to examine and photograph glochidia encysted on the gills. As in 2004, no Pit roach or hardhead were sacrificed.

2.6. Monitoring Juvenile Excystment in Freshwater Aquaria

On one fish sampling date per month from late March through September 2005, a subsample of the fish with visible *Anodonta* infections on the fins was transported to the laboratory and held in freshwater aquaria to monitor excystment of juvenile mussels (Neves et al. 1985; Hove et al. 2000). To minimize handling trauma, the gills of these fish were not inspected for glochidia. Fish were separated by species and held at densities ranging from 10 to 20 individuals per 10 gallon tank, each of which was supplied with filtered, aerated well water. To prevent fish from consuming juvenile mussels, mesh was used to separate the fish from the tank bottom. Water temperatures in the aquaria were checked twice a day and modified to approximate water temperatures in the Pit River. Every three days, the bottom of each tank was siphoned with a flexible hose (15 mm diameter) into a 0.053-mm mesh sieve, and the siphonate was examined for the presence of juvenile mussels using the methods described for stream drift. Dead juvenile mussels were distinguished from sloughed-off glochidia by the ciliated foot and/or appearance of shell growth, and were identified to genus based on size and shell morphology (Matteson 1955). Digital photographs were taken of representative specimens.

2.7. Monitoring River Discharge and Temperature

Daily discharge data for the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse were downloaded from the USGS website at http://nwis.waterdata.usgs.gov/ca/nwis. Discharge data for the Pit 1 Bypass were obtained from Pacific Gas and Electric Company.

In the Pit River reaches and Hat Creek, hourly water temperatures were recorded using underwater temperature loggers (OnSet Computer Corp., Boston, Mass.). In the Pit 4 Bypass, the temperature logger was located 5.5 river kilometers downstream of the primary study site; the United States Forest Service provided the 2004 data. A temperature logger was located at study sites in the Pit River downstream of Pit 1 Powerhouse, Pit 1 Bypass, and Hat Creek. In Fall River, bottom-strata water temperature data were obtained from a temperature logger located 1.0 river kilometer upstream of the study site (Todd Sloat Consulting, unpublished data).

3.0 Results

3.1. Mussel Collection and Observations of Habitat Use

3.1.1. Pit River Study Reaches

Anodonta were sparsely distributed in near-shore habitats typified by fine substrates and lower water velocities. They were more abundant near or under structures such as clumps of emergent vegetation or submerged logs that created velocity refuge, overhead cover, and/or areas of fine sediment deposition. In locations where sand and silt were abundant, these thin-shelled mussels were burrowed deeply into the substrate with just their siphons exposed (Figure 6). In more rocky or embedded substrates, they were often found lying on their sides, camouflaged by silt and/or algal growth on their shells. As a consequence, several *Anodonta* were crushed by wading anglers and sampling crews in 2004. In addition, many live *Anodonta* in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse were found stranded on shore amidst tangled tree roots days after sudden drops in river discharge (Figure 6).

Gonidea angulata were densely or sparsely distributed in a variety of substrate types and water velocities. They were found in association with *Anodonta* and/or *M. falcata*, as well as in locations seemingly unfit for other mussel species. For example, pockets of *G. angulata* were often found tightly wedged between boulders or in cracks of bedrock or diatomaceous earth (Figure 7). In locations where sand and silt were abundant, they were deeply burrowed and tightly anchored into the substrate with only their siphons exposed.

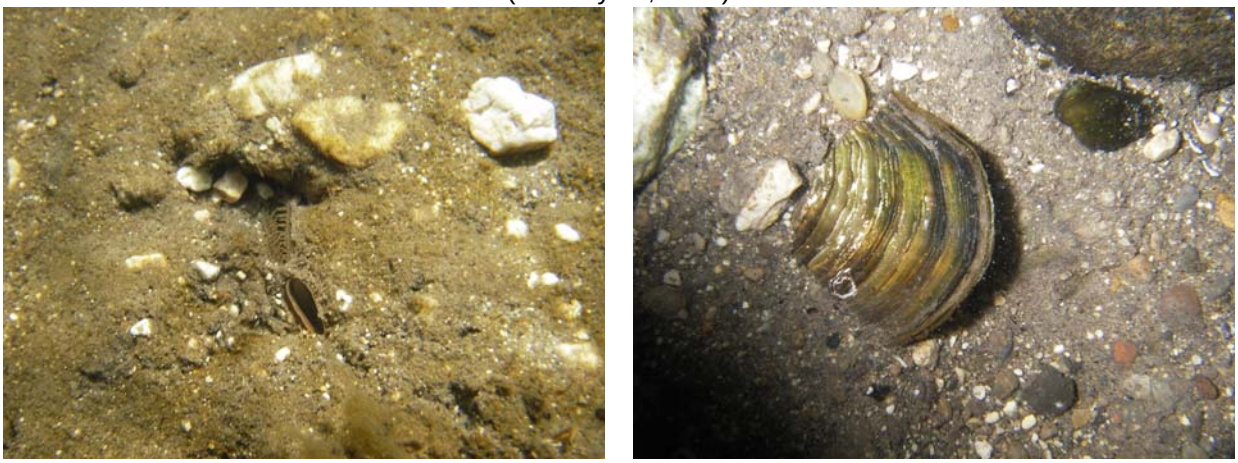
Margaritifera falcata were usually found in discrete beds, which consisted of numerous individuals tightly grouped within pockets of microhabitat typified by gravel and cobble substrates and higher water velocities (Figure 7). In some locations, they were found in waters so swift that collection was not possible. In the Pit River downstream of Pit 1 Powerhouse, they were more abundant in braided sections of the river, near the banks in swift-run habitats, or on the edges of mid-channel gravel bars. They were usually burrowed only a few centimeters into the gravel and appeared to rely on larger substrate and neighboring mussels for breaks in water velocity. The shells of *M. falcata* in this reach were more eroded than in the Pit 4 Bypass, and many appeared to have died as a consequence. The shells of many dead mussels were still anchored into the substrate, with only the holes in their shells to indicate that they were dead. Holes were also observed in the shells of two live mussels in this reach. Although the density of adult *M. falcata* within beds was often high (up to 88/m^2 at Malinda Gulch), no distinct beds were found in the Pit 1 Bypass and only a few large beds were found in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse. Like *Anodonta*, several live *M. falcata* in both reaches were found stranded on shore after sudden, significant drops in river discharge.

3.1.2. Hat Creek

In Hat Creek, 16 *Anodonta* were found in a 20-meter strip of near-shore habitat typified by sand and silt substrates (Figure 6). No *Anodonta* were found elsewhere in Hat Creek. During the summer, these mussels were found near the surface, but when water temperatures dropped below 9°C (48.2°F), they were burrowed much deeper into the substrate. No *G. angulata* were

Anodonta camouflaged by algae/silt in the Pit 4 Bypass

One of five *Anodonta* found stranded amidst tree roots in the Pit River downstream of Pit 1 Powerhouse (January 13, 2006)

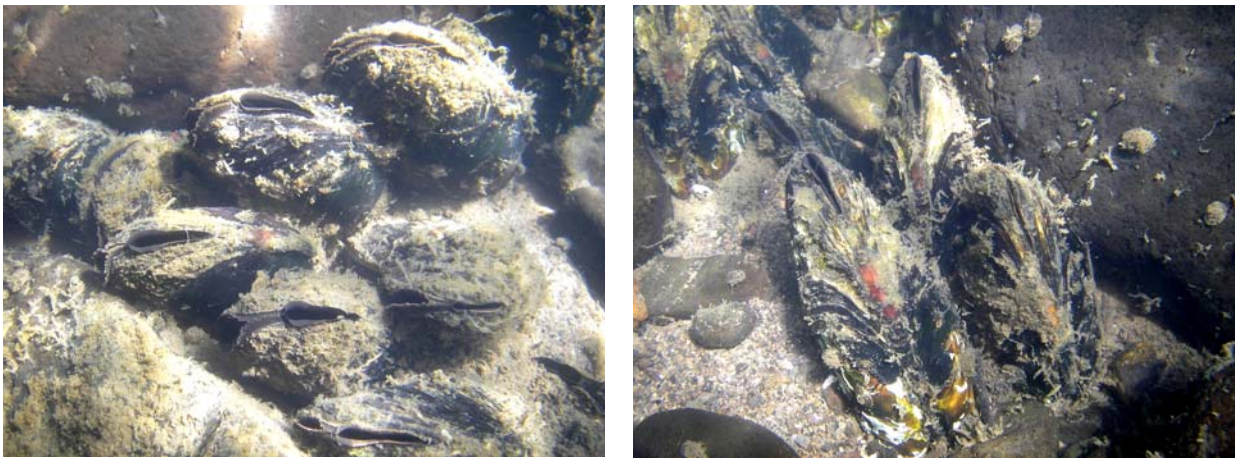


Anodonta burrowed in fine sediment in Hat Creek

Figure 6. *Anodonta* in situ in Pit River and Hat Creek

Gonidea angulata deeply burrowed with only siphons exposed in the Pit 4 Bypass

Gonidea angulata wedged between boulders or shelves of diatomaceous earth in the Pit River downstream of Pit 1 Powerhouse



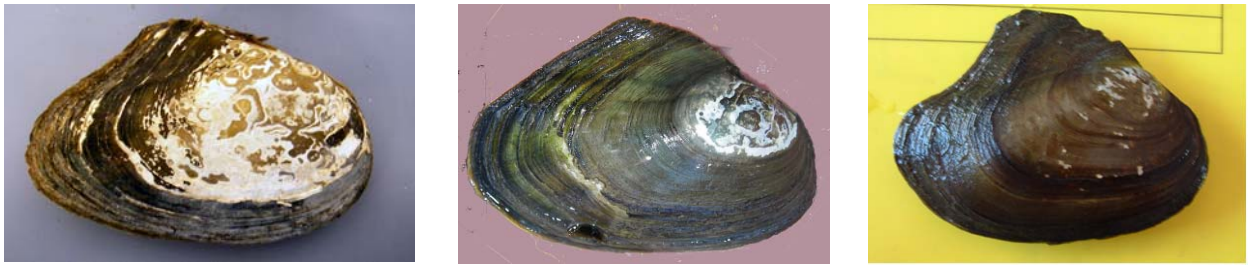
Margaritifera falcata tightly grouped in gravel between boulders in the Pit 4 Bypass

Figure 7. *Gonidea angulata* and *Margaritifera falcata* in situ in Pit River

found in Hat Creek, but *M. falcata* were found throughout Hat Creek in sand and gravel substrates. They were more abundant on the outside of river bends, where water velocities were higher, and were often tightly grouped between or downstream of large, woody debris.

3.1.3. Fall River

In Fall River, where sand and silt predominated, *Anodonta* and *G. angulata* were found at depths ranging from 1.5 to 4.5 meters, with distributions similar to those observed in the Pit River reaches. No *M. falcata* were found in Fall River or Fall River Lake. *Anodonta* were less abundant than *G. angulata* in Fall River, but were more abundant in Fall River Lake (Pit 1 Forebay). Male and female *Anodonta* collected from Fall River Lake had more inflated shell valves than those collected from Fall River and the Pit River reaches, as is typical of *A. oregonensis* (Figure 1). The shell wings of *Anodonta* from Fall River Lake, however, were more similar to *A. californiensis* and *A. nutalliana* (Figure 8). The shells of many *G. angulata* collected from Fall River and Fall River Lake were also unusual compared to the shells of those collected from the Pit River reaches. More than half of the *G. angulata* collected from the Fall River sites had twisted shell valves and/or valves stunted near the posterior (Figure 9).



Anodonta species from Pit River

Anodonta oregonensis from Big Lake (Fall River drainage)—see Figure 2

Anodonta species from Fall River Lake

Figure 8. *Anodonta* species collected in the Pit and Fall river drainages

Typical shell morphology of Pit River *Gonidea angulata*

Twisted and stunted shells of Fall River *Gonidea angulata*

Figure 9. *Gonidea angulata* shell morphologies in the Pit and Fall rivers

3.2. Mussel Sampling Data and Gravid Periods

3.2.1. Anodonta

During the 2004 field season, sex and gravidity status were determined for 217 of the 267 *Anodonta* collected in the Pit River reaches (Tables 1 and 2). Evidence of hermaphrodism was not observed in any of the gonad samples collected. Gametes were not obtained or were obscured by digestive material inadvertently obtained from the cecum in samples collected from 6 adults in the Pit 4 Bypass, 10 adults in the Pit River downstream of Pit 1 Powerhouse, and 4 adults in the Pit 1 Bypass. In addition, 16 adults collected in the Pit River downstream of Pit 1 Powerhouse and 14 collected in the Pit 1 Bypass during and after late July were not sampled to determine sex (due to time/budget constraints; see Section 2.3.2). Of the 217 mussels for which sex was determined, females and males accounted for 34% and 66% in the Pit 4 Bypass, 64% and 36% in the Pit River downstream of Pit 1 Powerhouse, and 50% and 50% in the Pit 1 Bypass. These percentages may be skewed somewhat towards the females in the Pit River

downstream of Pit 1 Powerhouse and Pit 1 Bypass, because sex was always determined for gravid mussels but not for non-gravid mussels. Even with this potential bias, females comprised 51% (n=110) of the *Anodonta* examined in all of the Pit River reaches and males (n=107) comprised 49% (Table 1).

Table 1. Number of female and male *Anodonta* collected in Pit River in 2004

Month	River Reach	Mussels Examined[1]	Gravid Females	Non-gravid Females	Males
Apr	Pit 4 Bypass	8	4	0	4
May	Pit 4 Bypass	19	7	0	12
Jun	Pit 4 Bypass	20	4	0	16
Jul	Pit 4 Bypass	18	4	1	13
Aug	Pit 4 Bypass	0	0	0	0
Sep	Pit 4 Bypass	4	0	2	2
Oct	Pit 4 Bypass	2	2	0	0
Nov	Pit 4 Bypass	0	0	0	0
	Total:	**71**	**21**	**3**	**47**
Apr	Pit River d/s Pit 1 PH	7	6	0	1
May	Pit River d/s Pit 1 PH	24	11	0	13
Jun	Pit River d/s Pit 1 PH	31	19	2	10
Jul	Pit River d/s Pit 1 PH	18	9	2	7
Aug	Pit River d/s Pit 1 PH	0	0	0	0
Sep	Pit River d/s Pit 1 PH	9	0	5	4
Oct	Pit River d/s Pit 1 PH	2	2	0	0
Nov	Pit River d/s Pit 1 PH	5	5	0	0
	Total:	**96**	**52**	**9**	**35**
Apr	Pit 1 Bypass	0	0	0	0
May	Pit 1 Bypass	5	2	0	3
Jun	Pit 1 Bypass	19	9	1	9
Jul	Pit 1 Bypass	18	8	0	10
Aug	Pit 1 Bypass	0	0	0	0
Sep	Pit 1 Bypass	2	0	0	2
Oct	Pit 1 Bypass	5	4	0	1
Nov	Pit 1 Bypass	1	1	0	0
	Total:	**50**	**24**	**1**	**25**
	Grand Total:	**217**	**97**	**13**	**107**

[1] Includes only unmarked (not previously collected) mussels for which sex was determined

Table 2. Developmental stages of *Anodonta* eggs/embryos in Pit River in 2004

Month	River Reach	Uncleaved Eggs	Developing Embryos[1]	Glochidia	Partially Empty[2]
Apr	Pit 4 Bypass	0	3	1	0
May	Pit 4 Bypass	3	2	2	0
Jun	Pit 4 Bypass	0	3	0	1
Jul	Pit 4 Bypass	0	1	1	2
Aug	Pit 4 Bypass	0	0	0	0
Sep	Pit 4 Bypass	0	0	0	0
Oct	Pit 4 Bypass	0	2	0	0
Nov	Pit 4 Bypass	0	0	0	0
	Total:	**3**	**11**	**4**	**3**
Apr	Pit River d/s Pit 1 PH	5	1	0	0
May	Pit River d/s Pit 1 PH	2	6	2	1
Jun	Pit River d/s Pit 1 PH	0	7	2	0
Jul	Pit River d/s Pit 1 PH	1	0	1	4
Aug	Pit River d/s Pit 1 PH	0	0	0	0
Sep	Pit River d/s Pit 1 PH	0	0	0	0
Oct	Pit River d/s Pit 1 PH	0	2	0	0
Nov	Pit River d/s Pit 1 PH	0	5	0	0
	Total:	**8**	**21**	**5**	**5**
Apr	Pit 1 Bypass	0	0	0	0
May	Pit 1 Bypass	1	1	0	0
Jun	Pit 1 Bypass	2	7	0	0
Jul	Pit 1 Bypass	2	3	2	1
Aug	Pit 1 Bypass	0	0	0	0
Sep	Pit 1 Bypass	0	0	0	0
Oct	Pit 1 Bypass	0	2	1	1
Nov	Pit 1 Bypass	0	1	0	0
	Total:	**5**	**14**	**3**	**2**
	Grand Total:	**16**	**46**	**12**	**10**

[1] Early stages of egg cleavage to beginning of larval shell development

[2] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia

Pit River Reaches

In 2004, 97 gravid females and 13 non-gravid females were collected in the Pit River reaches (Table 1). Gravid females were found during all months that *Anodonta* were collected except September in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse, and during August and September in the Pit 1 Bypass. No unmarked *Anodonta* were collected in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse in August, but gravidity appeared to taper off in all reaches in mid-July and resume in mid-to-late October. On most sampling dates,

some *Anodonta* had recently spawned while others incubated developing embryos or glochidia, or had partially empty marsupia. For example, at one Pit River site in May, 2 gravid females incubated uncleaved eggs, 6 incubated embryos in mid-to-late development, 2 incubated fully mature glochidia, and 1 appeared to be actively releasing glochidia, with glochidia visible in less than half of each fully distended marsupium (Table 2; Figure 10). This asynchronous reproduction was observed within all sampled populations and resulted in seemingly continuous and overlapping spawning and glochidial release periods.

During the 2005–06 field season, reproductive status was determined for all mussels collected, but sex was not determined for non-gravid mussels. Unmarked *Anodonta* were only collected in the Pit 4 and Pit 1 bypass reaches due to frequently high reservoir releases in the former reach and low mussel abundance in the accessible portions of the latter reach. Repeated sampling in the upper Pit River Canyon portion of the Pit 1 Bypass was not feasible. The data obtained from the bypass reaches and the Pit River downstream of Pit 1 Powerhouse were sufficient, however, to determine general patterns of spawning and glochidial release for Pit River *Anodonta*, which were similar to patterns observed in 2004. Gravid females were found in all Pit River reaches during all months that *Anodonta* were collected except August and September; gravidity appeared to taper off in mid-July and resume in mid-to-late October. *Anodonta* spawned and released glochidia asynchronously throughout the gravid periods (Table 3).

Hat Creek and Fall River

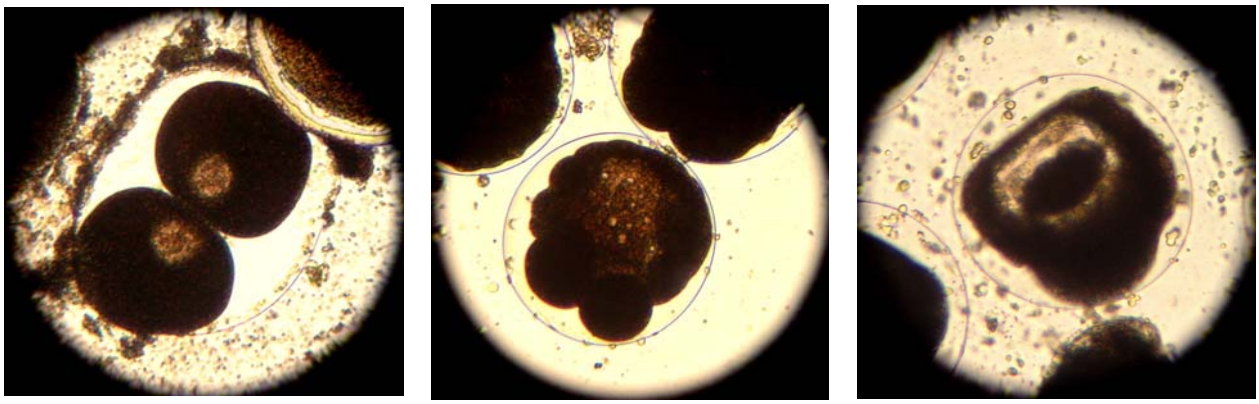
Mussel collection in these reaches was conducted in the 2005–06 field season only.

Reproductive timing in Hat Creek and Fall River was similar to that observed in the Pit River reaches. In lower Hat Creek, 18 *Anodonta* were collected in 2005–06 with one or more gravid females collected each month except August (Table 4). Upon initial collection, 7 of the 8 gravid females incubated uncleaved eggs or eggs in early development, and 1 collected in July appeared to be actively releasing glochidia.

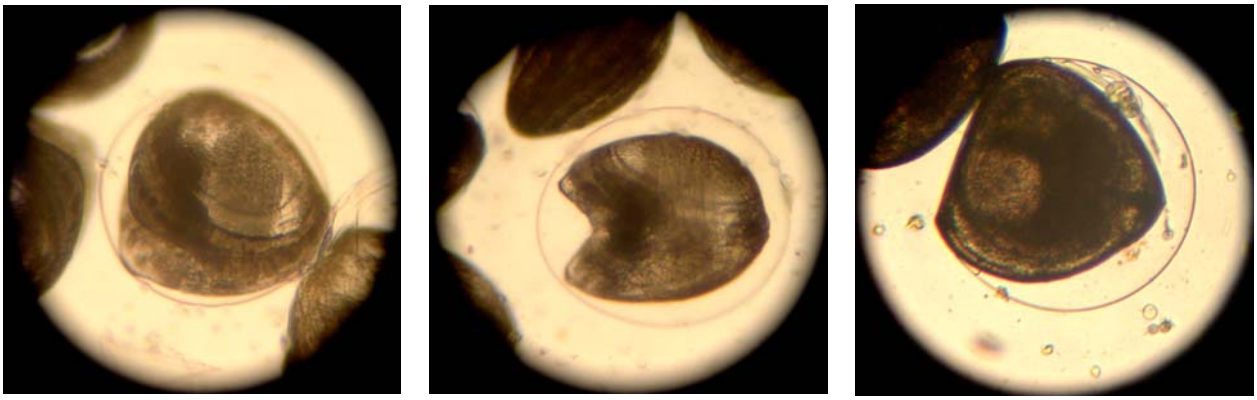
In Fall River, 89 *Anodonta* were collected in 2005–06, and gravid females were collected during all months except August and September. All gravid females collected on 21 July incubated glochidia or appeared to be actively releasing glochidia. Some also incubated or released glochidia in April and November in 2005 and January and February in 2006.

In Fall River Lake, 47 *Anodonta* were collected on 12 May, 27 July, and 10 November in 2005. In May, 14 of the 26 *Anodonta* collected were gravid: 8 incubated uncleaved eggs, 5 incubated developing embryos, and 1 incubated glochidia (Table 4). Five of the *Anodonta* with uncleaved eggs had aborted or were in the process of aborting white, cigar-shaped egg masses before they were collected. Chunks of aborted eggs were dangling from the exhalent siphons of these mussels or were lying in the sediment beside the mussels. The egg masses collected from the water column contained only unfertilized eggs. After the mussels were collected, some eggs were aborted in the mussel collection bucket and these were evidently fertilized during the 20-minute inspection period. Water collected from the bucket contained abundant mature spermatozoa. In July, none of the 8 *Anodonta* collected was gravid. In November, 7 of the 13

Anodonta were gravid: 1 incubated uncleaved eggs, 1 incubated developing embryos, 2 incubated glochidia, and 3 had nearly empty marsupia.



Development of *Anodonta* from early egg cleavage to late gastrula stage



Shell development within the vitelline membrane

Glochidia free of the vitelline membrane with well-defined, spined shell hooks that facilitate attachment to fish hosts. The shell lengths (anterior to posterior) and hinge lengths of 10 mature *Anodonta* glochidia averaged 0.246 ± 0.017 mm and 0.194 ± 0.015 mm, respectively.

Figure 10. Developing *Anodonta* embryos

Table 3. *Anodonta* collected in Pit River in 2005–06

Month	River Reach	Mussels Examined[1]	Gravid Females	Stage of Embryonic Development: Uncleaved Eggs	Developing Embryos[2]	Glochidia	Partially Empty[3]
Feb 2005	Pit 4 Bypass	----	-----	-----	-----	-----	-----
Mar	Pit 4 Bypass	1	1	1	0	0	0
Apr	Pit 4 Bypass	6	4	1	2	1	0
May	Pit 4 Bypass	----	-----	-----	-----	-----	-----
Jun	Pit 4 Bypass	3	2	0	2	0	0
Jul	Pit 4 Bypass	1	1	1	0	0	0
Aug	Pit 4 Bypass	5	0	0	0	0	0
Sep	Pit 4 Bypass	----	-----	-----	-----	-----	-----
Oct	Pit 4 Bypass	11	1	1	0	0	0
	Total:	**27**	**9**	**4**	**4**	**1**	**0**
Feb 2005	Pit River d/s Pit 1 PH	6	3	1	2	0	0
Mar	Pit River d/s Pit 1 PH	8	3	0	2	1	0
Apr	Pit River d/s Pit 1 PH	5	3	0	1	0	2
May	Pit River d/s Pit 1 PH	----	-----	-----	-----	-----	-----
Jun	Pit River d/s Pit 1 PH	----	-----	-----	-----	-----	-----
Jul	Pit River d/s Pit 1 PH	6	5	0	2	2	1
Aug	Pit River d/s Pit 1 PH	11	0	0	0	0	0
Sep	Pit River d/s Pit 1 PH	20	0	0	0	0	0
Oct	Pit River d/s Pit 1 PH	10	1	1	0	0	0
Nov	Pit River d/s Pit 1 PH	13	4	1	2	1	0
Dec	Pit River d/s Pit 1 PH	7	1	0	1	0	0
Jan 2006	Pit River d/s Pit 1 PH	7	2	1	0	0	1
Feb	Pit River d/s Pit 1 PH	6	3	0	1	1	1
Mar	Pit River d/s Pit 1 PH	-----	-----	-----	-----	-----	-----
Apr	Pit River d/s Pit 1 PH	-----	-----	-----	-----	-----	-----
	Total:	**99**	**25**	**4**	**11**	**5**	**5**
Feb 2005	Pit 1 Bypass	5	2	0	2	0	0
Mar	Pit 1 Bypass	8	5	1	2	2	0
Apr	Pit 1 Bypass	7	2	1	0	1	0
May	Pit 1 Bypass	----	-----	-----	-----	-----	-----
Jun	Pit 1 Bypass	----	-----	-----	-----	-----	-----
Jul	Pit 1 Bypass	----	-----	-----	-----	-----	-----
Aug	Pit 1 Bypass	----	-----	-----	-----	-----	-----
Sep	Pit 1 Bypass	18	0	0	0	0	0
Oct	Pit 1 Bypass	9	1	1	0	0	0
	Total:	**42**	**8**	**3**	**2**	**3**	**0**
	Grand Total:	**168**	**42**	**11**	**17**	**9**	**5**

[1] Number does not include marked mussels

[2] Early stages of egg cleavage to beginning of larval shell development

[3] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia

Table 4. *Anodonta* collected in Hat Creek and Fall River in 2005–06

Month	River Reach	Mussels Examined[1]	Gravid Females	Stage of Embryonic Development: Uncleaved Eggs	Developing Embryos[2]	Glochidia	Partially Empty[3]
Feb 2005	Hat Creek	-----	-----	-----	-----	-----	-----
Mar	Hat Creek	-----	-----	-----	-----	-----	-----
Apr	Hat Creek	2	2	2	0	0	0
May	Hat Creek	-----	-----	-----	-----	-----	-----
Jun	Hat Creek	-----	-----	-----	-----	-----	-----
Jul	Hat Creek	6	3	2	0	0	1
Aug	Hat Creek	4	0	0	0	0	0
Sep	Hat Creek	-----	-----	-----	-----	-----	-----
Oct	Hat Creek	3	1	1	0	0	0
Nov	Hat Creek	2	1	0	1	0	0
Dec	Hat Creek	-----	-----	-----	-----	-----	-----
Jan 2006	Hat Creek	-----	-----	-----	-----	-----	-----
Feb	Hat Creek	-----	-----	-----	-----	-----	-----
Mar	Hat Creek	1	1	0	1	0	0
Apr	Hat Creek	-----	-----	-----	-----	-----	-----
	Total:	**18**	**8**	**5**	**2**	**0**	**1**
Feb 2005	Fall River	-----	-----	-----	-----	-----	-----
Mar	Fall River	8	2	2	0	0	0
Apr	Fall River	17	8	2	2	3	1
May	Fall River	-----	-----	-----	-----	-----	-----
Jun	Fall River	3	1	0	1	0	0
Jul	Fall River	10	4	0	0	1	3
Aug	Fall River	6	0	0	0	0	0
Sep	Fall River	10	0	0	0	0	0
Oct	Fall River	8	1	0	1	0	0
Nov	Fall River	6	2	0	1	1	0
Dec	Fall River	-----	-----	-----	-----	-----	-----
Jan 2006	Fall River	5	2	0	0	0	2
Feb	Fall River	13	7	1	1	2	3
Mar	Fall River	-----	-----	-----	-----	-----	-----
Apr	Fall River	3	2	0	2	0	0
	Total:	**89**	**29**	**5**	**8**	**7**	**9**
Feb 2005	Fall River Lake	----	-----	-----	-----	-----	-----
May	Fall River Lake	26	14	8	5	1	0
Jul	Fall River Lake	8	0	0	0	0	0
Nov	Fall River Lake	13	7	1	1	2	3
	Total:	**51**	**21**	**9**	**6**	**3**	**3**
	Grand Total:	**154**	**58**	**19**	**16**	**10**	**13**

[1] Number does not include marked (i.e., previously examined and/or sampled) mussels

[2] Early stages of egg cleavage to beginning of larval shell development

[3] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia

3.2.2. Marked Anodonta

In 2004, 13 female *Anodonta* were reexamined in the Pit 4 Bypass, 25 were reexamined in the Pit River downstream of Pit 1 Powerhouse, and 8 were reexamined in the Pit 1 Bypass. The numbers reexamined on each date in each river reach were not standardized, because marked *Anodonta* were often difficult to relocate, even when their location was flagged. Marked females were gravid on all sampling dates except during August and September in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse, and during late August and September in the Pit 1 Bypass (Figure 11). One marked female was gravid and appeared to be actively releasing glochidia on 10 August in the Pit 1 Bypass. This female had dark-orange glochidia in less than half of each marsupium. Three marked females in the Pit River downstream of Pit 1 Powerhouse were sampled (i.e., fluid was collected from their marsupia) 27 days after their initial capture on 20 April. Two of these incubated eggs in the early stages of cell cleavage on 20 April and mature glochidia on 17 May, and one incubated uncleaved eggs on both dates.

In 2005–06, reexamined females totaled 13 in the Pit 4 Bypass, 16 in the Pit River downstream of Pit 1 Powerhouse, 5 in the Pit 1 Bypass, 8 in Hat Creek, and 8 in Fall River. Marked females were gravid during all months except August and September (Figures 12 and 13). Most examined in July incubated glochidia or had partially empty marsupia, and most examined in mid-to-late October incubated uncleaved eggs or embryos in early development. After the initial capture date, developmental stages were usually estimated by noting the color of developing eggs/embryos, as described in Section 2.3.2. A few females were sampled on subsequent dates, however, to confirm the estimated stages of development. All of the marked females appeared to produce multiple, consecutive broods throughout the periods of gravidity. During spring and summer, the interval between the appearance of uncleaved eggs or eggs in early development in the swollen marsupia of marked mussels and the appearance of early or mature glochidia ranged from 20 to 40 days, but the time required for development could not be determined, because the date of fertilization was not known. During the colder winter months, *Anodonta* continued to incubate eggs, embryos, or glochidia, but embryonic development may have been slowed, because some mussels appeared to be brooding eggs or embryos in mid-to-late development for over two months. The time required for development in winter could not be accurately assessed by visual observation and monthly examinations, but one female was fertilized sometime between mid- and late January, and two females released glochidia and spawned again during the months of December and January. In mid-March 2006, one of the caged females appeared emaciated and inactive, but still carried uncleaved eggs.

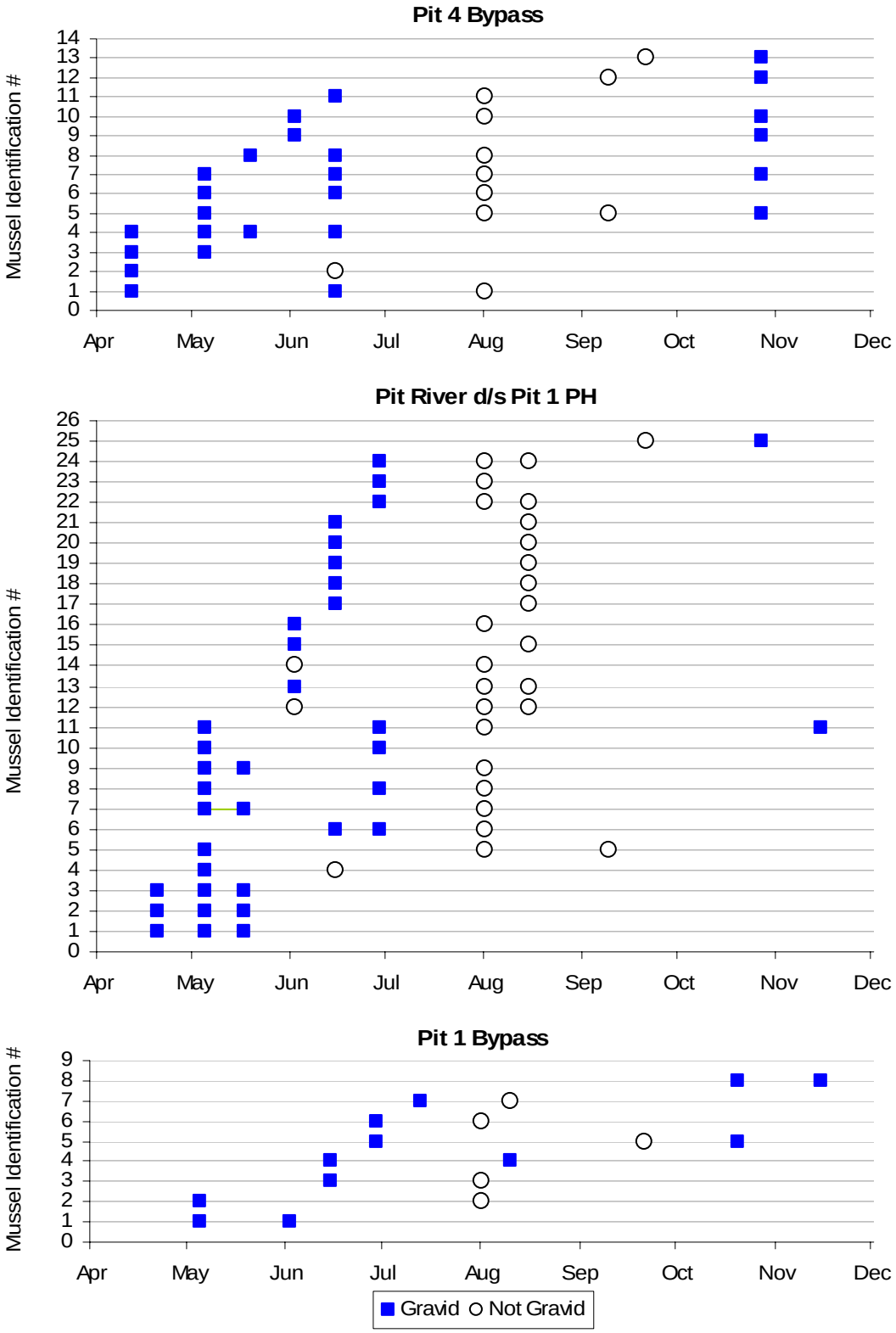


Figure 11. Gravidity status of marked *Anodonta* females in the Pit River in 2004

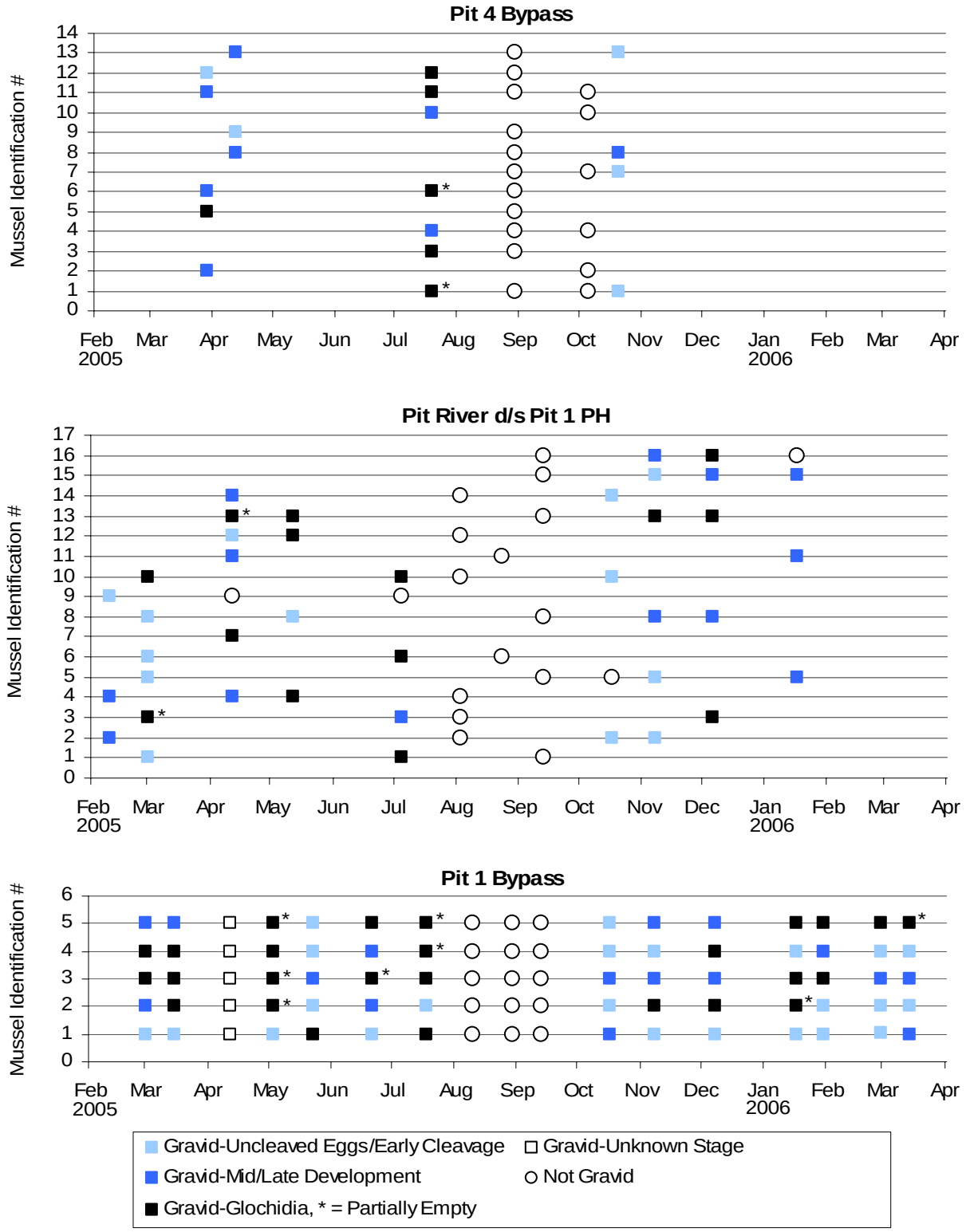


Figure 12. Gravidity status of marked *Anodonta* females and developmental stages of embryos in Pit River in 2005–06

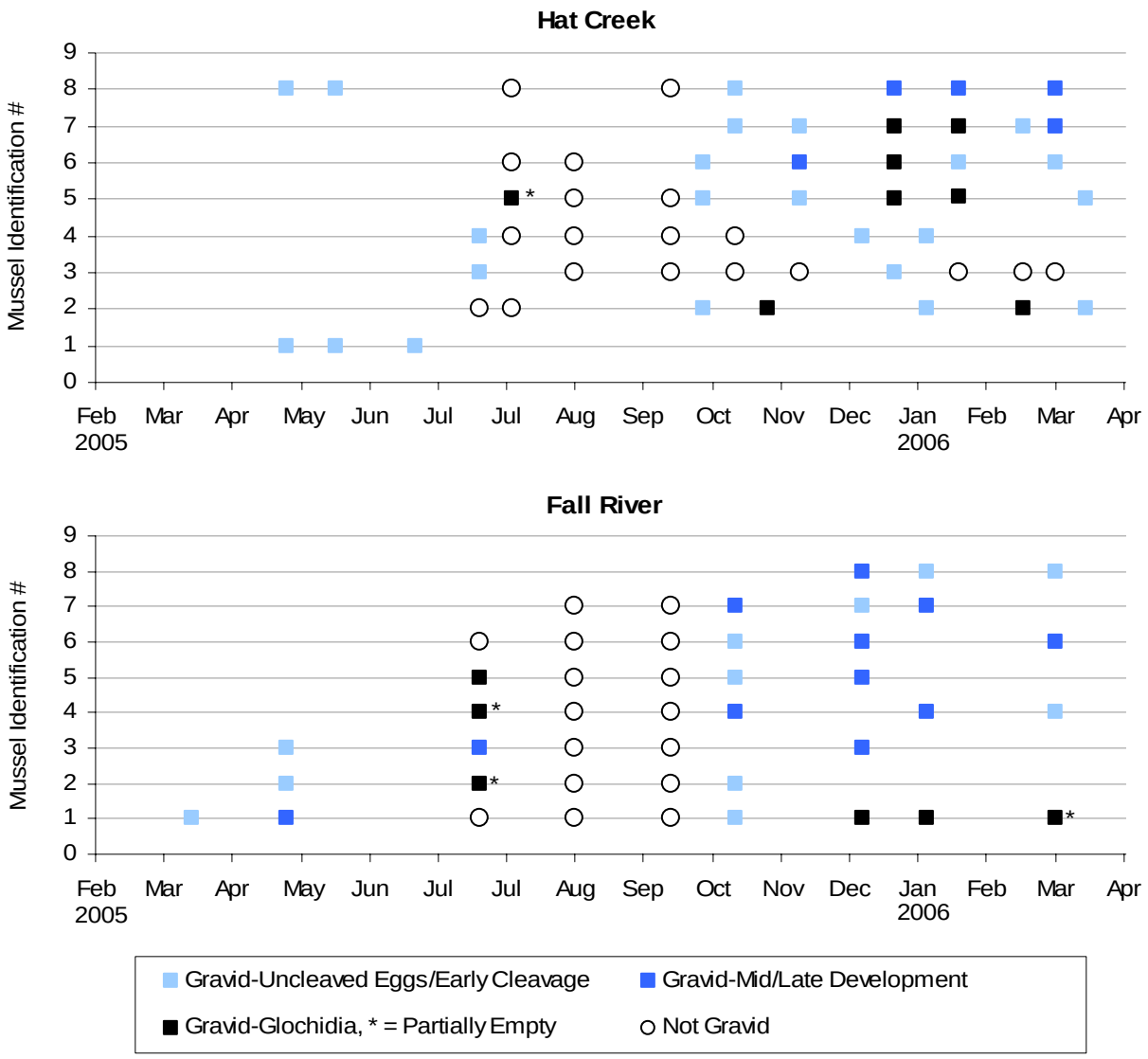


Figure 13. Gravidity status of marked *Anodonta* females and developmental stages of embryos in Hat Creek and Fall River in 2005–06

3.2.3. Gonidea angulata

In 2004, sex and gravidity status were determined for 188 of the 240 *G. angulata* collected in the Pit River reaches (Tables 5 and 6). (Sex and gravidity were not determined for all 240 *G. angulata* because gonadal fluid was not sampled after July 2004 and some of the fluid samples were inconclusive). Evidence of hermaphrodism was not observed in any of the gonad samples collected. Sex was not determined for 2 adults in the Pit 4 Bypass and 3 adults in the Pit River downstream of Pit 1 Powerhouse, because no gametes were obtained or observed in the gonad samples. In addition, 29 adults in the Pit 4 Bypass and 18 in the Pit River downstream of Pit 1 Powerhouse collected during and after late July were not sampled to determine sex (see Section

2.3.2). In the Pit 4 Bypass, 59% of the collected mussels for which sex was determined were female and 41% were male. In the Pit River downstream of Pit 1 Powerhouse, 60% were female and 40% were male. Only 17 *G. angulata* were collected in the Pit 1 Bypass; 47% were female and 53% were male. In total, females comprised 59% (n=110) and males comprised 41% (n=78) of *G. angulata* collected in the Pit River reaches (Table 5).

Of the 110 females collected in the three reaches in 2004, 80 were gravid and 30 were not gravid (Table 5). Gravid females were found in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse from the first sampling date in early April to mid-July. No gravid females were found during or after late July. In the Pit 1 Bypass, gravid females were found on both collection dates in late May and early June. High flow conditions prevented mussel collection in the Pit 1 Bypass in April, and no unmarked *G. angulata* were found in this reach after early June. As observed in *Anodonta*, the timing of spawning and glochidial release in *G. angulata* was not synchronized among individuals. Gravid females incubated embryos at varying stages (i.e., from mid- to late development) throughout the gravid period and glochidia in May, June, and July (Table 6; Figure 14). Only one *G. angulata* with partially empty marsupia was collected in April. The marsupia of this individual were mostly deflated, but small, beige-colored patches in one of the four marsupia contained some active, mature glochidia. During the gravid period, 4 or 5 females carrying embryos in mid-to-late development in each of the Pit River reaches were reexamined two weeks after they were first sampled and most were still gravid. Those that were still gravid appeared to have aborted and re-spawned after they were sampled, because they carried uncleaved eggs or eggs in early development.

In 2005, spawning and glochidial release periods for *G. angulata* were similar to those observed in 2004. Gravid *G. angulata* were found in the Pit 4 Bypass from late March to late June (no mussels were collected in this reach during the first 3 weeks in July), and in the Pit River downstream of Pit 1 Powerhouse from late March to mid-July (Table 7). Spawning and glochidial release occurred throughout the gravid periods in each reach, and these events were not synchronized among individuals. In the Pit 1 Bypass, 1 of the 20 *G. angulata* collected in late March was gravid with uncleaved eggs, and 8 of the 20 collected in late April were gravid with developing embryos or glochidia (Figure 14). High spring flows prevented collection of unmarked mussels in all of the Pit River reaches during March and April in 2006.

In Fall River, 360 *G. angulata* were collected during the 2005–06 field season. Gravid females were found from late April to mid-July in 2005 and beginning in late April in 2006 (Table 8). No gravid females were found in mid-March or early April in 2005 or 2006. In Fall River Lake, 50 *G. angulata* were collected on 12 May, 27 July, and 10 November in 2005. Gravid females were only found in May, when 1 incubated uncleaved eggs, 4 incubated embryos in mid-to-late development, and 1 had partially empty marsupia. No *G. angulata* were observed aborting their marsupial contents prior to or after collection, as was observed for *Anodonta* in this location.

Table 5. Number of male and female *Gonidea angulata* collected in Pit River in 2004

Month	River Reach	Mussels Examined[1]	Gravid Females	Non-gravid Females	Males
Apr	Pit 4 Bypass	9	6	1	2
May	Pit 4 Bypass	20	11	2	7
Jun	Pit 4 Bypass	20	12	0	8
Jul	Pit 4 Bypass	20	6	2	12
Aug	Pit 4 Bypass	0	0	0	0
Sep	Pit 4 Bypass	7	0	5	2
Oct	Pit 4 Bypass	0	0	0	0
Nov	Pit 4 Bypass	0	0	0	0
	Total:	**76**	**35**	**10**	**31**
Apr	Pit River d/s Pit 1 PH	9	3	2	4
May	Pit River d/s Pit 1 PH	36	22	2	12
Jun	Pit River d/s Pit 1 PH	20	8	3	9
Jul	Pit River d/s Pit 1 PH	20	6	4	10
Aug	Pit River d/s Pit 1 PH	0	0	0	0
Sep	Pit River d/s Pit 1 PH	10	0	7	3
Oct	Pit River d/s Pit 1 PH	0	0	0	0
Nov	Pit River d/s Pit 1 PH	0	0	0	0
	Total:	**95**	**39**	**18**	**38**
Apr	Pit 1 Bypass	0	0	0	0
May	Pit 1 Bypass	12	4	2	6
Jun	Pit 1 Bypass	5	2	0	3
Jul	Pit 1 Bypass	0	0	0	0
Aug	Pit 1 Bypass	0	0	0	0
Sep	Pit 1 Bypass	0	0	0	0
Oct	Pit 1 Bypass	0	0	0	0
Nov	Pit 1 Bypass	0	0	0	0
	Total:	**17**	**6**	**2**	**9**
	Grand Total:	**188**	**80**	**30**	**78**

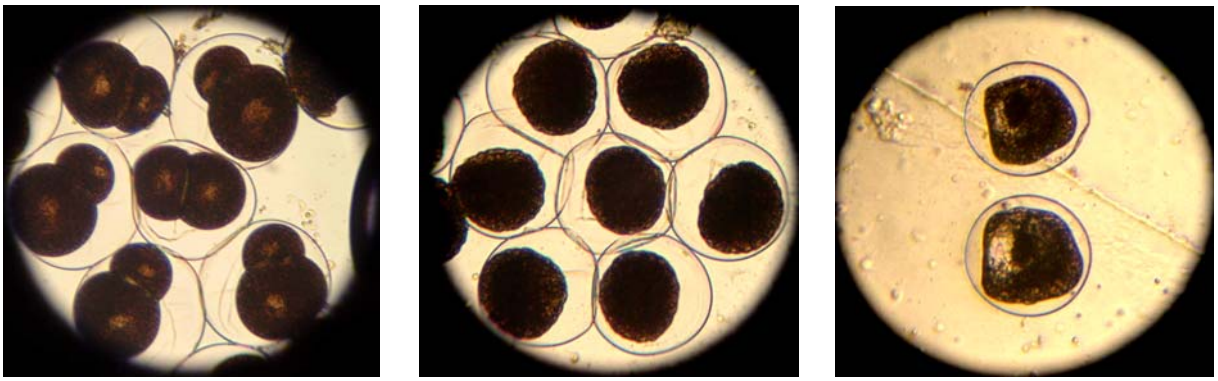
[1] Includes only unmarked (not previously collected) mussels for which sex was determined

Table 6. Developmental stages of *Gonidea angulata* eggs/embryos in Pit River in 2004

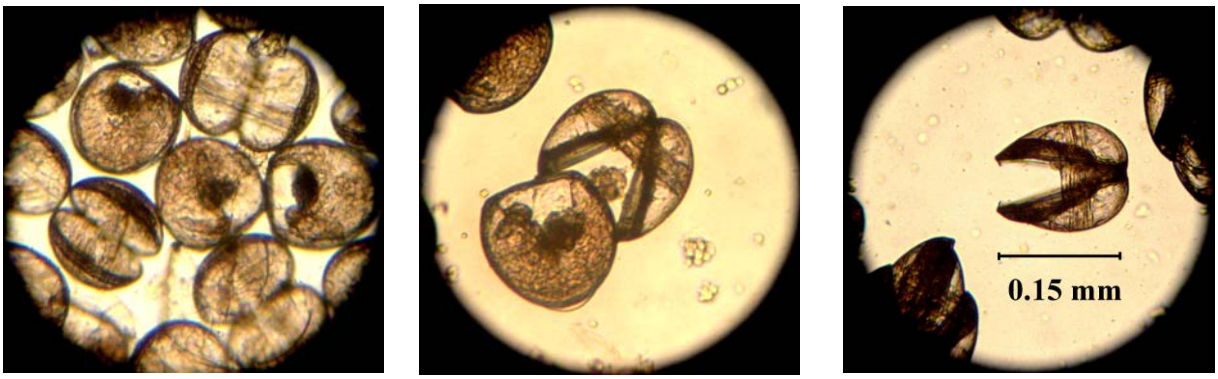
Month	River Reach	Uncleaved Eggs	Developing Embryos[1]	Glochidia	Partially Empty[2]
Apr	Pit 4 Bypass	2	4	0	0
May	Pit 4 Bypass	0	11	0	0
Jun	Pit 4 Bypass	0	12	0	0
Jul	Pit 4 Bypass	0	5	1	0
Aug	Pit 4 Bypass	0	0	0	0
Sep	Pit 4 Bypass	0	0	0	0
Oct	Pit 4 Bypass	0	0	0	0
Nov	Pit 4 Bypass	0	0	0	0
	Total:	**2**	**32**	**1**	**0**
Apr	Pit River d/s Pit 1 PH	0	2	0	0
May	Pit River d/s Pit 1 PH	2	16	4	0
Jun	Pit River d/s Pit 1 PH	0	6	2	0
Jul	Pit River d/s Pit 1 PH	0	4	1	1
Aug	Pit River d/s Pit 1 PH	0	0	0	0
Sep	Pit River d/s Pit 1 PH	0	0	0	0
Oct	Pit River d/s Pit 1 PH	0	0	0	0
Nov	Pit River d/s Pit 1 PH	0	0	0	0
	Total:	**2**	**28**	**7**	**1**
Apr	Pit 1 Bypass	0	0	0	0
May	Pit 1 Bypass	0	4	0	0
Jun	Pit 1 Bypass	0	2	0	0
Jul	Pit 1 Bypass	0	0	0	0
Aug	Pit 1 Bypass	0	0	0	0
Sep	Pit 1 Bypass	0	0	0	0
Oct	Pit 1 Bypass	0	0	0	0
Nov	Pit 1 Bypass	0	0	0	0
	Total:	0	**6**	**0**	**0**
	Grand Total:	**4**	**66**	**8**	**1**

[1] Early stages of egg cleavage to beginning of larval shell development

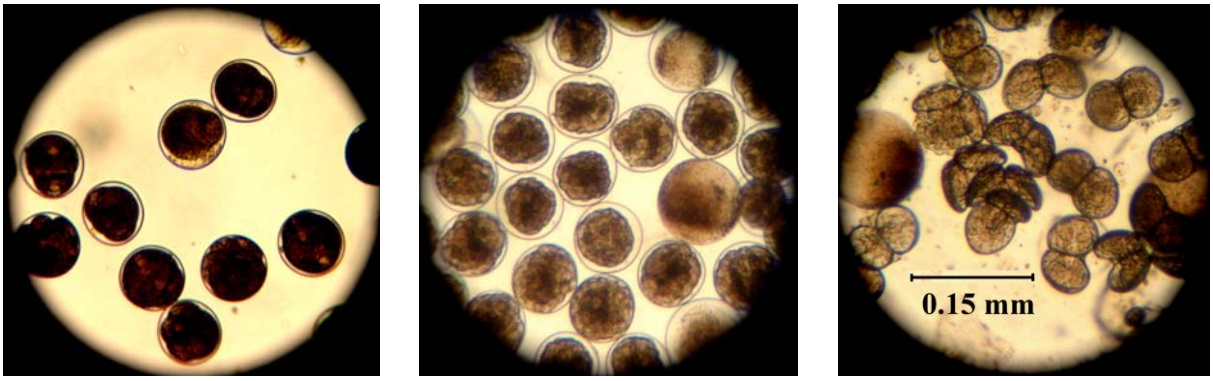
[2] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia



Development of *Gonidea angulata* from early egg cleavage to gastrula stage



Early and mature glochidia of *Gonidea angulata*.
The shell lengths (anterior to posterior) and hinge lengths of 10 mature *G. angulata* glochidia averaged 0.153 ± 0.007 mm and 0.114 ± 0.013 mm, respectively.



Development of *Margaritifera falcata* from early egg cleavage to early glochidia.
The shell lengths (anterior to posterior) and hinge lengths of 10 *M. falcata* glochidia averaged 0.057 ± 0.013 mm and 0.041 ± 0.014 mm, respectively.

Figure 14. Developing *Gonidea angulata* and *Margaritifera falcata* embryos

Table 7. *Gonidea angulata* collected in Pit River in 2005–06

Month	River Reach	Mussels Examined[1]	Gravid Females	Stage of Embryonic Development: Uncleaved Eggs	Developing Embryos[2]	Glochidia	Partially Empty[3]
Feb 2005	Pit 4 Bypass	-----	-----	-----	-----	-----	-----
Mar	Pit 4 Bypass	10	2	0	2	0	0
Apr	Pit 4 Bypass	10	2	1	1	0	0
May	Pit 4 Bypass	-----	-----	-----	-----	-----	-----
Jun	Pit 4 Bypass	20	8	1	5	2	0
Jul	Pit 4 Bypass	40	0	0	0	0	0
Aug	Pit 4 Bypass	20	0	0	0	0	0
Sep	Pit 4 Bypass	-----	-----	-----	-----	-----	-----
Oct	Pit 4 Bypass	20	0	0	0	0	0
Nov	Pit 4 Bypass	-----	-----	-----	-----	-----	-----
Dec	Pit 4 Bypass	-----	-----	-----	-----	-----	-----
	Total:	**120**	**12**	**2**	**8**	**2**	**0**
Feb 2005	Pit River d/s Pit 1 PH	40	0	0	0	0	0
Mar	Pit River d/s Pit 1 PH	40	2	2	0	0	0
Apr	Pit River d/s Pit 1 PH	20	9	2	7	0	0
May	Pit River d/s Pit 1 PH	10	5	0	3	1	1
Jun	Pit River d/s Pit 1 PH	20	10	1	4	4	1
Jul	Pit River d/s Pit 1 PH	20	3	0	1	1	1
Aug	Pit River d/s Pit 1 PH	40	0	0	0	0	0
Sep	Pit River d/s Pit 1 PH	20	0	0	0	0	0
Oct	Pit River d/s Pit 1 PH	10	0	0	0	0	0
Nov	Pit River d/s Pit 1 PH	10	0	0	0	0	0
Dec	Pit River d/s Pit 1 PH	10	0	0	0	0	0
Jan 2006	Pit River d/s Pit 1 PH	-----	-----	-----	-----	-----	-----
Feb	Pit River d/s Pit 1 PH	10	0	0	0	0	0
Mar	Pit River d/s Pit 1 PH	-----	-----	-----	-----	-----	-----
Apr	Pit River d/s Pit 1 PH	-----	-----	-----	-----	-----	-----
	Total:	**240**	**29**	**5**	**15**	**6**	**3**
Feb 2005	Pit 1 Bypass	-----	-----	-----	-----	-----	-----
Mar	Pit 1 Bypass	20	1	1	0	0	0
Apr	Pit 1 Bypass	20	8	0	5	3	0
	Total:	**40**	**9**	**1**	**5**	**3**	**0**
	Grand Total:	**400**	**50**	**8**	**28**	**11**	**3**

[1] Number does not include marked mussels

[2] Early stages of egg cleavage to beginning of larval shell development

[3] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia

Table 8. *Gonidea angulata* collected in Fall River in 2005–06

Month	River Reach	Mussels Examined[1]	Gravid Females	Stage of Embryonic Development			
				Uncleaved Eggs	Developing Embryos[2]	Glochidia	Partially Empty[3]
Feb 2005	Fall River	-----	-----	-----	-----	-----	-----
Mar	Fall River	40	0	0	0	0	0
Apr	Fall River	40	5	1	4	0	0
May	Fall River	20	7	1	4	0	2
Jun	Fall River	20	11	2	4	4	1
Jul	Fall River	20	1	0	0	0	1
Aug	Fall River	20	0	0	0	0	0
Sep	Fall River	20	0	0	0	0	0
Oct	Fall River	20	0	0	0	0	0
Nov	Fall River	20	0	0	0	0	0
Dec	Fall River	-----	-----	-----	-----	-----	-----
Jan 2006	Fall River	20	0	0	0	0	0
Feb	Fall River	20	0	0	0	0	0
Mar	Fall River	60	0	0	0	0	0
Apr	Fall River	60	3	3	0	0	0
	Total:	**380**	**27**	**7**	**12**	**4**	**4**
Feb 2005	Fall River Lake	-----	-----	-----	-----	-----	-----
May	Fall River Lake	20	7	1	4	0	2
Jul	Fall River Lake	20	0	0	0	0	0
Nov	Fall River Lake	20	0	0	0	0	0
	Total:	**60**	**7**	**1**	**4**	**0**	**2**
	Grand Total:	**440**	**34**	**8**	**16**	**4**	**6**

[1] Number does not include marked (i.e., previously examined and/or sampled) mussels

[2] Early stages of egg cleavage to beginning of larval shell development

[3] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia

3.2.4. Margaritifera falcata

During the 2004 field season, sex and gravidity status were determined for 114 of the 173 *M. falcata* collected in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse (Tables 9 and 10). Evidence of hermaphrodism was not observed in any of the gonad samples collected. Sex was not determined for 5 adults collected in the Pit 4 Bypass and 4 adults collected in the Pit River downstream of Pit 1 Powerhouse, because gametes were not obtained or observed in the gonad samples. In addition, 33 adults collected in the Pit 4 Bypass and 17 adults collected in the Pit River downstream of Pit 1 Powerhouse during and after late July were not sampled to determine sex (see Section 2.3.2). In the Pit 4 Bypass, 47% of the collected mussels for which sex was determined were female and 53% were male. In the Pit River downstream of Pit 1 Powerhouse, 33% were female and 67% were male. In total, males comprised 58% (n=66) and females 42% (n=48) of *M. falcata* for which sex was determined in the Pit River reaches (Table 9).

Table 9. *Margaritifera falcata* collected in Pit River in 2004

Month	River Reach	Mussels Examined[1]	Gravid Females	Non-gravid Females	Males
Apr	Pit 4 Bypass	10	3	2	5
May	Pit 4 Bypass	17	0	5	12
Jun	Pit 4 Bypass	18	3	10	5
Jul	Pit 4 Bypass	20	0	8	12
Aug	Pit 4 Bypass	0	0	0	0
Sep	Pit 4 Bypass	7	0	3	4
Oct	Pit 4 Bypass	0	0	0	0
Nov	Pit 4 Bypass	0	0	0	0
	Total:	**72**	**6**	**28**	**38**
Apr	Pit River d/s Pit 1 PH	0	0	0	0
May	Pit River d/s Pit 1 PH	19	0	8	11
Jun	Pit River d/s Pit 1 PH	12	0	3	9
Jul	Pit River d/s Pit 1 PH	7	0	3	4
Aug	Pit River d/s Pit 1 PH	0	0	0	0
Sep	Pit River d/s Pit 1 PH	4	0	0	4
Oct	Pit River d/s Pit 1 PH	0	0	0	0
Nov	Pit River d/s Pit 1 PH	0	0	0	0
	Total:	**42**	**0**	**14**	**28**
	Grand Total:	**114**	**6**	**42**	**66**

[1] Includes only unmarked (not previously collected) mussels for which sex was determined

Table 10. Developmental stages of *Margaritifera falcata* eggs/embryos in 2004

Month	River Reach	Uncleaved Eggs	Developing Embryos[1]	Glochidia	Partially Empty[2]	Unknown Stage
Apr	Pit 4 Bypass	3	0	0	0	0
May	Pit 4 Bypass	0	0	0	0	0
Jun	Pit 4 Bypass	0	1	0	0	2
Jul	Pit 4 Bypass	0	0	0	0	0
Aug	Pit 4 Bypass	0	0	0	0	0
Sep	Pit 4 Bypass	0	0	0	0	0
Oct	Pit 4 Bypass	0	0	0	0	0
Nov	Pit 4 Bypass	0	0	0	0	0
	Total:	**3**	**1**	**0**	**0**	**2**
Apr	Pit River d/s Pit 1 PH	0	0	0	0	0
May	Pit River d/s Pit 1 PH	0	0	0	0	0
Jun	Pit River d/s Pit 1 PH	0	0	0	0	0
Jul	Pit River d/s Pit 1 PH	0	0	0	0	0
Aug	Pit River d/s Pit 1 PH	0	0	0	0	0
Sep	Pit River d/s Pit 1 PH	0	0	0	0	0
Oct	Pit River d/s Pit 1 PH	0	0	0	0	0
Nov	Pit River d/s Pit 1 PH	0	0	0	0	0
	Total:	**0**	**0**	**0**	**0**	**0**
	Grand Total:	**3**	**1**	**0**	**0**	**2**

[1] Early stages of egg cleavage to beginning of larval shell development

[2] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia

Table 11. *Margaritifera falcata* collected in Pit River in 2005–06

				Stage of Embryonic Development			
Month	**River Reach**	**Mussels Examined[1]**	**Gravid Females**	**Uncleaved Eggs**	**Developing Embryos[2]**	**Glochidia**	**Partially Empty[3]**
Feb 2005	Pit 4 Bypass	----	----	----	----	----	----
Mar	Pit 4 Bypass	80	0	0	0	0	0
Apr	Pit 4 Bypass	23	6	6	0	0	0
May	Pit 4 Bypass	----	----	----	----	----	----
Jun	Pit 4 Bypass	60	0	0	0	0	0
Jul	Pit 4 Bypass	40	0	0	0	0	0
Aug	Pit 4 Bypass	40	0	0	0	0	0
Sep	Pit 4 Bypass	----	----	----	----	----	----
Oct	Pit 4 Bypass	40	0	0	0	0	0
Nov	Pit 4 Bypass	----	----	----	----	----	----
Dec	Pit 4 Bypass	----	----	----	----	----	----
	Total:	**283**	**6**	**6**	**0**	**0**	**0**
Feb 2005	Pit River d/s Pit 1 PH	60	0	0	0	0	0
Mar	Pit River d/s Pit 1 PH	40	0	0	0	0	0
Apr	Pit River d/s Pit 1 PH	40	0	0	0	0	0
May	Pit River d/s Pit 1 PH	40	0	0	0	0	0
Jun	Pit River d/s Pit 1 PH	40	3	2	1	0	0
Jul	Pit River d/s Pit 1 PH	22	1	0	0	1	0
Aug	Pit River d/s Pit 1 PH	40	0	0	0	0	0
Sep	Pit River d/s Pit 1 PH	----	----	----	----	----	----
Oct	Pit River d/s Pit 1 PH	----	----	----	----	----	----
Nov	Pit River d/s Pit 1 PH	20	0	0	0	0	0
Dec	Pit River d/s Pit 1 PH	----	----	----	----	----	----
	Total:	**302**	**4**	**2**	**1**	**1**	**0**
	Grand Total:	**585**	**10**	**8**	**1**	**1**	**0**

[1] Number does not include marked mussels

[2] Early stages of egg cleavage to beginning of larval shell development

[3] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia

Only 6 gravid *M. falcata* were found in the Pit 4 Bypass in 2004; 3 in early April and 3 in mid-June (Table 9). Gravidity was barely noticeably, because the marsupia were only slightly inflated and did not differ in coloration from the marsupia of non-gravid mussels. All of the gravid mussels sampled in early April incubated uncleaved eggs (Table 10). These mussels were reexamined in early May and none was gravid. One gravid *M. falcata* sampled in mid-June incubated embryos at the blastula stage of development (Figure 14). The other gravid females collected in mid-June were not sampled, because their marsupia did not appear to be sufficiently inflated to allow fluid collection. No gravid *M. falcata* were found in the Pit 4 Bypass during early or late May or early or late June. In the Pit River downstream of Pit 1 Powerhouse, no *M. falcata* were found during April and none of the 63 collected from May through September was gravid.

In 2005, gravid *M. falcata* were collected in the Pit 4 Bypass throughout April and in the Pit River downstream of Pit 1 Powerhouse from early June through early July (Table 11). In the Pit 4 Bypass, 6 of the 23 *M. falcata* collected during April were gravid and all incubated uncleaved eggs. One of the gravid females aborted a long white string of uncleaved eggs about 10 minutes after examination. High flows prevented mussel collection in the Pit 4 Bypass throughout May, so the duration of the gravid period could not be precisely determined in this reach. None of the 80 *M. falcata* collected during March or the 60 collected in late June was gravid, however. In the Pit River downstream of Pit 1 Powerhouse, 2 of the 20 *M. falcata* collected in early June were gravid with uncleaved eggs, 1 of the 20 collected in late June was gravid with developing embryos, and 1 of the 22 collected in early July was gravid with glochidia (Figure 14).

In Hat Creek, 760 *M. falcata* were collected during 2005–06 and only 1 of the 50 examined on July 7, 2005 was gravid with embryos in the early stages of cell cleavage (Table 12). After this date, frequent and extensive snorkel surveys were conducted in conjunction with mussel collections, but release of conglutinates containing glochidia was never observed and no gravid mussels were found.

Table 12. *Margaritifera falcata* collected in Hat Creek in 2005–06

				Stage of Embryonic Development			
Month	**River Reach**	**Mussels Examined[1]**	**Gravid Females**	**Uncleaved Eggs**	**Developing Embryos[2]**	**Glochidia**	**Partially Empty[3]**
Feb 2005	Hat Creek	60	0	0	0	0	0
Mar	Hat Creek	60	0	0	0	0	0
Apr	Hat Creek	80	0	0	0	0	0
May	Hat Creek	60	0	0	0	0	0
Jun	Hat Creek	60	0	0	0	0	0
Jul	Hat Creek	60	1	0	1	0	0
Aug	Hat Creek	60	0	0	0	0	0
Sep	Hat Creek	----	----	----	----	----	----
Oct	Hat Creek	40	0	0	0	0	0
Nov	Hat Creek	40	0	0	0	0	0
Dec	Hat Creek	40	0	0	0	0	0
Jan 2006	Hat Creek	----	----	----	----	----	----
Feb	Hat Creek	60	0	0	0	0	0
Mar	Hat Creek	60	0	0	0	0	0
Apr	Hat Creek	80	0	0	0	0	0
	Total:	**760**	**1**	**0**	**1**	**0**	**0**

[1] Number does not include marked mussels

[2] Early stages of egg cleavage to beginning of larval shell development

[3] Marsupia containing mostly clear fluid with some degraded or unreleased glochidia

3.3. Stream Drift Sampling and Periods of Glochidial Release

Anodonta glochidia were absent or in very low abundance in samples of stream drift collected during the 2005–06 field season (i.e., April 2005 to May 2006). Eleven *Anodonta* glochidia were collected during the months of March, April, and May in the Pit River reaches and in May and July in Fall River (Figures 15 and 16). These glochidia were found amidst particles ranging from 0.15 mm to 0.30 mm in diameter on dates when abundant vegetation and/or benthic substrates were collected in the nets. Collections of clean surface water never yielded *Anodonta* glochidia.

In 2005, *G. angulata* glochidia were abundant in stream drift samples collected during May and June in the Pit 4 Bypass (stream drift was not sampled in early July), late April through early July in the Pit River downstream of Pit 1 Powerhouse, mid-April through mid-July in the Pit 1 Bypass, and early April through mid-July in Fall River (Figures 15 and 16). Numbers of glochidia per liter of sample water examined ranged from 30–99 in the Pit 4 Bypass, 21–991 in the Pit River downstream of Pit 1 Powerhouse, 643–2067 in the Pit 1 Bypass, and 8–619 in Fall River. The highest concentrations were in samples collected during June in the Pit 4 Bypass, Pit River downstream of Pit 1 Powerhouse, and Fall River; and in early May in the Pit 1 Bypass. Stream drift was not sampled in the Pit 1 Bypass in June. In 2006, *G. angulata* glochidia were collected in the Pit River downstream of Pit 1 Powerhouse and Fall River the last week in April.

The glochidia of *M. falcata* were not observed in any of the stream drift samples collected during the 2005–06 field season.

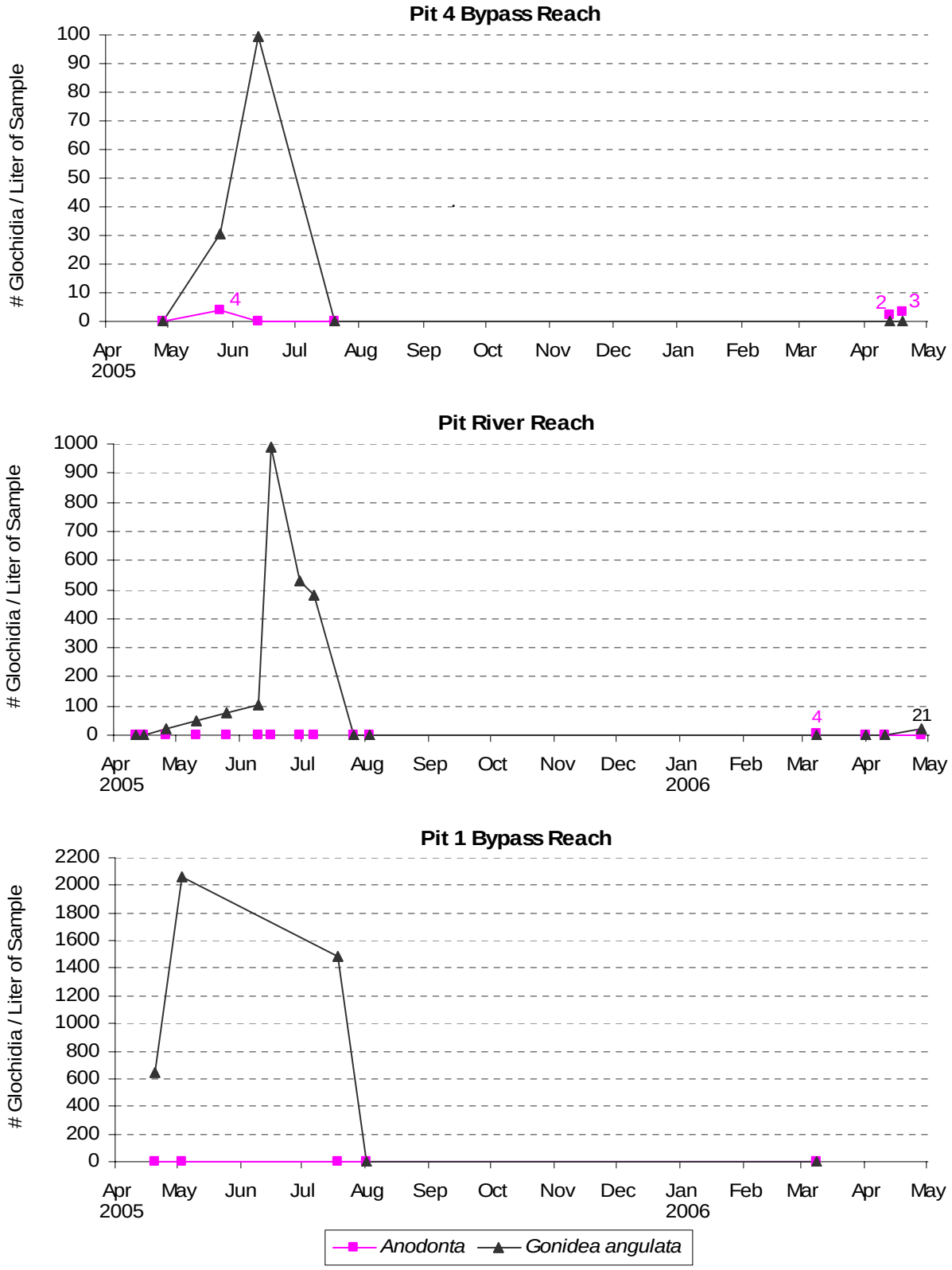


Figure 15. Sample concentration of glochidia in stream drift from Pit River in 2005–06

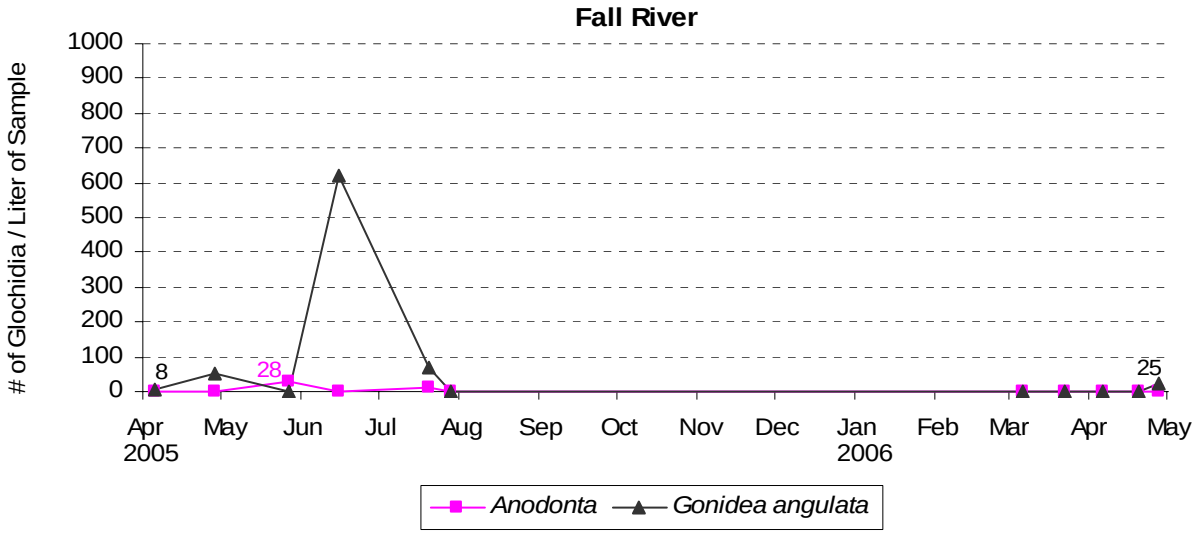


Figure 16. Sample concentration of glochidia in stream drift from Fall River in 2005–06

3.4. Fish Sampling and Periods of Glochidial Encystment

In 2004, only 54 fish were collected in the Pit 4 Bypass during April through June, 30 were collected in the Pit 4 Bypass in late August, and 34 were collected in the Pit River downstream of Pit 1 Powerhouse in May and June. Glochidia were not observed on any of the fish collected.

With the use of a backpack electroshocker in 2005–06, 176 fish were collected in the Pit 4 Bypass, 506 were collected in the Pit River downstream of Pit 1 Powerhouse, and 135 were collected in the Pit 1 Bypass (Table 13). Without the electroshocker, only 6 fish were collected in Hat Creek, 23 in Fall River, and 11 in Fall River Lake in 2005–06.

3.4.1. Anodonta Encystment

In 2005–06, *Anodonta* glochidia were observed on fish collected in the Pit 4 Bypass from late March through July 2005, and from early November 2005 through April 2006; none of the 32 fish collected in August 2005 was infected with glochidia (Figure 17). In the Pit River downstream of Pit 1 Powerhouse, *Anodonta* glochidia were observed on fish collected from late March through July 2005; early December 2005; and February, April, and early May 2006. None of the 147 fish collected from early August to early November 2005 was infected. At Big Eddy in the Pit 1 Bypass (i.e., *Anodonta* study site), *Anodonta* glochidia were observed on fish collected from early April to early August 2005 (no fish were collected in March) and from early November 2005 to mid-January 2006. None of the 50 fish collected at this site from late August through October 2005 was infected with glochidia. In Hat Creek, only one Pit sculpin collected in March 2006 was infected with *Anodonta* glochidia (Figure 18). None of the 34 fish collected in Fall River and Fall River Lake in July and August 2005 was infected with *Anodonta* glochidia.

Table 13. Number and size range of fish by species collected in all locations in 2005–06

Common and Taxonomic Names	Size Range (mm fork length)	Pit 4 Bypass	Pit River	Pit 1 Bypass	Hat Creek	Fall River	Fall River Lake	All Sites
Native Fish Species								
Hardhead (*Mylopharodon conocephalus*)	25–200	90	192	11	0	20	8	**321**
Pit roach (*Lavinia symmetricus mitrulus*)	55–93	7	2	0	0	0	0	**9**
Pit sculpin (*Cottus pitensis*)	35–84	20	84	0	4	0	0	**108**
Rainbow trout (*Oncorhynchus mykiss*)	30–260	7	41	0	2	0	0	**50**
Sacramento pikeminnow (*Ptychocheilus grandis*)	56–175	13	55	11	0	0	0	**79**
Sacramento sucker (*Catostomus occidantalis*)	35–150	6	66	0	0	3	0	**75**
Speckled dace (*Rhinichthys osculus*)	32–82	27	12	0	0	0	0	**39**
Tule perch (*Hysterocarpus traski*)	47–115	6	35	2	0	0	0	**43**
	Total Fish:	**176**	**487**	**24**	**6**	**23**	**8**	**724**
Non-native Fish Species								
Black crappie (*Pomoxis nigromaculatus*)	200–230	0	3	0	0	0	0	**3**
Bluegill (*Lepomis macrochirus*)	20–155	0	1	5	0	0	0	**6**
Common carp (*Cyprinus carpio*)	90–450	0	0	2	0	0	0	**2**
Golden shiner (*Notemigonus crysoleucas*)	83	0	1	0	0	0	0	**1**
Green sunfish (*Lepomis cyanellus*)	20–130	0	14	80	0	0	0	**94**
Largemouth bass (*Micropterus salmoides*)	30–130	0	0	23	0	0	1	**24**
Mosquitofish (*Gambusia affinis*)	23–32	0	0	0	0	0	2	**2**
Smallmouth bass (*Micropterus dolomieui*)	115	0	0	1	0	0	0	**1**
	Total Fish:	**0**	**19**	**111**	**0**	**0**	**3**	**133**
	Grand Total:	**176**	**506**	**135**	**6**	**23**	**11**	**857**

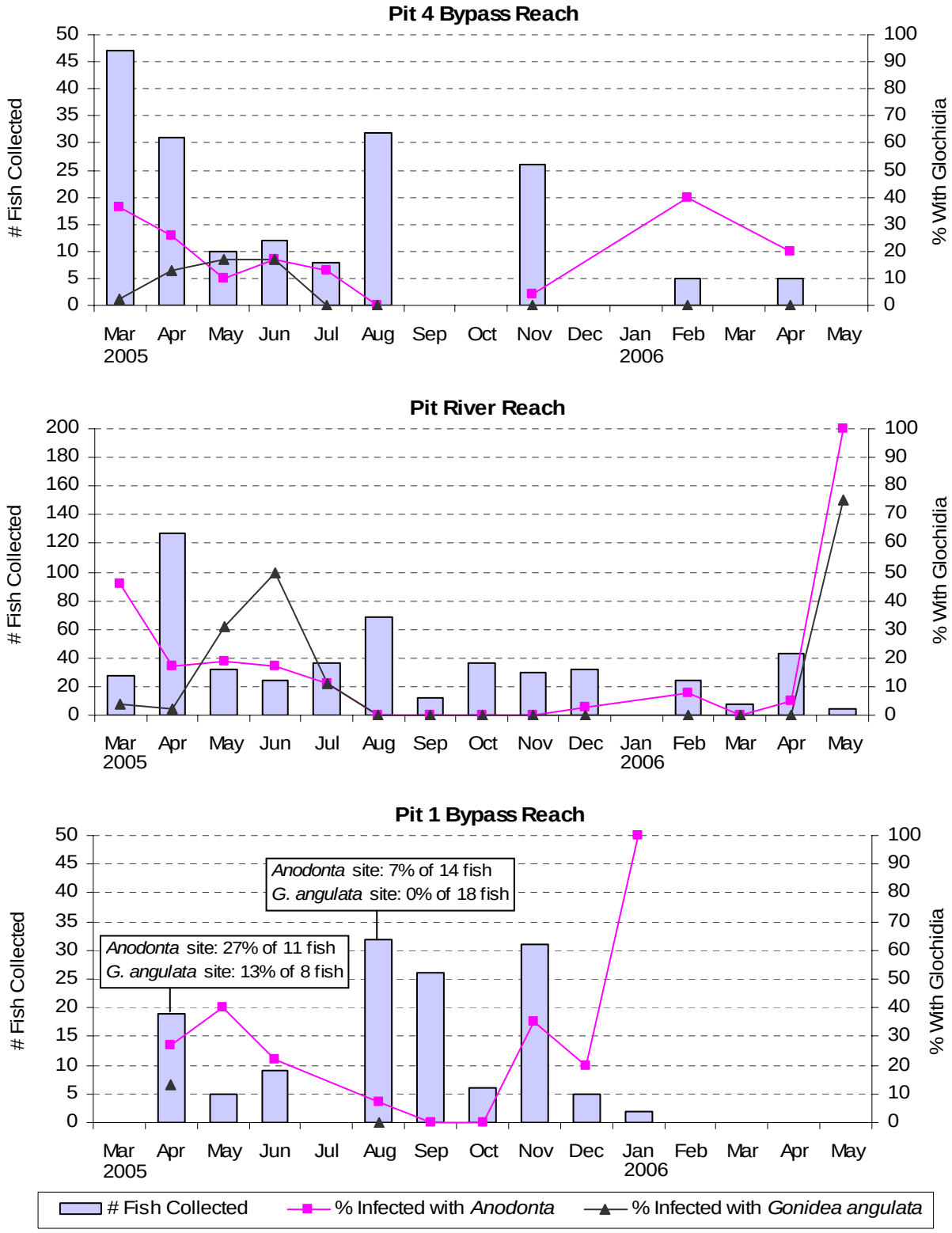


Figure 17. Number of fish collected and percentage infected with glochidia in Pit River in 2005–06. In the Pit 1 Bypass, fish were only collected at the *G. angulata* study site in April and August.

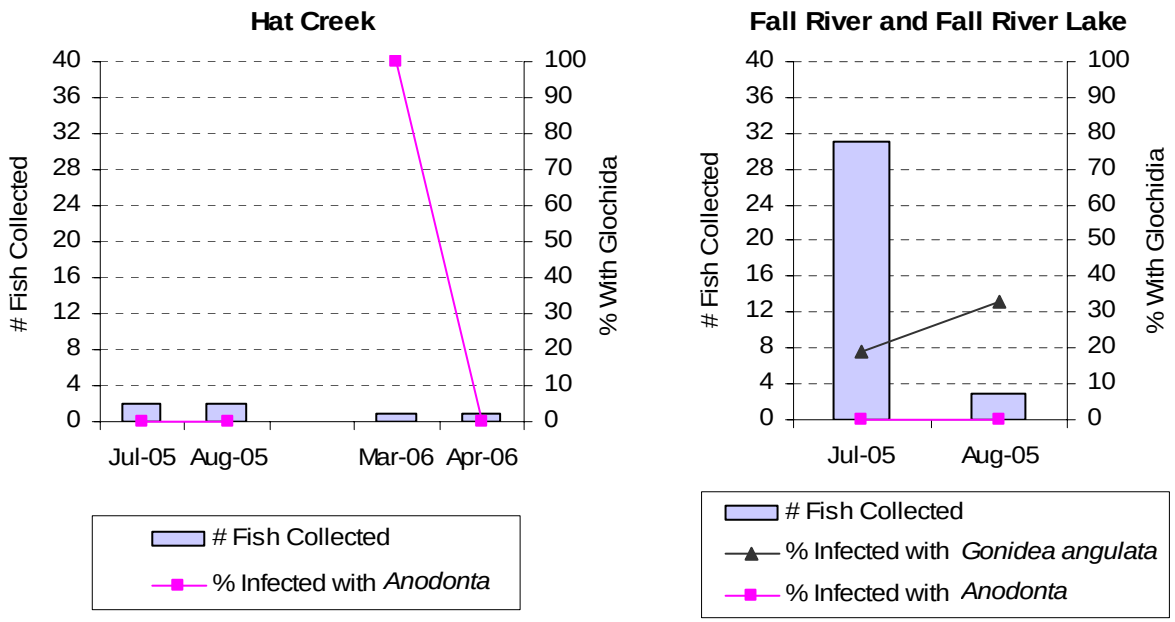


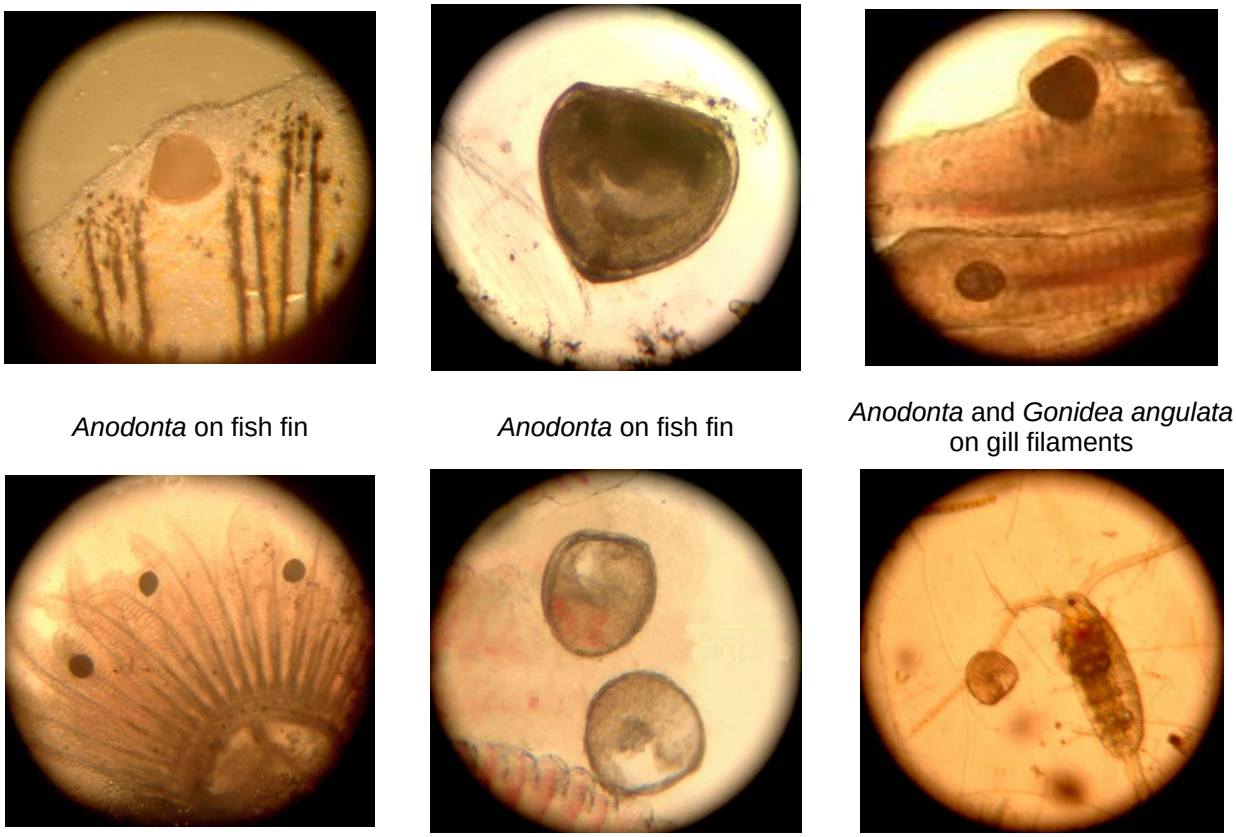
Figure 18. Number of fish collected and percentage infected with glochidia in Hat Creek and Fall River in 2005–06

Fish species infected with *Anodonta* glochidia included native members of Cyprinidae (hardhead, Pit roach, Sacramento pikeminnow, speckled dace); Catastomidae (Sacramento sucker); Cottidae (Pit sculpin); Embiotocidae (tule perch); Salmonidae (rainbow trout); and non-native members of Centrarchidae (green sunfish, black crappie, bluegill, largemouth bass) (Table 14). In the Pit 1 Bypass, *Anodonta* glochidia were also observed on 2 bullfrog tadpoles that were collected from the cages containing marked *Anodonta* adults. The relatively large glochidia of *Anodonta*, which averaged 0.246 ± 0.017 mm total length (anterior to posterior), were more prevalent on fish fins, but some were also found attached to the gill arches or filaments of fish larger than 100 mm fork length (Figure 19). The majority of collected fish were less than 100 mm fork length and numbers of *Anodonta* glochidia on these fish ranged from 1 to 18 and averaged 2 per fish.

Table 14. Percentage of each fish species infected with glochidia during periods of mussel encystment in 2005–06

Native Fish Species	*Anodonta*				*Gonidea angulata*			
	# Fish Examined During Encystment Period[1]	% of Fish Infected with Glochidia	# Glochidia per Fish (Range)	# Glochidia per Fish (Average)	# Fish Examined During Encystment Period[2]	% of Fish Infected with Glochidia	# Glochidia per Fish (Range)	# Glochidia per Fish (Average)
Hardhead	321	17	1–12	1.8	240	5	1–12	3.7
Pit roach	9	67	1–4	1.7	8	38	1–6	2.7
Pit sculpin	82	13	1	1.8	48	29	1–16	4.7
Rainbow trout	50	2	1	1.0	42	5	1–4	2.5
Sacramento pikeminnow	64	16	1–7	2.3	48	13	1–4	2.3
Sacramento sucker	55	4	1	1.0	38	0		
Speckled dace	39	10	1	1.0	28	0		
Tule perch	29	28	1–3	1.0	26	42	1–44	10.1
Total:	**649**	**15**	**1–12**	**1.7**	**478**	**10**	**1–44**	**5.3**
Non-Native Fish Species								
Black crappie	3	100	2–4	3.7	3	100	10–20	16.3
Bluegill	6	33	1–2	1.5	6	0		
Common carp	2	0			2	0		
Golden shiner	1	0			1	0		
Green sunfish	93	18	1–18	5.2	42	2	1–13	13.0
Largemouth bass	19	5	1	1.0	24	0		
Mosquitofish	2	0			2	50	1	1.0
Smallmouth bass	1	0			1	0		
Total:	**127**	**18**	**1–18**	**4.6**	**81**	**6**	**1–20**	**12.6**
Grand Total:	**776**	**15**	**1–18**	**2.2**	**559**	**9**	**1–44**	**6.0**

[1] Includes fish collected in all study reaches during all months in 2005–06 except September and October
[2] Includes fish collected in the Pit River downstream of Pit 1 Powerhouse and Fall River from March through August 2005 and from the Pit River downstream of Pit 1 Powerhouse on 8 May 2006



Anodonta on fish fin | *Anodonta* on fish fin | *Anodonta* and *Gonidea angulata* on gill filaments

Gonidea angulata on fish gill | *Gonidea angulata* on fish gill | *Gonidea angulata* on copepod antenna

Glochidia on fish were in the size range observed for glochidia in the marsupium samples (*Anodonta* = 0.246 ± 0.017 mm; *G. angulata* = 0.153 ± 0.007 mm)

Figure 19. Mussel glochidia attached to fish and other aquatic organisms

3.4.2. Gonidea angulata Encystment

In 2005, *G. angulata* glochidia were observed on fish collected in the Pit 4 Bypass from late March to early June. Fish were not collected in this reach in late June or early July, and none of the fish collected in late July was infected. In the Pit River downstream of Pit 1 Powerhouse, *G. angulata* glochidia were observed on fish from late March to late July (Figure 17). In the Pit 1 Bypass, fish were only collected at the *G. angulata* study site in April and August. At this site, the glochidia of *G. angulata* were observed on 1 of the 8 fish collected in April, but none of the 18 fish collected in August. At the Fall River study sites, 2 of the 23 fish collected in Fall River and 4 of the 8 fish collected in Fall River Lake in late July were infected with *G. angulata* glochidia (Figure 18). One of the 3 fish collected at the Fall River Lake boat ramp on 8 August was also infected. In 2006, *G. angulata* glochidia were not observed on fish collected in the Pit River reaches until the first week in May, when 3 of the 4 Pit sculpin collected were infected.

Fish species infected with *G. angulata* glochidia included native members of Cyprinidae (hardhead, Sacramento pikeminnow, Pit roach); Cottidae (Pit sculpin); Embiotocidae (tule perch); Salmonidae (rainbow trout); and non-native members of Centrarchidae (green sunfish, black crappie); and Poeciliidae (mosquitofish) (Table 14). One *G. angulata* glochidium was also observed attached to the antenna of a copepod that was collected in one of the Fall River stream drift samples (Figure 19). In locations where *Anodonta* and *G. angulata* adults occurred together, the glochidia of both species were often found on a single host. The glochidia of *G. angulata* averaged 0.153 ± 0.007 mm total length (anterior to posterior) and were only found on the gills of host fish, where their numbers ranged from 1 to 44 and averaged 6 per fish.

3.4.3. Margaritifera falcata Encystment

The glochidia of *M. falcata* were not observed on any of the fish collected in the Pit River or Hat Creek during the 2005–06 field season.

3.5. Juvenile Excystment in Freshwater Aquaria

Water temperatures in the aquaria were highly variable during April 2005, with daily temperatures ranging from 7.4°C to 18.6°C (45.3°–65.5°F). Thereafter, small aquarium heaters were used to prevent water temperatures from dropping below 10°C (50°F) and additions of cold well water into a circulating water bath were used to prevent temperatures from exceeding 24°C (75.2°F). Water temperatures, which were checked three times a day, ranged from 10°C to 18°C (50°–64.4°F) in May, 12°C to 22°C (53.6°–71.6°F) in June, 20°C to 24°C (68°–75.2°F) in July and August, and 16°C to 19°C (61°–66°F) in September. Water temperatures after April were within the range of those recorded in the Pit River reaches in 2005.

3.5.1. Anodonta Excystment

Sixteen transformed juvenile *Anodonta* were collected from aquarium tanks containing hardhead, Sacramento pikeminnow, Pit sculpin, tule perch, and green sunfish (Table 15). Live and/or dead juvenile mussels were first collected on 16 April and were last collected on 5 August. Two sloughed-off glochidia that had not transformed into juveniles were also collected from the tule perch and green sunfish tanks in June. No juvenile mussels or untransformed glochidia were collected from a tank containing 10 Sacramento suckers. These fish had visible *Anodonta* infections on their fins when first collected, but not after two weeks in the tanks, when their fins were highly tattered. Fish without visible infections of *Anodonta*, including some rainbow trout and mosquitofish, were collected in early and late August and held in aquaria through September, but no glochidia or juvenile mussels were collected from tanks of these fish. In the examination dishes, live juvenile *Anodonta* were relatively sedentary and could only be differentiated from glochidia when their foot or shell growth was visible (Figure 20). The prominent shell hooks of *Anodonta* glochidia were still visible immediately after excystment, but became obscured by shell growth after several days.

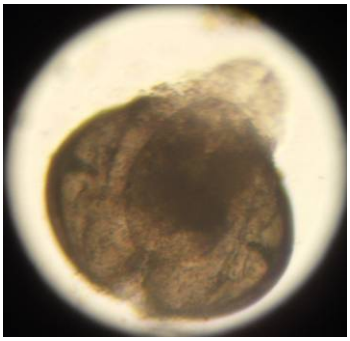
3.5.2. *Gonidea angulata* Excystment

Thirty-two transformed juvenile *G. angulata* were collected from aquarium tanks containing hardhead, Pit sculpin, and tule perch (Table 15). Live and/or dead juveniles were first collected on 27 June and last collected on 29 July, and 30 of the 32 juveniles were obtained from three tanks of Pit sculpin that were collected on 21 June. Pit sculpin were the most abundant fish collected during electroshocking in June and July, so more individuals of this species were monitored in the freshwater aquaria. Three sloughed-off glochidia that had not transformed into juvenile mussels were collected from the hardhead tank and one glochidium was collected from a tank containing two mosquitofish. No juveniles or untransformed glochidia were obtained from tanks of Sacramento pikeminnow, green sunfish, Sacramento sucker, or rainbow trout. These fish may not have been infected with *G. angulata* glochidia when they were collected. In the examination dishes, *G. angulata* juveniles had a distinct shape and coloration that differentiated them from glochidia (Figure 20). In addition, *G. angulata* juveniles were more active than *Anodonta* juveniles and could be seen moving along the bottom of the examination dishes.

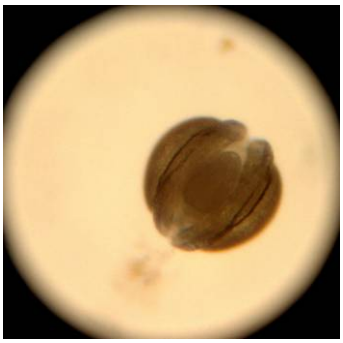
3.5.3. *Margaritifera falcata* Excystment

No transformed juvenile *M. falcata* or untransformed glochidia were obtained from the freshwater aquaria in 2005.

Live *Anodonta*

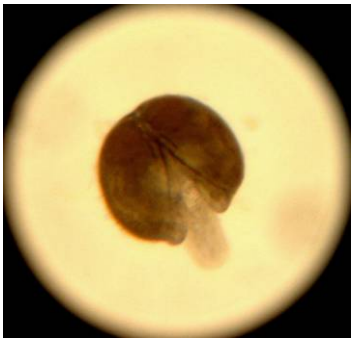


Dead *Anodonta* with extended foot

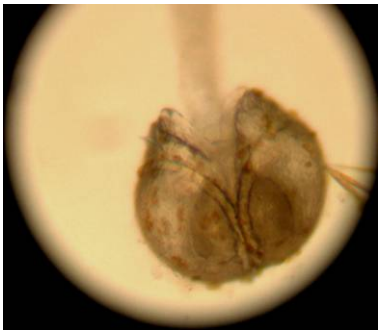


Live *Gonidea angulata* with retracted foot

Shell hinge of live *Gonidea angulata*



Live *Gonidea angulata* with extended foot



Live *Gonidea angulata* showing visible shell growth

Figure 20. Excysted juvenile mussels

Table 15. Summary of freshwater aquaria data from March through September 2005

Tank	Fish Species	Date Fish Collected[1]	Collection Site	Date Tank Siphoned[2]	*Anodonta* Glochidia[3]	*Anodonta* Juveniles	*Gonidea angulata* Glochidia	*Gonidea angulata* Juveniles
1	hardhead	3/31/05	Pit 4 Bypass	4/16/05	0	1 dead	0	0
1	hardhead	3/31/05	Pit 4 Bypass	4/19/05	0	2 alive	0	0
1	hardhead	4/12/05	Pit 4 Bypass	5/6/05	0	1 dead	1	0
1	hardhead	6/21/05	Pit River d/s Pit 1 PH	7/12/05	0	0	2	1 alive
				Total:	**0**	**4**	**3**	**1**
2	pikeminnow	3/31/05	Pit 4 Bypass	4/16/05	0	1 alive, 4 dead	0	0
2	pikeminnow	3/31/05	Pit River d/s Pit 1 PH	5/9/05	0	1 dead	0	0
				Total:	**0**	**6**	**0**	**0**
3	tule perch	5/26/05	Pit River d/s Pit 1 PH	6/6/05	1	0	0	0
3	tule perch	5/26/05	Pit River d/s Pit 1 PH	7/1/05	0	1 dead	0	0
3	tule perch	6/21/05	Pit River d/s Pit 1 PH	7/7/05	0	0	0	1 alive
3	tule perch	7/20/05	Pit 4 Bypass	8/5/05	0	1 dead	0	0
				Total:	**1**	**2**	**0**	**1**
4	Pit sculpin	6/21/05	Pit River d/s Pit 1 PH	6/27/05	0	0	0	4 dead
4	Pit sculpin	6/21/05	Pit River d/s Pit 1 PH	6/29/05	0	0	0	10 live, 1 dead
4	Pit sculpin	6/21/05	Pit River d/s Pit 1 PH	7/6/05	0	0	0	1 live, 2 dead
4	Pit sculpin	6/21/05	Pit River d/s Pit 1 PH	7/11/05	0	0	0	5 dead
5	Pit sculpin	6/21/05	Pit River d/s Pit 1 PH	7/11/05	0	1 alive	0	6 live
6	Pit sculpin	6/30/05	Pit River d/s Pit 1 PH	7/29/05	0	1 dead	0	1 dead
				Total:	**0**	**2**	**0**	**30**
7	green sunfish	4/13/05	Pit 1 Bypass	4/16/05	0	1 dead	0	0
7	green sunfish	6/21/05	Pit River d/s Pit 1 PH	6/27/05	1	0	0	0
7	green sunfish	6/21/05	Pit River d/s Pit 1 PH	7/1/05	0	1 alive	0	0
				Total:	**1**	**2**	**0**	**0**
8	mosquitofish	8/8/05	Fall River Lake	8/9/05	0	0	1	0
				Grand Total:	**2**	**16**	**4**	**32**

[1] Does not include all collection dates; [2] Does not include all siphonate dates; [3] Untransformed glochidia shed by the host

3.6. River Discharge and Temperature in Relation to Mussel Gravid Periods

Gravid periods are discussed for *Anodont*a in all reaches; for *G. angulata* in the Pit River reaches and Fall River; and for *M. falcata* in Pit 4 Bypass, Pit River downstream of Pit 1 Powerhouse, and Hat Creek.

3.6.1. Pit River 2004

Mean daily discharges, mean daily water temperatures, and mussel gravid periods in the three Pit River reaches from April through November 2004 are shown in Figure 21. A natural high-flow event in the Pit River in mid-February, two months prior to the beginning of the field season, resulted in a nearly six-fold increase in Pit River discharge downstream of the Pit 1 Powerhouse and a 30-fold increase over the normal base-flow release in the Pit 4 Bypass. There are no discharge data for the Pit 1 Bypass during this period. Mean daily water temperatures in each reach peaked in early July, with temperatures reaching 21.1°C (70°F) in the Pit 4 Bypass, 21.4°C (70.5°F) in the Pit River downstream of Pit 1 Powerhouse, and 24.3°C (75.7°F) in the Big Eddy pool of the Pit 1 Bypass.

Pit 4 Bypass 2004

In the Pit 4 Bypass, discharge remained high throughout February, then dropped and stabilized around 200 cfs until the end of September. The field season began in early April, at which time some individuals of all mussel species were gravid. Water temperatures during the month of April ranged from 10.6°C to 14.1°C (51.1°–57.4°F) and averaged 12.5°C (54.5°F). The spring/summer gravid periods for *Anodonta* and *G. angulata* ended soon after water temperatures peaked around mid-July. *Anodonta* resumed spawning in mid-to-late October during or after a brief period of intermittent high flows. By this time, mean daily water temperatures had dropped below 13°C. Gravid *M. falcata* were not found in late April or May, but a few individuals spawned in mid-June, when water temperature averaged 18°C (64.4°F).

Pit River Downstream of Pit 1 Powerhouse 2004

In the Pit River downstream of Pit 1 Powerhouse, discharge fluctuated on a daily basis with an hourly average discharge between 206 cfs and 866 cfs throughout 2004. Potential effects of these flow fluctuations on mussel reproduction were not specifically investigated, but reproductive timing for *Anodonta* and *G. angulata* did not appear to differ significantly (differences of less than two weeks could not be determined) between the Pit River downstream of Pit 1 Powerhouse and the more hydrologically stable Pit 4 Bypass. After the February high-flow event, mean daily discharge in the Pit River downstream of Pit 1 Powerhouse declined steadily from March to late June and remained at about 1000 cfs from July to early October. The spring/summer gravid periods for *Anodonta* and *G. angulata* ended as the hydrograph reached base flow and after water temperatures peaked around mid-July. The non-gravid interval for *Anodonta* coincided with the duration of base flows. *Anodonta* resumed spawning sometime in October, when discharge began to increase above base flow and after mean daily water temperature dropped below 13°C (55.4°F). No gravid *M. falcata* were found in this reach in 2004.

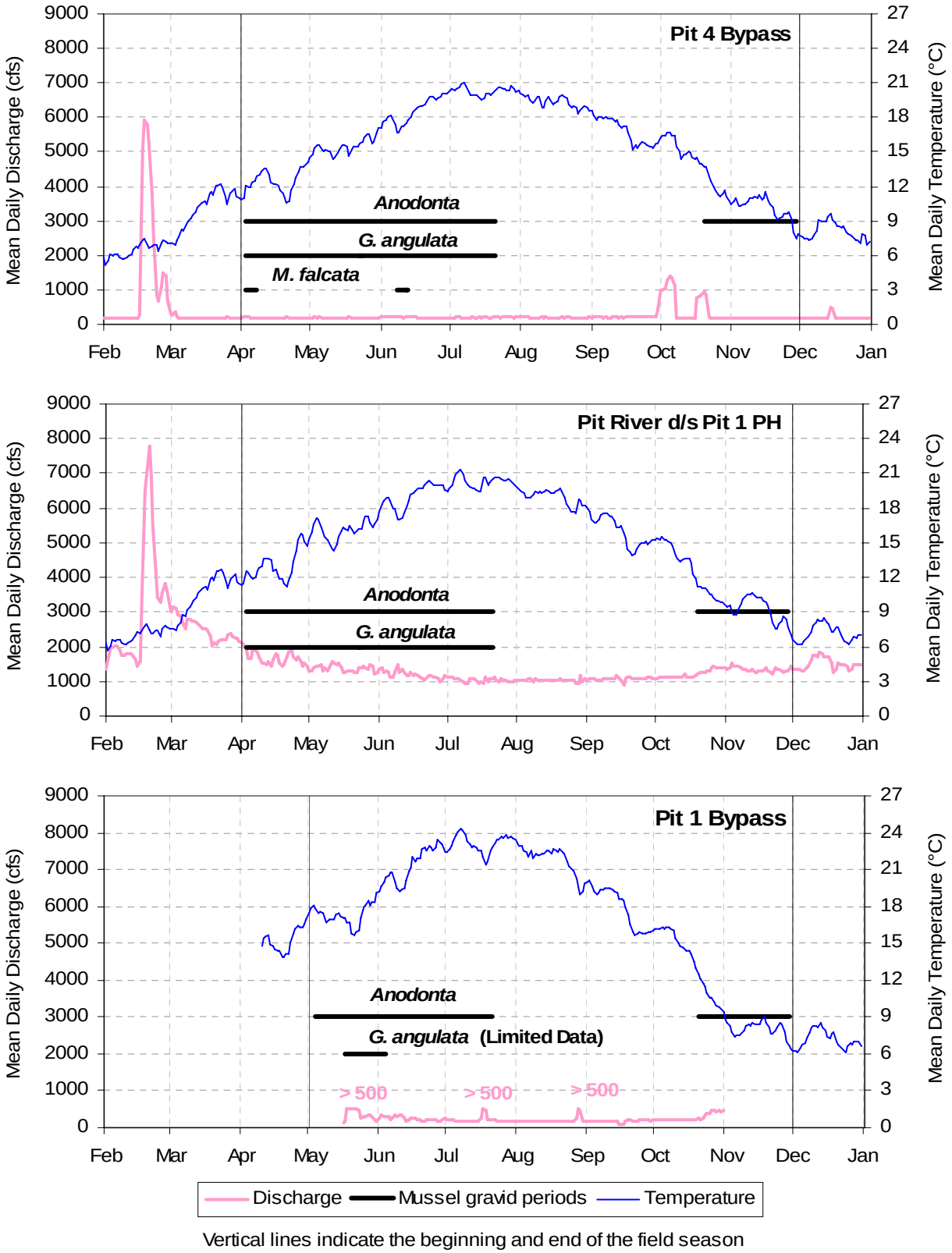


Figure 21. Pit River discharges, temperatures, and mussel gravid periods in 2004

Pit 1 Bypass 2004

In the Big Eddy pool of the Pit 1 Bypass, discharge less than 500 cfs was determined using a stage-discharge rating curve (PG&E 2004). Discharges greater than 500 cfs could not be determined using this curve. Daily average discharge fluctuations ranging from 200 cfs to greater than 500 cfs occurred from 16 May to 3 July as a result of Muck Valley Hydroelectric Project operations. Discharge also exceeded 500 cfs during three flushing/whitewater pulsed flow events that occurred during 18–23 May, 17–18 July, and 28–29 August. After 3 July, mean daily discharge stabilized at about 200 cfs, except during the pulsed flow events. Water temperatures in Big Eddy averaged about 2°C (3.6°F) higher than in the other study reaches during most of the field season, but *Anodonta* gravid periods were similar (within two weeks) in all reaches. The duration of gravidity was not determined for *G. angulata* in this reach, but gravid *G. angulata* were collected 1 day before and 15 days after the May pulsed flow event.

3.6.2. Pit River 2005

Mean daily discharges, mean daily water temperatures, and mussel gravid periods in the three Pit River reaches from February through December 2005 are shown in Figure 22. Two discharge peaks occurred in all reaches, one throughout the month of May and one during the last few days of December. The high flows in May reached 7340 cfs in the Pit 4 Bypass and 7500 cfs in the Pit River downstream of Pit 1 Powerhouse, and the high flows in December exceeded 9000 cfs in both reaches. Discharge data are not available for the Pit 1 bypass before June or after September 2005.

Mean daily water temperatures peaked in all of the Pit River reaches in mid-July, with temperatures reaching 22.0°C (71.6°F) in the Pit 4 Bypass, 22.2°C (72°F) in the Pit River downstream of Pit 1 Powerhouse, and 23.9°C (75°F) in the Big Eddy pool of the Pit 1 Bypass. Peak temperatures occurred about two weeks later and were about 1°C (1.8°F) higher in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse, and 0.4°C (0.7°F) lower in the Pit 1 Bypass in 2005 than in 2004.

Pit 4 Bypass 2005

In the Pit 4 Bypass, mean daily discharge was about 200 cfs throughout February, fluctuated between 200 cfs and 2000 cfs from March through April, exceeded 5000 cfs during May, and stabilized around 200 cfs from mid-June through September. Mussel collection began in February, when only *Anodonta* were gravid. Spawning in *G. angulata* began in late March during the second 2000 cfs discharge spike. Mean daily water temperatures exceeded 12°C (53.6°F) for a period of 2 weeks prior to the spike, but averaged 10°C (50°F) during the spike. Stream drift data confirmed that some *Anodonta* and *G. angulata* released glochidia in the Pit 4 Bypass during the May discharge peak. The spring/summer gravid periods for *Anodonta* and *G. angulata* ended as water temperatures peaked around mid-July. As observed in 2004, *Anodonta* resumed spawning in mid-to-late October, after water temperatures dropped below 13°C (55.4°F). *Anodonta* glochidia were found encysted on fish by early November. Spawning in *M. falcata* began or was first detected in early April after the two-week period of 12°C (53.6°F) water

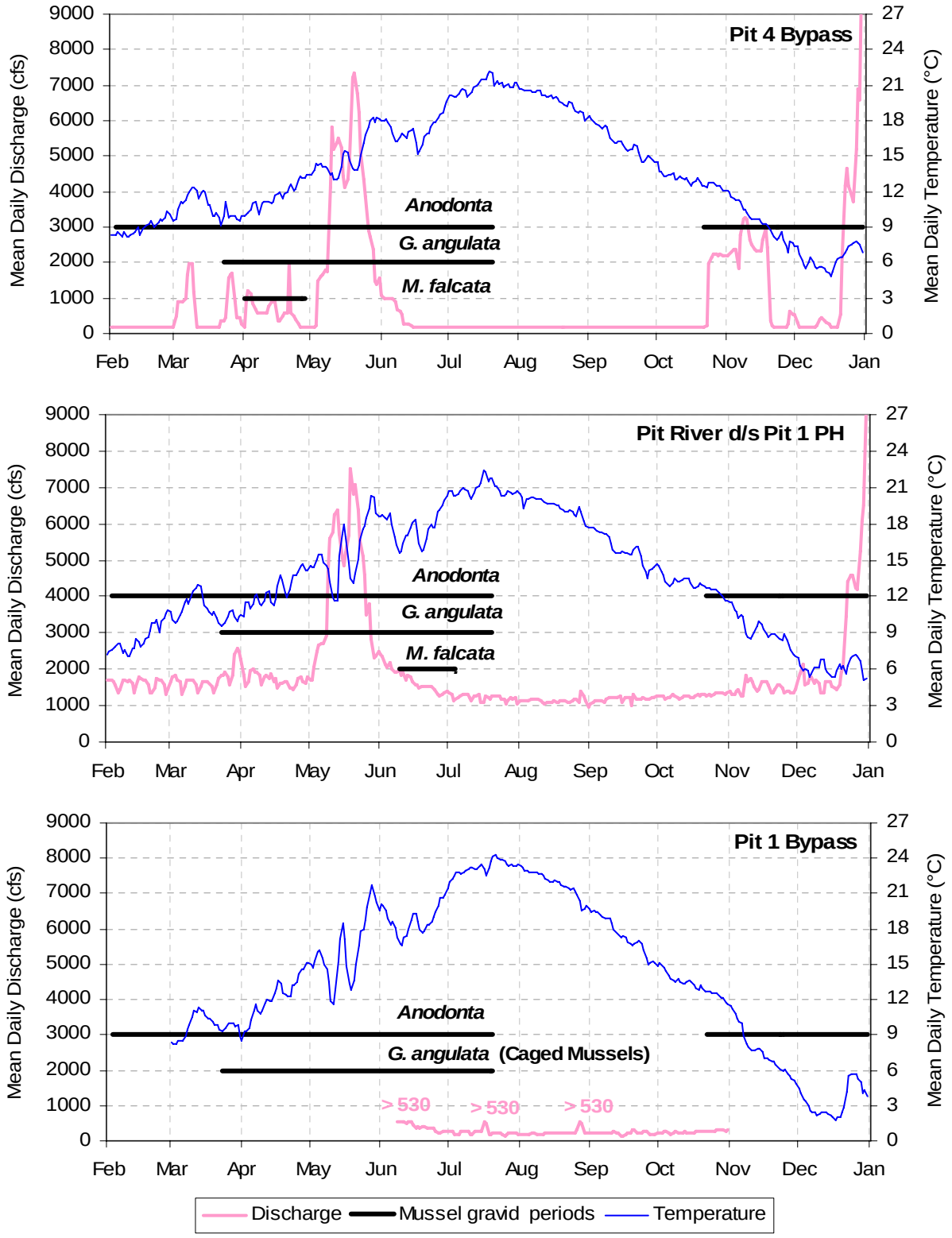


Figure 22. Pit River discharges, temperatures, and mussel gravid periods in 2005

temperatures. During the roughly three-week period of *M. falcata* gravidity, water temperatures ranged between 10.0°C and 13.2°C (50°–55.8°F) and averaged 11.8°C (53.2°F). Mussel collection was not possible during the May discharge peak, so the precise duration of *M. falcata* gravidity could not be determined in this reach.

Pit River Downstream of Pit 1 Powerhouse 2005

In the Pit River downstream of Pit 1 Powerhouse, temperature regimes and periods of gravidity for *Anodonta* and *G. angulata* were similar to those in the Pit 4 Bypass. Seasonal discharge regimes were also similar, although total discharge exceeded that in the Pit 4 Bypass during periods of regulated releases from Pit 4 Dam and discharge fluctuated throughout the day as a result of hydroelectric peaking. Mussel collection was limited during the period of high flows in May, but some marked *Anodonta* and unmarked *G. angulata* collected from a backwater habitat were gravid before, during, and after May; and the marked *Anodonta* continued to incubate embryos in various stages of development during the period of high flows. The spring/summer gravid periods for *Anodonta* and *G. angulata* ended as the hydrograph reached base flow and water temperatures peaked around mid-July. As observed in 2004, the duration of the non-gravid interval for *Anodonta* roughly coincided with the duration of base flows. Gravid *M. falcata* that had just spawned were found in early June, after the May discharge peak subsided. Water temperatures exceeded 18°C (64.4°F) for a period of three weeks prior to the onset of spawning. During the roughly four-week period of *M. falcata* gravidity, mean daily water temperatures ranged between 15.8°C and 20.9°C (60.4°–69.6°F) and averaged 18.5°C (10.3°F).

Pit 1 Bypass 2005

In the Big Eddy pool of the Pit 1 Bypass, the discharge recorder was not installed until 7 June, but discharge in the lower bypass reach ranged from 950–6200 cfs from 16 May to 6 June (PG&E 2005). In Big Eddy, discharge exceeded 530 cfs (rating curve was updated in 2005) during most of June and during flushing/whitewater flows on 30 May–5 June, 15–17 July and 26–28 August. Hourly discharge fluctuations of 200–300 cfs occurred through July as a result of Muck Valley Hydroelectric Project operations.

Water temperatures in Big Eddy were about 1.5°C (2.7°F)higher than in the other reaches from May through October, but gravid periods for marked *Anodonta* in the Pit 1 Bypass were similar (within 2 weeks) to gravid periods for marked and unmarked *Anodonta* in the other Pit River reaches. The 5 caged *Anodonta* in the Pit 1 Bypass were examined during the July pulsed flow event; 1 had just spawned, 1 was gravid with mature glochidia, 1 had glochidia in about 50% of each marsupium, and 2 had nearly empty marsupia (see Figure 12). Water temperatures in the Pit 1 Bypass were also measured at the *G. angulata* study site in the lower Pit River Canyon (see Figure 4), where an influx of several springs lowered the temperature by about 1°C (1.8°F) during the summer months and raised the temperature by about 1°C during the winter months. Temperatures at this site averaged 0.5°C (0.9°F) higher than in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse from May through October. Spawning in *G. angulata* began in late March and glochidial release ended in mid-July.

3.6.3. Hat Creek, Fall River, and Fall River Lake 2005

Hat Creek and Fall River 2005

Compared to the Pit River study reaches, river discharge regimes in Hat Creek and Fall River are relatively stable. In Hat Creek, daily discharge averaged 460 cfs from January through September in 2005. During seasonal runoff, peak discharges of 500–550 cfs occurred. Discharge data are not available for Fall River, but only one stream contributes seasonal snowmelt runoff to the spring-dominated system.

Mean daily water temperatures in Hat Creek were about 3°C (5.4°F) cooler during the summer months than in Fall River, where water temperatures were similar to those in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse (Figure 23). In Hat Creek and Fall River, *Anodonta* gravid periods were similar to those observed in the Pit River reaches. In Fall River, gravid *G. angulata* were found in late April and some glochidia were collected in stream drift in early April. At least one *M. falcata* in Hat Creek spawned in early July; about four weeks after females spawned in the Pit River downstream of Pit 1 Powerhouse and four months after females spawned in the Pit 4 Bypass. Mean daily water temperatures during the early July spawning event in Hat Creek averaged 16°C (60.8°F), but did not exceed 18°C (64.4°F) prior to the spawning event, as did water temperatures during the June 2005 spawning event in the Pit River downstream of Pit 1 Powerhouse.

Fall River Lake 2005

In Fall River Lake, water temperatures were measured on the day of mussel collection. In mid-May, when some *Anodonta* were observed aborting their marsupial contents, lake levels were down and *Anodonta* were collected in less than 1 meter of water that was 27.3°C (81.1°F) at 1:00 PM. None of the *G. angulata* collected on this date appeared to be aborting, but they were collected in 1.5–2 meters of water in a more shaded portion of the lake, where water temperature may have been cooler. By late July, lake levels were higher and water temperature at the *Anodonta* collection site was cooler—23.1°C (73.6°F) at 2:30 PM—but none of the 8 *Anodonta* collected was gravid. In early November, water temperature was 10.1°C (50.2°F) and 7 of the 13 *Anodonta* collected were gravid.

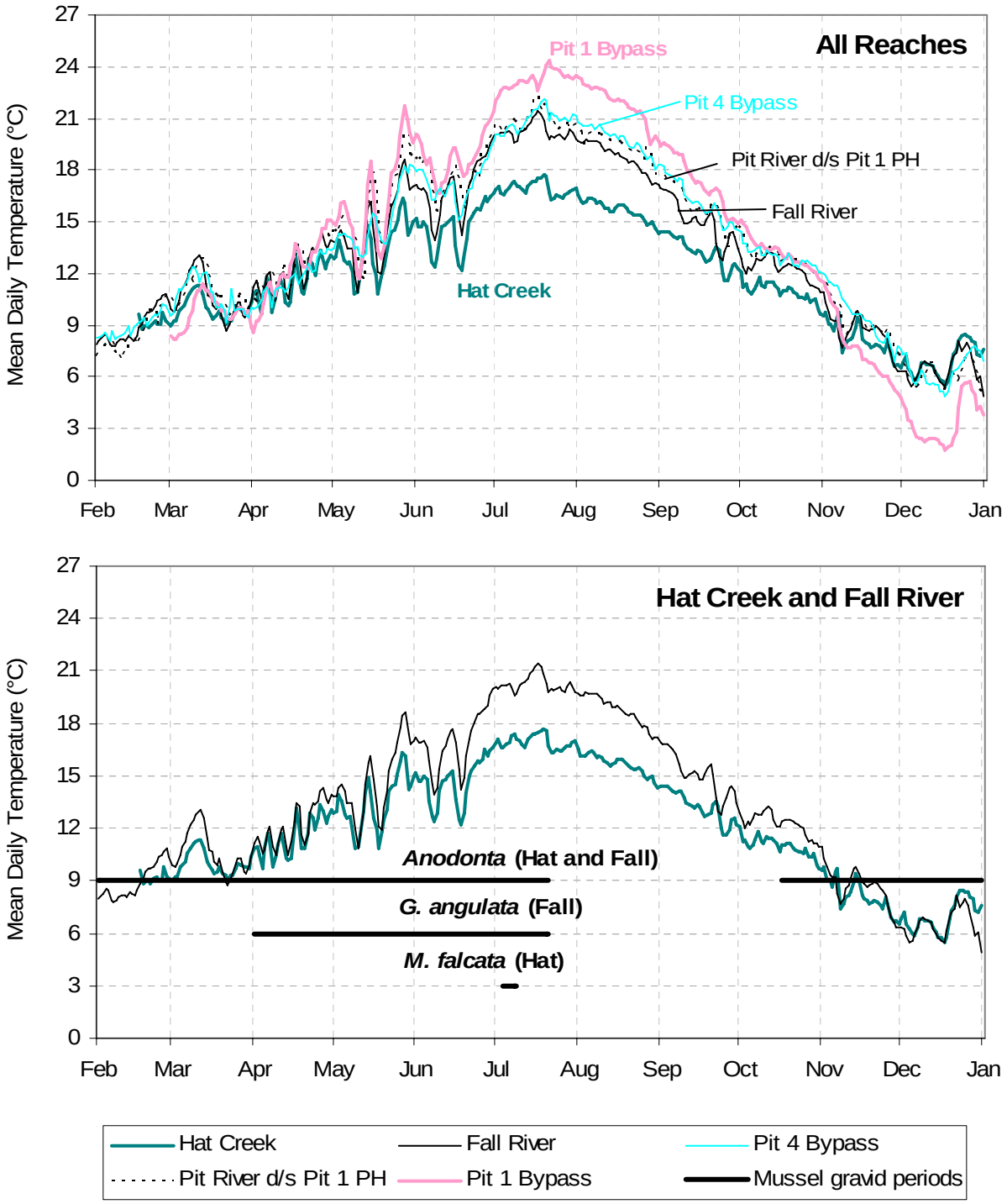


Figure 23. Water temperatures and mussel gravid periods in Hat Creek and Fall River in 2005, with temperature data from other reaches for comparison

3.6.4. Pit River 2006

Mean daily discharges, mean daily water temperatures, and mussel gravid periods in the Pit River downstream of Pit 1 Powerhouse; and water temperatures and mussel gravid periods in the Pit 1 Bypass are shown in Figure 24 for the period of January through April 2006. Sustained high discharges in the Pit 4 Bypass prevented mussel collection and limited fish and stream drift sampling, so discharge and water temperature data are not presented for this reach. In the Pit 1 Bypass, water temperatures were recorded but stream discharge data were not collected prior to May.

Pit River Downstream of Pit 1 Powerhouse 2006

In the Pit River downstream of Pit 1 Powerhouse, mean daily discharge peaked at 12,100 cfs in early January, then dropped down to 2500 cfs by the end of the month. Mean monthly discharge increased from February through April via a series of pulsed releases that ranged from 2000 to 6000 cfs and occurred in early February, early March, late March, and mid-to-late April. Mean daily water temperatures in the Pit River downstream of Pit 1 Powerhouse fluctuated with changes in flow, but increased from a low of 3°C (5.4°F) in early January to a high of 16°C (60.8°F) in late April.

With the aid of SCUBA, some mussels were collected in a complex side-channel habitat in the Pit River downstream of Pit 1 Powerhouse, where they were protected from the much higher water velocities in the main channel. Mussels were collected at this site during the recession of high flows in mid-January and during the period of increasing flows in early February. *Anodonta* incubated eggs, embryos, or glochidia or released glochidia on both dates. Stream drift collected at this site indicated that some *Anodonta* released glochidia during the period of increasing flows in early March, and some fish collected at this site had recent infections of *Anodonta* glochidia in April and May. Five live *Anodonta* were found stranded at this site in mid-January during the abrupt decrease in river discharge and none of these mussels was gravid (see Figure 6).

Some *G. angulata* were collected at this location in early February, but none was gravid. Infections of *G. angulata* glochidia were not observed on fish until early May.

Pit 1 Bypass 2006

In the Pit 1 Bypass, marked *Anodonta* maintained in wire cages were gravid throughout January and February, when river discharge was visibly high (discharge data were not collected in this reach prior to May) and water temperatures ranged from 2°C to 7°C (35.6°–44.6°F). Some male and female *Anodonta* spawned during this period and some females released glochidia.

3.6.5. Hat Creek and Fall River 2006

In Hat Creek, water temperatures during January averaged 2°C (3.6°F) higher than in the Pit River downstream of Pit 1 Powerhouse and 4°C (7.2°F) higher than in the Pit 1 Bypass (Figure 25). By mid-April, temperatures in Hat Creek and the Pit River reaches were similar, with daily means ranging from 9°C to 11°C (48.2°–51.8°F). Marked *Anodonta* in Hat Creek continued to spawn and release glochidia from January through April, but no evidence of *M. falcata*

spawning was observed. In Fall River, no evidence of *G. angulata* spawning or glochidial release was observed until the last week in April, when some gravid mussels were found and glochidia were collected in stream drift.

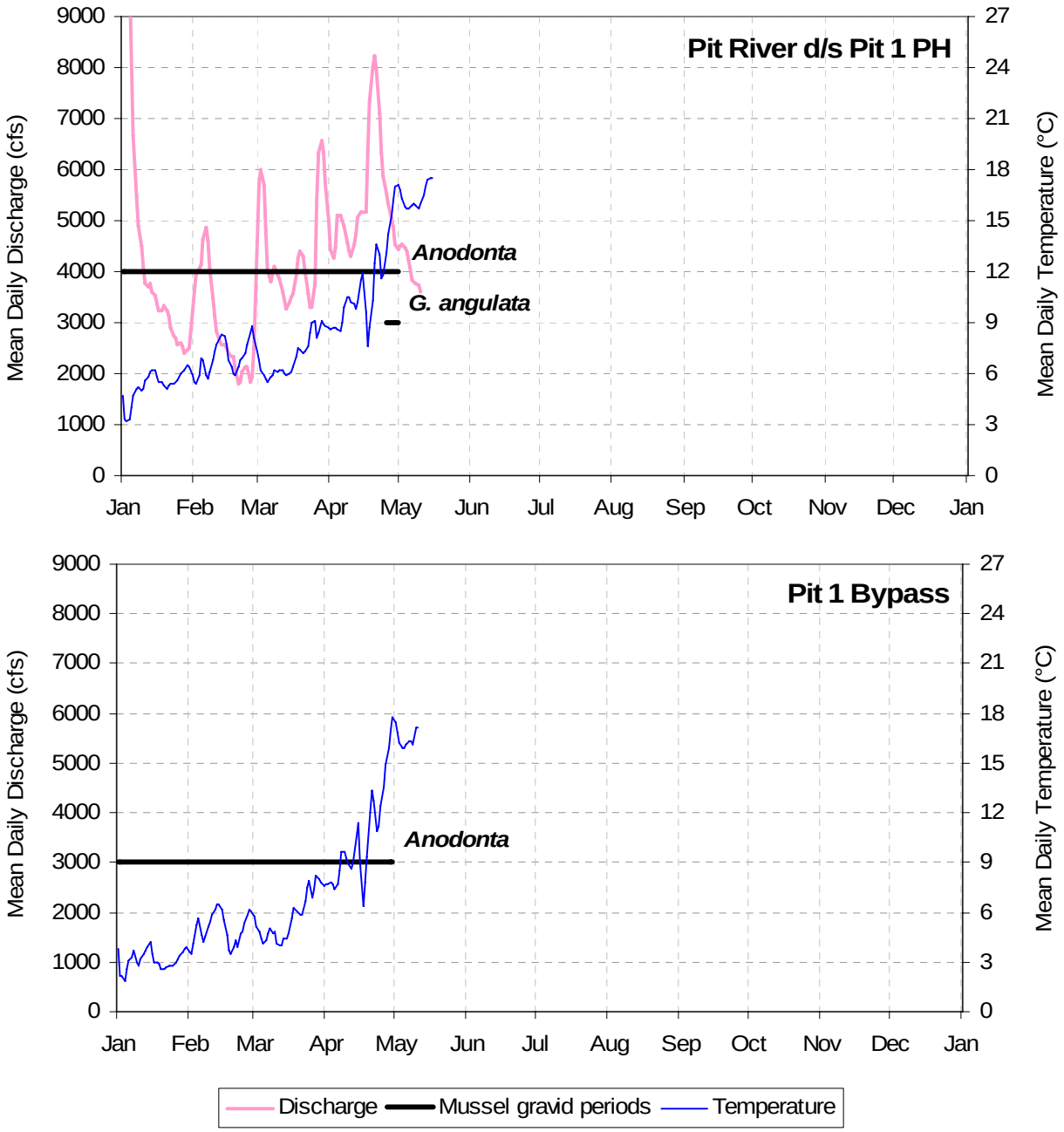


Figure 24. Pit River discharges, temperatures, and mussel gravid periods in 2006

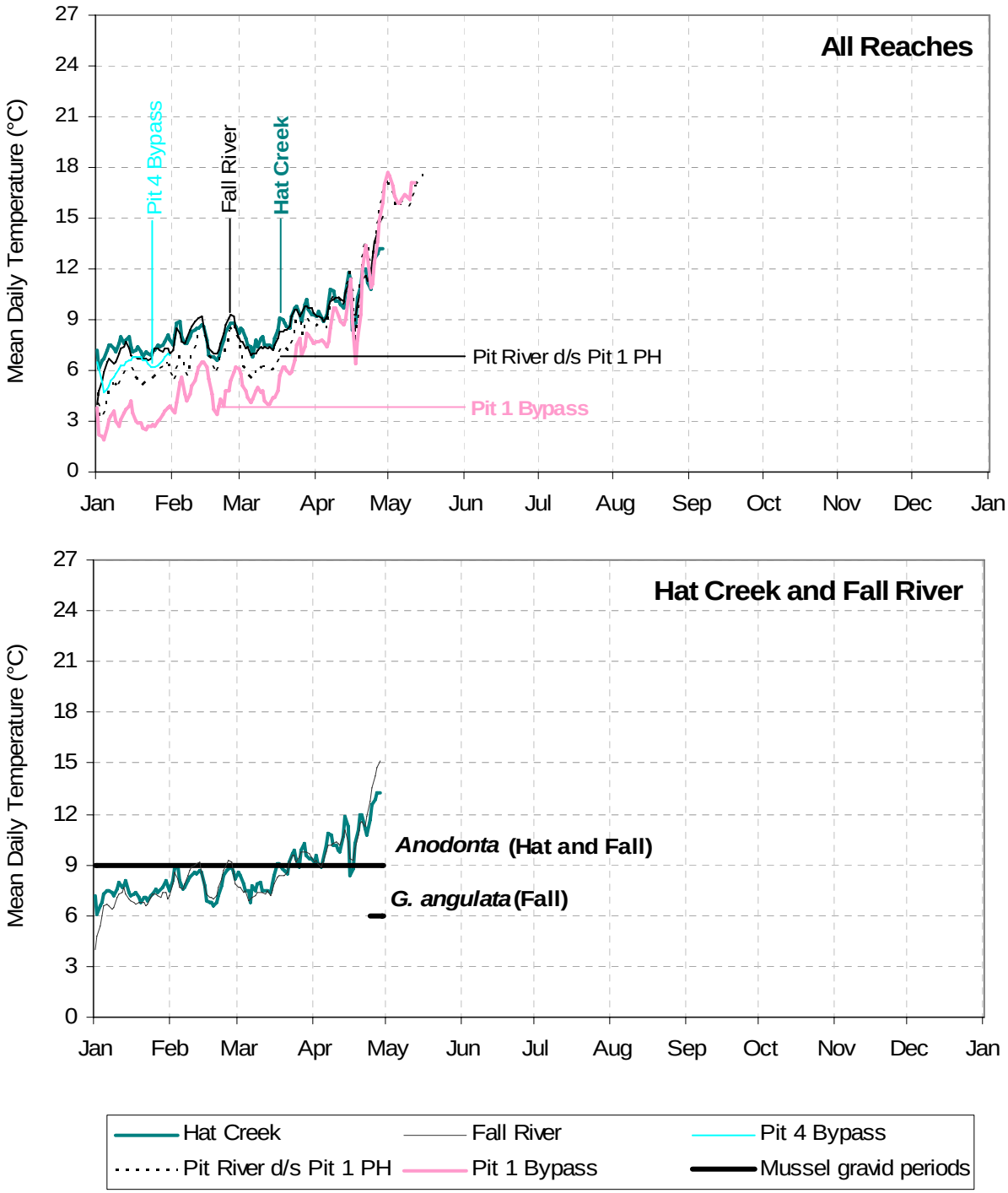


Figure 25. Water temperatures and mussel gravid periods in Hat Creek and Fall River in 2006, with temperature data from other reaches for comparison

4.0 Conclusions and Recommendations

4.1. Mussel Reproductive Timing in the Lower Pit River Drainage

Anodonta in all reaches spawned and released glochidia asynchronously throughout most of the 2004 and 2005–06 field seasons, except from roughly late July to mid-October, and their glochidia were observed on fish during all months except September and October. Marked females that were reexamined in 2005–06 produced multiple, consecutive broods throughout the periods of gravidity. During the monitoring in freshwater aquaria, which extended from late March through September 2005, juvenile *Anodonta* dropped off of host fish during the months of April through July. Sixteen juvenile mussels were collected from aquarium tanks containing native hardhead, Sacramento pikeminnow, tule perch, Pit sculpin, and non-native green sunfish. Two sloughed-off glochidia that had not transformed into juveniles were also collected from the tanks containing tule perch and green sunfish.

Gonidea angulata in the Pit River reaches and Fall River (this species was not found in Hat Creek) spawned and released glochidia asynchronously from late March/early April to mid-July in 2004 and 2005, and beginning in late April 2006. In 2005, *G. angulata* glochidia were more abundant in stream drift samples collected during June in the Pit 4 Bypass, Pit River downstream of Pit 1 Powerhouse, and Fall River. In the Pit 1 Bypass, stream drift was not sampled in June, but glochidia were more abundant in samples collected in early May than in mid-April or mid-July. Glochidia were observed on fish from late March to late July/early August. Juvenile *G. angulata* dropped off of host fish during the months of June and July in 2005. Thirty juveniles were collected from three tanks of Pit sculpin, one juvenile was collected from a tank containing hardhead, and one juvenile was collected from a tank containing tule perch. Three sloughed-off glochidia that had not transformed into juvenile mussels were also collected from the hardhead tank and one glochidium was collected from a tank containing two mosquitofish.

The beginning of the non-gravid interval for *Anodonta* and the end of the gravid period for *G. angulata* occurred as (or shortly after) the hydrograph reached base flow and water temperatures peaked—around mid-July in 2004 and 2005. *Anodonta* resumed spawning in mid-to-late October in both years, when flows began to increase and water temperatures dropped below 13 C (55.4°F). *Gonidea angulata* resumed spawning the following spring under variable flow conditions and after water temperatures exceeded 10–12 C (50°–53.6°F).

Few gravid ***Margaritifera falcata*** were found during this study, and the timing of gravidity differed between river reaches and years. In the Pit 4 Bypass, *M. falcata* were gravid in early April and mid-June in 2004, when discharge was stable (200 cfs) and water temperatures ranged from 10°C to 16°C (50°–60.8°F); and throughout April in 2005, when discharges ranged from 200–2000 cfs and water temperatures averaged 12°C (53.6°F). In the Pit River downstream of Pit 1 Powerhouse, gravid females were not found in 2004, but were found from early June to early July in 2005. Those collected in early June had just spawned, and spawning occurred after a 5000-cfs drop in average daily discharge, when water temperature averaged 18°C (64.4°F). In lower Hat Creek, a single *M. falcata* spawned in early July 2005, but no other gravid *M. falcata*

were found in this reach. The glochidia of *M. falcata* were not observed in samples of stream drift or on fish collected in any river reach in 2005-06.

4.2. Discussion of Results

4.2.1. Anodonta

The prolonged gravid periods and short non-gravid intervals observed for *Anodonta californiensis* and/or *A. nutalliana* in the lower Pit River drainage have been observed in other North American *Anodonta* species (Lefevre and Curtis 1912; Heard 1975). Prolonged incubation periods, with glochidia carried over the winter and released in the spring, are considered typical of *Anodonta* and other long-term brooders (Ortmann 1911, 1912; Neves and Widlak 1988). In this study, however, incubation times were much shorter and glochidial release occurred throughout the year, even during the colder, winter months. Marked female *Anodonta* reexamined during this study produced multiple, consecutive broods throughout the gravid periods, and there was no evidence of abortion after handling. The appearance of the partially empty marsupia of some gravid *Anodonta* indicated that glochidia were released singly or in small groups over an extended period of time rather than all at once, as occurs for *M. falcata* (Murphy 1942; Jeanette Howard, Confederated Tribes of the Umatilla Indian Reservation, personal communication). Release of *Anodonta* glochidia was never directly observed, but some *Anodonta* species release glochidia in mucous threads or webs that entangle nearby fishes (Wood 1974). In this study, mucous threads were observed in samples of glochidia obtained from the marsupia of gravid females.

Given the asynchronous spawning and glochidial release observed for *Anodonta* in the Pit River, excystment of juvenile *Anodonta* was likely asynchronous as well. Some encysted *Anodonta* with characteristics indicative of juveniles (e.g., appearance of two adductor muscles [D'Eliscu 1972; Fisher and Dimock 2002]) were observed on fish during all seasons, and newly excysted juveniles were collected from the freshwater aquaria periodically from April through July. In the Pit River, asynchronous excystment of juveniles may have occurred throughout the observed parasitic periods. During the winter, however, colder water temperatures could have prolonged the duration of encystment and limited or prevented excystment (Zale and Neves 1982; Watters and O'Dee 2000).

The asynchronous, repetitive reproductive cycles of *Anodonta* may make them less vulnerable to seasonal pulsed flow events than the other Pit River mussel species. In the event that fertilization, encystment on host fish, or settlement of newly excysted juvenile mussels is reduced during a pulsed flow event, only a portion of the year class would be affected. In addition, *Anodonta* in the Pit River and elsewhere are host generalists that can encyst and transform on many different host species (D'Eliscu 1972; Trdan and Hoeh 1982). Host generalists are more opportunistic than host specialists, and have evolved to cope with random fluctuations in host population size (Watters 1997). The effects of pulsed flows on fish movements are not fully understood and are the subject of ongoing investigations, but if pulsed flows result in redistribution of fish species, *Anodonta* may be able to transform on any available host.

Water temperature differences among reaches and between years did not noticeably alter the duration of gravidity for *Anodonta* by more than two weeks, except in Fall River Lake. At this location, water temperature as high as 27.3°C (81.1°F) at one site may have caused the observed abortion of egg masses and premature onset of the non-gravid period. Thermal stress has been shown to cause abortion in other unionid species (Aldridge and McIvor 2003). Consequently, seasonal pulsed flows that lower water temperature below a certain critical threshold may be beneficial for *Anodonta* in lentic habitats. In lotic habitats, however, *Anodonta* may be exposed to higher water velocities during seasonal pulsed flows that can result in stranding or displacement of adults. Mussels stranded on the banks were found after recession of high-flow events during this study and in previous studies in the Pit River (Ellis 1996). In addition, *Anodonta* exposed to high water velocities can be easily dislodged from fine sediments and flushed downstream or temporarily exposed to predators, as was also observed during this study. Their thin shells are easily cracked or crushed, making them potentially easy prey for predators such as minks, river otters, and muskrats. Moreover, these thinner-shelled mussels may be more susceptible to desiccation while stranded (Matteson 1955).

Where suitable refuge from high velocities was available, such as the complex side channel habitats in the Pit River downstream of Pit 1 Powerhouse, many marked *Anodonta* were found in approximately the same location before and after pulsed or seasonal high-flow events, and gravid females continued to incubate eggs, embryos, or glochidia. Some *Anodonta* in these lower-velocity habitats, however, were found stranded on the shore after sudden drops in river discharge. Scouring or stranding caused by pulsed flows may pose a bigger threat to *Anodonta* than reproductive interference, especially in habitats without sufficient velocity refuges. In the South Fork Eel River in California, *Anodonta* were found almost exclusively in habitats that protected them from the highest flow-induced stresses (Howard and Cuffey 2003). The availability of velocity refuges in different river systems may determine if and to what degree *Anodonta* species are affected by pulsed flows.

4.2.2. Gonidea angulata

The spring/summer gravid period observed for *G. angulata* in the lower Pit River drainage and the prevalence of *G. angulata* glochidia in stream drift during this time has been observed in other unionid mussels that brood in all four gills and have unhooked glochidia (Neves and Widlak 1988; Howells 2000; Haag and Warren 2003). The number of broods produced by each female *G. angulata* during the gravid period could not be assessed by visual examination, because females that were sampled and reexamined in 2004 appeared to have aborted their marsupial contents and respawned soon after they were sampled. This tendency to abort in response to disturbance has been observed in other short-term brooders (Yeager and Neves 1986; Howells 2000; Haag and Warren 2003). The relatively short gravid period observed for *G. angulata* and the apparent spike in glochidial release towards the end of the gravid period, however, suggest that females of this species normally produce just one brood per year.

In the Pit River reaches and Fall River, *G. angulata* utilized more diverse habitats than *Anodonta* or *M. falcata*, and were found in locations where the other mussel species were absent. Their propensity to burrow deeply and anchor firmly in either fine or coarse substrate makes them

less susceptible to being flushed downstream during pulsed flow events than the other mussel species. In the event that *G. angulata* are scoured from their burrows or stranded on dry land, their thicker shells relative to *Anodonta* may also provide more protection from predators and/or desiccation (Matteson 1955). On the other hand, reproduction in *G. angulata* may be more impacted by pulsed flows than reproduction in *Anodonta*, because *G. angulata* have shorter gravid periods. Any adverse impacts associated with a seasonal pulsed flow event could affect a higher proportion of the *G. angulata* year class, especially if the high-event occurred during peak periods of spawning, glochidial release, or juvenile excystment.

In 2005, *G. angulata* glochidia were most abundant in stream drift samples collected during June in the Pit 4 Bypass, Pit River downstream of Pit 1 Powerhouse, and Fall River. Stream drift was not collected in the Pit 1 Bypass in June, but glochidia were more numerous in the drift in early May than in mid-April or mid-July. Glochidial release in this reach may have peaked during June as well, given that *G. angulata* gravid periods did not differ significantly between river reaches. Further evidence for a June peak in glochidial release was that percentages of fish infected with *G. angulata* glochidia were highest during June. The apparent peak in glochidial release observed in June, and the absence of infected fish in all of the Pit River reaches after late July, suggest that peak juvenile excystment in the Pit River drainage probably occurred from late June to late July. This estimated period of peak juvenile excystment was supported by the freshwater aquaria data. Excysted juvenile *G. angulata* were observed in siphonate samples from fish tanks during late June through July, but not during April, May, early June, or August. The June to July peaks in glochidial release and juvenile excystment observed in 2005 may have occurred in 2004 as well, since the duration of gravidity did not differ significantly between years.

In the Pit 1 Bypass, pulsed flows for channel maintenance/recreation occurred in mid-May, mid-July, and late August in 2004 and late May/early June, mid-July, and late August in 2005. The May/June pulsed flows may have occurred during the period of peak glochidial release, the mid-July pulsed flows likely occurred during the period of peak juvenile excystment, and the late August pulsed flows likely occurred when most juvenile mussels were settled on the stream bottom.

Pulsed flows that occur during periods of glochidial release could reduce encystment potential if interactions between host fish and glochidia are reduced. The degree to which pulsed flows affect glochidia/fish interaction may depend on the species of host fish, as well as the mode of glochidial release. For example, the planktonic glochidia of *G. angulata* were widely dispersed and abundant in the water column during peak glochidial release and could thus attach to any fish near or downstream of the mussel bed, whereas the heavier, non-planktonic glochidia of *Anodonta* probably had to rely on close proximity of host fishes for successful encystment (Wood 1974; Michaelson and Neves 1995). Pulsed flows that result in movement of host fish out of adult mussel habitat, therefore, might impact *Anodonta* encystment more than *G. angulata* encystment. On the other hand, the planktonic glochidia of *G. angulata* may be more susceptible to getting swept into unsuitable environments during high flows than the non-planktonic glochidia of *Anodonta* (Wood 1974). In this study, however, percentages of fish infected with *G. angulata* glochidia in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse were

equal to or higher than those infected with *Anodonta* glochidia during high- and low-flow conditions in May and June 2005. Sampling of mussels, stream drift, and fish was limited in the Pit 1 Bypass, where scheduled pulsed flow events occurred, so any potential impacts on glochidial release/encystment associated with these pulsed flows could not be evaluated for *Anodonta* or *G. angulata*.

Pulsed flows that occur immediately after juvenile excystment could displace settling juveniles before they can burrow into or attach to the substratum (Holland-Bartels 1990). Recruitment of mussels in three regulated rivers in Kentucky was thought to be limited by unnaturally high discharges that occurred during periods of juvenile excystment (Hardison and Layzer 2001). The mid-July and late August pulsed flows in the Pit 1 Bypass could have disrupted settlement of juvenile mussels during the two to three days when discharge spiked above the 150-cfs base flow. The proportion of juveniles potentially displaced was probably limited by the short duration of the pulsed flows. Prolonged and more frequent pulsed flows would likely have a greater impact on *G. angulata* recruitment.

The mid-July pulsed flow event in the Pit 1 Bypass reach may have been more detrimental to juvenile mussels than the late August pulsed flow event. Most juveniles dropped off of their hosts by early August 2005, so juveniles were probably larger and more developed in late August than in mid-July. The potential for settled juveniles 3–6 weeks old to be displaced by pulsed flows would likely depend on the magnitude of the flows and the size and anchoring ability of the juveniles. Discharge data for the Pit 1 Bypass were limited in 2004 and 2005, and the anatomical characteristics of juvenile *G. angulata* have not yet been described. This information, as well as juvenile growth rate data, will be important for quantifying potential losses associated with individual pulsed flow events in the Pit 1 Bypass.

In 2005, the glochidia of *G. angulata* were encysted on many different species of fish and transformed on three species of fish in three different families. Although more juvenile *G. angulata* were collected from tanks of Pit sculpin than from tanks containing other fish species, there were three times more Pit sculpin monitored in the aquaria (three tanks of 10 fish) than other fish species during the period of peak excystment in June and July. In addition, all of the Pit sculpin were collected during mid-to-late June, when more glochidia were in the water column. Consequently, these fish may have had higher numbers of encysted glochidia than fish collected prior to June. Although Pit sculpin are clearly important hosts for *G. angulata*, the relative importance of other host fish species to *G. angulata* could not be accurately determined in this study, because the presence and number of *G. angulata* glochidia on the fish held in aquaria was not known.

4.2.3. Margaritifera falcata

The reproductive data obtained for *M. falcata* in this study were limited compared to the data obtained for *Anodonta* and *G. angulata*, because gravid *M. falcata* were rarely found. In 2004, males made up 58% of the *M. falcata* collected in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse, which might indicate that sex ratios in the Pit River are skewed slightly towards males. This could explain the low number of gravid mussels found in the Pit River

reaches. Even if sex ratios were similarly skewed in Hat Creek and only 42% of the mussels collected per month were female, numbers of gravid females collected in this location should have been much higher given the large sample sizes. Assuming 42% of the 60 adult *M. falcata* collected in Hat Creek in July were female (25), then only 4% of those females were gravid. It may be that the majority of the female population did not spawn and was resting in 2005–06, as described for European populations of *Margaritifera margaritifera* (Bauer 1987a). Females may have had inadequate nutrition or environmental variables may have been unsuitable for spawning during this study. These potential explanations warrant further investigation.

In 2005, reproductive timing in *M. falcata* was not consistent either between rivers or among reaches, with the onset of spawning occurring in early April in the Pit 4 Bypass, early June in the Pit River downstream of Pit 1 Powerhouse, and early July in Hat Creek. Between-river differences in the timing of spawning and glochidial release in North American and European species of *Margaritifera* have mostly been attributed to water temperature, with spawning occurring earlier in warmer rivers (Meyers et al. 1980; Hastie and Young 2003). Spawning *M. falcata* have been observed in April in the South Fork Eel River in California, when water temperatures exceeded 10°C (50°F) (J. Howard, Confederated Tribes of the Umatilla Indian Reservation, personal communication), and mid-May in the Truckee River, California, when water temperatures exceeded 7.2°C (45°F) (Murphy 1942). In the Pit River, water temperatures were similar in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse in 2005, so between-reach differences may have been more related to river discharge, a combination of river discharge and temperature, or other habitat variables that affected the microclimate within the mussel beds.

Periods of juvenile excystment for *M. falcata* could not be estimated in this study, because infections of *M. falcata* glochidia on fish were not observed. Unlike the glochidia of *Anodonta* and *G. angulata*, which do not appear to grow while attached to their hosts, the glochidia of *Margaritifera* species grow significantly after encystment and are often highly visible without magnification (Murphy 1942; Meyers and Millemann 1977; Fustish and Millemann 1978; Karna and Millemann 1978; Meyers et al. 1980). It is likely, therefore, that *M. falcata* glochidia would have been detected if fish infected with these glochidia were collected.

The fact that no fish with infections of *M. falcata* were collected could have been due to limitations in the fish sampling methods. For example, electroshocking was not permitted in Hat Creek, where *M. falcata* were most abundant, because of the presence of the fully protected rough sculpin; and electroshocking in the Pit River reaches was necessarily restricted to nearshore habitats, where no or few rainbow trout (a known *M. falcata* host) were collected during periods of *M. falcata* gravidity. Alternatively, the lack of detection of *M. falcata* glochidia on fish collected in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse could reflect a truly low incidence of infection. This latter possibility is supported by the low percentages of females that were observed to be spawning in all reaches, and the lack of *M. falcata* glochidia collected in stream drift before, during, and after observed spawning periods. The methods used to sample stream drift in this study have been successfully employed by other researchers to detect the timing and duration of *Margaritifera margaritifera* glochidial release (Hastie and Young 2003), and the nets used in this study were functioning properly (i.e., were not clogged

with sediment or vegetation) during the expected periods of glochidial release in the Pit River downstream of Pit 1 Powerhouse and Hat Creek. Regardless of the reasons for the lack of detection of *M. falcata* infections, the probability of detection could be improved by collecting fish from all habitats, including deeper runs or pools, and by increasing the abundance and diversity of fish collected.

The duration of the parasitic period for *M. falcata* has been reported to extend for 36 days when daily water temperatures averaged 14.2 °C (57.6°F) (Murphy 1942), but the duration of encystment may be shorter at higher water temperatures (Zale and Neves 1982; Watters and O'Dee 2000). In the warmer waters of the Pit River, juvenile excystment (assuming that at least some host fish were infected with *M. falcata* glochidia) may have occurred less than 36 days after encystment, and encystment may have occurred in late April, May, June, or July, depending on location. Water temperatures in Hat Creek were warmer than 14.2° but cooler than in the Pit River during the observed spawning event in July, so encystment could have occurred in late July or August, and excystment could have occurred in late August or September. Glochidial release and encystment in *M. falcata* can occur within a matter of days (Murphy 1942), so the duration of excystment may be similarly brief and dependent on water temperature.

The short, synchronized periods of spawning, glochidial release, and juvenile excystment and the dependence of these events on water temperature and/or flow (Zale and Neves 1982; Watters and O'Dee 2000; Hastie and Young 2003) probably make *M. falcata* more vulnerable to seasonal pulsed flows or other abrupt alterations in river discharge than *Anodonta* or *G. angulata*. The variability in reproductive timing observed for *M. falcata* in the Pit River reaches also suggests an increased sensitivity to river discharge and/or water temperature fluctuations. In addition, their preference for higher water velocities may make them more susceptible to shell erosion and displacement during periods of high flow. The low abundance of *M. falcata* in the turbulent and turbid waters of the Pit River reaches compared to the relatively smoothly flowing, clear waters of Hat Creek may reflect a preference for more stable habitats. In the South Fork Eel River, Howard and Cuffey (2003) reported that high discharges provide more of a constraint on the distribution and persistence of *M. falcata* than do summer low flows. This is likely true for Pit River *M. falcata* as well, since minimum flow requirements have been established for all regulated reaches. The persistence of *M. falcata* in the Pit River may depend on successful management strategies that minimize abrupt and significant discharge fluctuations, especially during periods when *M. falcata* adults may be spawning and releasing glochidia, and juveniles may be excysting from their fish hosts.

4.3. Evaluation of Study Methods

The methods used to determine reproductive timing in this study proved effective, but more time-consuming and costly than anticipated. Mussel collection and sampling were useful for pinpointing the onset of spawning and determining the duration of gravid periods. Mussel collection was also easier to do than fish sampling under certain circumstances, for instance, in areas where electroshocking was not allowed. Mussels were disturbed and removed from their

positions during collection, however, and sampling was invasive. In addition, mussel collection was often not possible during periods of high river flow.

The combination of stream drift and fish sampling was effective in determining periods of glochidial release and encystment on host fish during periods of high and low flow, and was less invasive than mussel collection and/or sampling. Stream drift sampling, however, was not useful for determining glochidial release in *Anodonta*. In addition, drift nets could quickly become clogged and cease to sample drift under high-flow or high-turbidity conditions, and analysis of drift samples was very time-consuming. In rivers where electroshocking is not restricted and river access is not limited, the combination of stream drift and fish sampling may prove the most cost-effective means of determining periods of glochidial release and encystment on host fish for all mussel species.

The methods used to estimate periods of juvenile excystment and determine host fish species were effective, but somewhat limited. A larger and more sophisticated aquaculture system would hold more fish, improve temperature regulation, and allow researchers to compare the timing of excystment between tanks of naturally infected or artificially infected fish held at different water temperatures.

4.4. Recommendations

In regulated reaches of the Pit River, pulsed flows that occur during September and October would be less likely to interfere with mussel reproduction and settlement of newly excysted juveniles than those that occur during April through August, when adults of all mussel species may be spawning and/or releasing glochidia, and juveniles may be dropping off of their hosts to settle on the stream bottom. Seasonal pulsed flows in the Pit 1 Bypass, which have only been in effect since 2003, and maintenance outages in the Pit 4 Bypass and Pit River downstream of Pit 1 Powerhouse should be planned before or after peak periods of glochidial release and/or juvenile excystment for *G. angulata* and *M. falcata*. These critical reproductive events may occur for both species during the months of June, July, and August.

This study on mussel reproductive timing did not attempt to measure or describe the effects of pulsed flows on mussel reproduction or physiology. The license-prescribed flushing/whitewater flows in the Pit 1 Bypass, however, have the potential to adversely impact recruitment in *G. angulata* and the few *M. falcata* present in the reach by reducing interactions between host fish and glochidia during glochidial release, and by preventing settlement of newly excysted juveniles. Newly excysted juveniles may be especially vulnerable to scouring flows, and scheduling the summer pulsed flows after August or preferably September, if possible, would allow juveniles time to grow to a size where they will be better able to withstand such flows.

Continued monitoring and assessment of specific impacts of pulsed flows on freshwater mussels, especially in the Pit 1 Bypass, is recommended. Effects of the pulsed flows on habitat and displacement of adults should also be monitored. Because the timing of mussel reproduction observed in the Pit River may differ in other California drainages, reproductive

data from rivers throughout the state should be gathered and compared to determine if site-specific, regional, or statewide flow recommendations would be appropriate.

4.5. Benefits to California

The energy demands of California are great and ever increasing, and hydroelectric projects continue to supply the state with significant amounts of energy. Relicensing of these projects requires that potential impacts on fish and aquatic biota be considered in the planning and implementation of flow regimes. Awareness of freshwater mussels in western rivers is increasing, and managing regulated waterways to ensure the long-term health of mussel populations is likely to become increasingly important.

This report benefits California by increasing the knowledge base regarding native freshwater mussel biology and ecology. The research provides the groundwork for future monitoring and assessment of pulsed flows in the Pit River drainage, and for research of mussel reproductive timing in other regulated and unregulated rivers within California. Project experience with different sampling and monitoring methods should help researchers to fine-tune future work aimed at assessing reproductive timing of mussels in other California drainages.

Data from this and future studies on mussel reproductive timing will help managers of hydroelectric facilities, industry regulators, and policy makers assess the potential impacts of seasonal pulsed flow releases on reproduction of California mussel populations and plan these events to minimize adverse effects on mussel reproduction.

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6.0 Glossary

Anodonta	Mussel genus in the family Unionidae. In the Pit River drainage, species in this genus may include *A. californiensis*, *A. nuttalliania* (a.k.a. *wahlamatensis*), and *A. oregonensis* (rare in Pit River, but found in a few locations in Fall River).
Brood	The young of mussels. In this report, refers to eggs, embryos, or glochidia.
Brooding	The act of incubating eggs, embryos, or glochidia in the female marsupia. In this report, the brooding period is the same as the incubation or gravid period.
Conglutinate	Sticky mass of mature glochidia and unfertilized eggs that dangles from the siphons of certain species of mussels (e.g., *Margaritifera falcata*) during glochidial release, presumably to attract host fish and increase the likelihood of contact between glochidia and host fish.
Encystment	The attachment of glochidia to host fish, when host tissue forms a cyst around the site of attachment.
Excystment	When transformed juvenile mussels break out of the cyst that surrounds them and drop off of their hosts.
Glochidia	Larvae of freshwater mussels that are released from the female mussel to parasitize a host, which is typically a fish, before transforming into a juvenile mussel.
Glochidial release	When mature mussel glochidia are expelled from the female marsupia, either singly through pores in the marsupia, or as a conglutinate. This process is also referred to as spat.
Gonidea angulata	Mussel species in the family Unionidae. This is a unique species assigned to its own genus and found only in western North America.
Gravid	Carrying developing young or eggs. In this report, gravid mussels are those that are brooding (i.e., incubating) eggs, embryos, or glochidia, or have partially empty marsupia containing clear fluid with some as yet unreleased or partially degraded glochidia. Gravidity is the state of being gravid.
Host fish	A species of fish that does not have a natural immunity to glochidial infection and serves as a host for mussel glochidia throughout the transformation process.

Margaritifera falcata	Mussel species in the family Margaritiferidae. Also referred to as *M. m. falcata*, Gould 1850.
Marsupia	The gills of female mussels that are modified to form brood pouches, where eggs, embryos, or glochidia are incubated during gravidity. Singular: marsupium.
Parasitic period	When glochidia are encysted on host fish. In this report, refers to the season or seasons when each mussel species is found on collected fish.
Spawning	When females move eggs into their marsupia and males expel sperm into the water column.
Pulsed flows	Flows that are released from hydroelectric plants as byproducts of load-following electrical production, for recreational use, or for sediment/ vegetation management.
Transformation	When glochidia attached to host fish transform into juvenile mussels.