Spawning, Fertilization, and Larval Development of *Potamocorbula amurensis* (Mollusca: Bivalvia) from San Francisco Bay, California[1]

MARY HELEN NICOLINI[2,3] AND DEBORAH L. PENRY[2]

ABSTRACT: In *Potamocorbula amurensis* time for development to the straight-hinge larval stage is 48 hr at 15°C. *Potamocorbula amurensis* settles at a shell length of approximately 135 μm 17 to 19 days after fertilization. Our observations of timing of larval development in *P. amurensis* support the hypothesis of earlier workers that its route of initial introduction to San Francisco Bay was as veliger larvae transported in ballast water by trans-Pacific cargo ships. The length of the larval period of *P. amurensis* relative to water mass residence times in San Francisco Bay suggests that it is sufficient to allow substantial dispersal from North Bay to South Bay populations in concordance with previous observations that genetic differentiation among populations of *P. amurensis* in San Francisco Bay is low. *Potamocorbula amurensis* is markedly euryhaline at all stages of development. Spawning and fertilization can occur at salinities from 5 to 25 psu, and eggs and sperm can each tolerate at least a 10-psu step increase or decrease in salinity. Embryos that are 2 hr old can tolerate salinities from 10 to 30 psu, and by the time they are 24 hr old they can tolerate the same range of salinities (2 to 30 psu) that adult clams can. The ability of *P. amurensis* larvae to tolerate substantial step changes in salinity suggests a strong potential to survive incomplete oceanic exchanges of ballast water and subsequent discharge into receiving waters across a broad range of salinities.

Potamocorbula amurensis (Schrenck, 1867) was first introduced to San Francisco Bay in 1986 from estuaries in eastern Asia, and its numbers have increased dramatically in all regions of the bay (Carlton et al. 1990). It has displaced some members of the previous benthic community (Nichols et al. 1990) and is now a dominant component in some areas of the bay (Nichols et al. 1990, Alpine and Cloern 1992). The high population densities and rapid individual and population growth rates of *P. amurensis* within San Francisco Bay and its inferred ability to exploit a vari-

ety of food resources (e.g., phytoplankton, bacteria, detritus, larvae) (Alpine and Cloern 1992, Werner and Hollibaugh 1993, Kimmerer et al. 1994) suggest that it has become an important consumer component in carbon and nutrient cycling in the bay (Hollibaugh and Werner 1991). Understanding the life history of *P. amurensis* is therefore central to understanding the ecology of populations in San Francisco Bay.

Most research on *P. amurensis* in San Francisco Bay has focused on adults. Field studies indicate that some populations of *P. amurensis* spawn throughout the year and that newly settled clams become reproductive within a few months (Parchaso 1995), but beyond that there is little information on reproduction, development, or survival of early life history stages of *P. amurensis* in San Francisco Bay. We have induced *P. amurensis* to spawn in the laboratory and here describe development from fertilization through settlement.

[2] Department of Integrative Biology, University of California, Berkeley, California 94720-3140 (phone: 510-643-5915; fax: 510-643-6264; E-mail: dpenry@socrates.berkeley.edu).
[3] Current address: U.S. Geological Survey, Placer Hall, 6000 J Street, Sacramento, California 98519.

TABLE 1

Collection and Maintenance of Laboratory Brood Stock

COLLECTION DATE	LOCATION[a]	FIELD SALINITY (psu)	HOLDING TIME IN LABORATORY[b] (weeks)	HOLDING SALINITY[c] AFTER ACCLIMATION (psu)
19 December 1996	Sta. 30	18	12	25
19 March 1997[d]	Sta. 33	18	12	5, 15, 25
17 June 1997	Sta. 29	31	4	2, 12, 22, 32
2 October 1997	Sta. 29	32	4	32
3 October 1997	Sta. 30	11	4	15
19 May 1998	Sta. 30	15	3	15
22 June 1998	Sta. 33	15	1	15

[a] Coordinates of USGS stations in San Francisco Bay: Sta. 29 (37° 34.8′ N, 122° 14.7′ W), Sta. 30 (37° 33.3′ N, 122° 11.4′ W), and Sta. 33 (37° 30.5′ N, 122° 7.3′ W).
[b] Length of time clams were held after collection before being used in spawning inductions.
[c] Salinity at which clams were maintained after step-wise acclimation from the field salinity.
[d] Clams were used in spawning on 28 May 1997, 11 June 1997, and 17 July 1997.

We also present the results of tests of salinity tolerances of gametes, embryos, and larvae. In estuaries fluctuations in salinity on tidal and seasonal time scales are one of the most important physiological and ecological factors affecting survival of estuarine organisms (Beadle 1972). In San Francisco Bay the range of *P. amurensis* extends from almost freshwater (<1 psu) to full-strength seawater (about 32–33 psu) (Carlton et al. 1990). Adult clams tolerate weeks of exposure to salinities ranging from from 0 to 35 psu, but long-term survival is highest at salinities from 5 to 25 psu (pers. obs.). The fact that populations of *P. amurensis* in North San Francisco Bay spawn in both the wet season and the dry season and populations in South San Francisco Bay spawn throughout the year (Parchaso 1995) suggests that the survival of gametes, larvae, and juveniles requires the ability to tolerate substantial fluctuations in salinity as well.

MATERIALS AND METHODS

Collection and Maintenance of Adult Clams for Brood Stock

Adult *P. amurensis* were collected from San Francisco Bay (Table 1). Some clams were maintained at field salinities, and some were transferred through a series of salinity steps (3 to 5 psu, every 1 to 4 days) to acclimate them to other salinities (2, 5, 10, 15, 25, and 30 psu). Natural, filtered seawater from Bodega Bay, California, was diluted with deionized water to obtain water of various salinities. Brood stock clams were held from 1 to 12 weeks at 15°C. From December 1996 through April 1998 all brood stock were in aquariums with a fine sand sediment. Beginning in May 1998, all clams were held in 1-liter beakers with no sediment to facilitate adult stock maintenance. Every 1 to 3 days clams were fed a mixture of natural surface sediment flocs (Decho and Luoma 1991) and the laboratory-cultured phytoplankton *Rhodomonas salina, Isochrysis galbana,* and *Phaeodactylum cornutum.*

Induction of Spawning and Fertilization

Spawning was induced by transferring clams to filtered water (with *no* change in salinity) for 24 to 32 hr. The physical shock of being placed in filtered water is similar to other spawning inductions with physical stress such as heat shock and rough handling (Loosanoff and Davis 1963). About 30 individual clams were placed in separate 400-ml beakers, and the water was changed after 3 to 5 hr. Spawning typically occurred overnight. If spawning did not take place the water was

changed again, and spawning usually occurred by early afternoon of the second day. Male clams produced enough sperm to turn a 300-ml volume of clear seawater to a thin, white consistency. Female clams released a small mound of light-cream-colored eggs. Typically the mound of eggs was found near the posterior end of the clam adjacent to the siphons.

Gamete viability was assessed under a compound microscope at 400×. Viable sperm were active and motile, and viable oocytes had complete, solid yolks and complete outer membranes. The gametes were then subsampled to estimate the oocyte and sperm stock available for fertilization. We used a density of 10 oocytes per milliliter of seawater and an oocyte to sperm ratio of 1:5. Sperm were added to the oocytes, and the fertilization containers were gently mixed with a perforated Plexiglas plunger every 5 min. Once fertilization was evident (i.e., when a minimum of 70% of oocytes had polar bodies) excess sperm were removed by gently backwashing through a 65-μm Nitex mesh.

Larval Development

The timing of progress through developmental stages (e.g., fertilized egg, two-cell stage, four-cell stage, blastula, trochophore, straight-hinge veliger, newly metamorphosed juvenile [Loosanoff and Davis 1963]) was established with clams spawned in the first induction (March 1997, Table 1). Fertilization success and embryological development were assessed under a compound microscope at 400×. Embryological development was monitored for 48 hr after fertilization, and larval and juvenile development were followed for 4 weeks. All developing embryos, larvae, and juveniles were maintained at 15°C on a 16:8 light:dark cycle. Larvae were fed a mixture of *Isochrysis galbana* and *Rhodomonas salina* after 48 hr, and the water was changed every other day. Transfer from the controlled-environment chamber (15°C) to the laboratory (22°C) for observation resulted in no obvious developmental problems. Larvae in all stages of development

were documented using still photos and video images.

Salinity Experiments with Gametes and Embryos

The first salinity tolerance experiment was performed in May 1997. Spawning was induced using clams collected in March 1997. Clams were acclimated to 5, 15, and 25 psu and held at these salinities for 1 week before spawning. Spawning was always induced at the same salinities in which clams were held. Ten clams were placed in separate 400-ml beakers, and the water was changed after 5 hr. Spawning occurred overnight in the 15-psu treatment (one male) and at 25 psu (three males and one female). No clams spawned at 5 psu. The eggs at 25 psu were fertilized with sperm from 15- and 25-psu treatments.

At 48 hr, some of the straight-hinge veliger larvae that developed in the 15-psu treatment were transferred to 10 psu, and some of the larvae that developed at 25 psu were transferred to 32 psu. There was no initial count of larvae transferred into each salinity, so we cannot quantify survival, but many of the larvae transferred to differing salinities survived and continued to develop normally.

The second salinity tolerance experiment was conducted in June 1997 with clams collected in March 1997. Clams were acclimated to and then held at 5, 15, and 25 psu for 4 weeks before spawning was induced at these salinities. Gametes spawned at 5, 15, and 25 psu were transferred to 5, 10, 15, and 25 psu for fertilization. Replicates of each fertilization cross were assessed at 44 hr for normal development to straight-hinge veliger larvae.

The third salinity tolerance experiment was conducted in June 1998 with brood stock collected in May 1998. These clams were held at 18°C for 12 hr before spawning, and spawning induction was conducted at 15 psu with 30 individuals. Gametes from this spawn induction in June 1998 were used for fertilization and postfertilization salinity tolerance experiments.

Fertilization and postfertilization salinity

TABLE 2

SPAWNING INDUCTIONS

| COLLECTION DATE | DATE OF SPAWN INDUCTION | MEAN LENGTH OF SPAWNED CLAMS (μm) | | SPAWNING SALINITY (psu) | SPAWNING OBSERVED | |
		MALE	FEMALE		MALE	FEMALE
19 December 1996	11 March 1997	N/A[a]	N/A	25	1	1
19 March 1997	28 May 1997	14	0	15	1	0
		14	15	25	3	1
19 March 1997	11 June 1997		13	5	0	3
		13.5	15	15[b]	2	1
			16	15	0	1
			14	25	0	2
19 March 1997	16 July 1997	14.5		5	2	0
		14	13	15	1	2
			11	25	0	1
17 June 1997	16 July 1997	—	—	2, 12, 22, 32	0	0
2 October 1997	4 November 1997	—	—	32	0	0
3 October 1997	4 November 1997	—	—	15	0	0
19 May 1998	9 June 1998	N/A	N/A	15	2	4
22 June 1998	30 June 1998	N/A	N/A	15	1	1

[a] N/A, not available.
[b] Transferred from 5 to 15 psu 1 week before spawn induction.

tolerance experiments were conducted with three distinct developmental stages at salinities of 2, 10, 15, 25, and 30 psu. Gametes, 2-hr-old embryos (after two to three cell cleavages), and 12-hr-old ciliated blastulas were transferred from 15 psu to 2, 10, 15, 25, and 30 psu to test for fertilization success and development with a salinity stress. All treatments were run in triplicate. A sperm to egg ratio of 5 : 1 (fertilization treatment) or an embryo concentration of approximately two to three embryos per milliliter (development treatments) was used. The end point of all these salinity exposures was normal development to straight-hinge veliger larvae at 40 hr.

RESULTS

Spawning and Fertilization

Potamocorbula amurensis can produce viable gametes in salinities ranging from 5 to 25 psu (Table 2). Oocytes were negatively buoyant, and the number of oocytes released per female was quite variable. For example, in the 9 June 1998 spawning induction fe-

males produced from 45,000 to about 220,000 viable oocytes, and the number produced was independent of female size. Oocyte diameter generally ranged between about 60 and 70 μm (average = 68 μm). Sperm ranged in length (head to tail) from about 1 to 2 μm (average = 1.5 μm) and remained active for up to 12 hr at 15°C.

The timing of development in *P. amurensis* was determined at 15°C (Figure 1a–d). Polar bodies were apparent within 15 min after the introduction of active sperm, and first cleavage was first noticed about 45 min after the appearance of polar bodies. Ciliated blastulas were visible 12 hr after fertilization, and most embryos had reached the trochophore stage by 24 hr (Figure 1a) and the straight-hinge stage by 48 hr (Figure 1b).

Larval Growth, Settlement, and Metamorphosis

On day 5 after fertilization larvae averaged 102 μm in length (SD = 6 μm), and by day 10 they averaged 128 μm (SD = 25 μm). Larvae swam actively in the water column from day 2 to day 7. After day 7 veligers
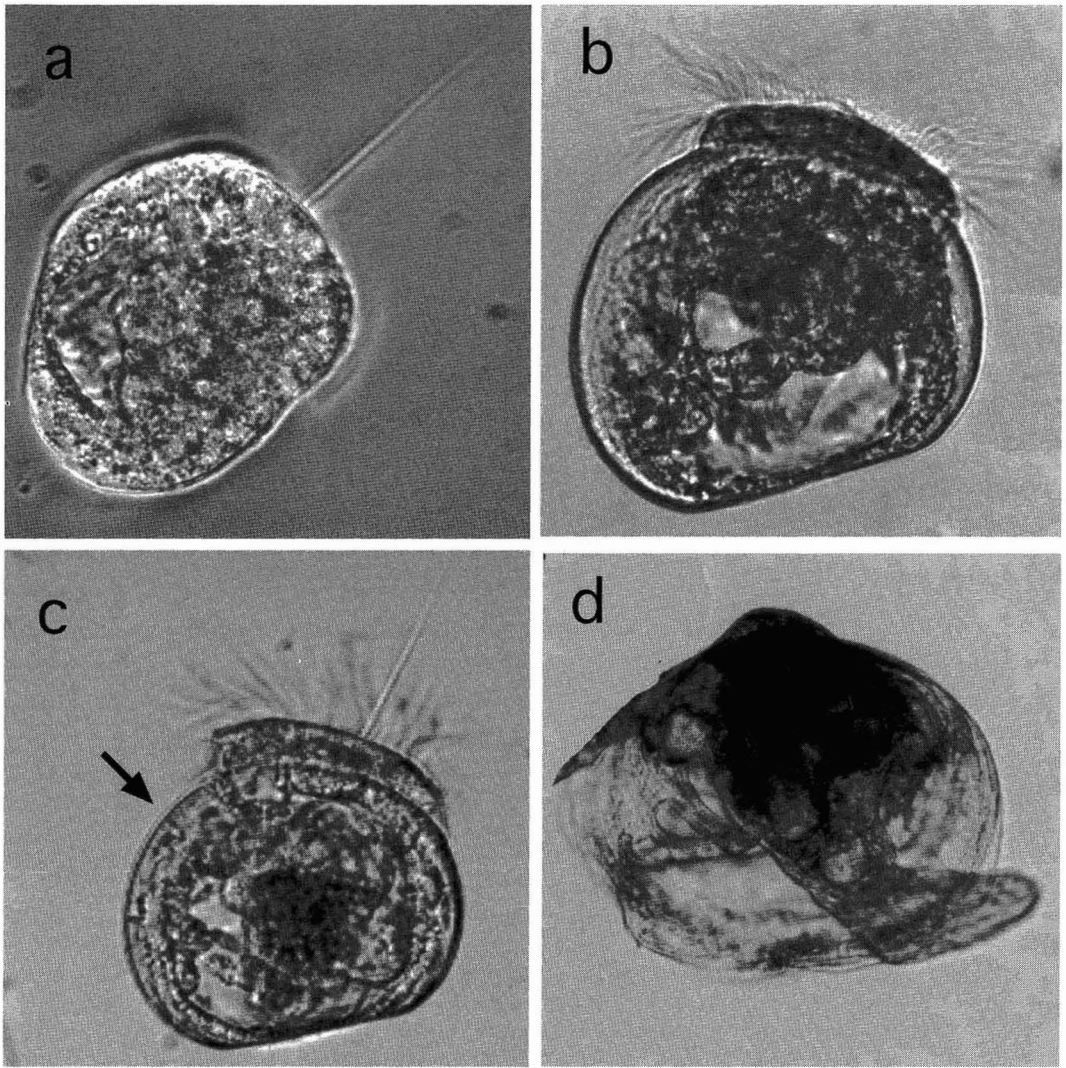
FIGURE 1. *a. Potamocorbula amurensis* trochophore, 24 hr after fertilization, approximate length 70 µm. The apical tuft is visible as well as the characteristic encircling ciliary band. *b.* At 48 hr after fertilization, straight-hinge veliger larvae swim actively and begin feeding. This image shows the rows of compound cilia around the edge of the oval velum. Approximate length is 75 µm. *c.* Veliger larva 4 days after fertilization, approximate length 100 µm. The edge of the larval shell (indicated by arrow) is more easily seen in this image. *d.* Juvenile, 26 days after fertilization, approximate length 300 µm.

swam intermittently and when not swimming were observed inactive on the bottom of the rearing container with their velar lobes retracted. From day 7 to day 17 veligers actively filtered phytoplankton and underwent further internal differentiation. All internal organs, pumping heart, and digestive system could be viewed through the translucent shells. By day 15 larvae were 135 µm (SD = 33 µm) in length on average and began to settle 17 to 19 days after fertilization. The first larvae to settle and metamorphose (on day 17) were the ones cultured at 15 psu.

Cues from adults do not appear to be

necessary for settlement and metamorphosis. Larvae settled in clean laboratory glassware containing seawater that was unlikely to have carried any chemical cues from adults. Seawater used in the rearing containers was collected from Bodega Bay, a bay north of San Francisco Bay without a population of *P. amurensis,* and by the time larvae began to settle, the seawater in the rearing containers had been changed at least seven or eight times.

At metamorphosis the larva absorbed its velar lobes and simultaneously developed its foot. A veliger larva with a partial velum and an extended foot was observed. Upon settlement adhesive mucus was secreted by the foot, rendering the entire foot "sticky." Newly settled clams attached to the bottom as well as to one another. Byssal threads were also observed holding several juveniles together in clumps. Metamorphosed clams moved about actively by pushing with the extended foot and using the foot to burrow. By day 27 (10 days after settlement) clams were 379 μm in length (SD = 67 μm). Fused siphons and gills appeared at metamorphosis. Inhalant and exhalent siphons became apparent within 3 to 4 weeks after metamorphosis. Also at 3 to 4 weeks development of

the chambered gill cavity became apparent, and movement of the ctenida could be viewed through the translucent shell.

Salinity Tolerance Experiments with Gametes and Embryos

The first salinity tolerance experiment (28 May 1997) was conducted with gametes produced by clams collected in March 1997. We observed that sperm could tolerate a 10-psu increase in salinity from 15 to 25 psu. Two crosses were performed: (1) sperm produced by a male at 15 psu were crossed with eggs produced by one female at 25 psu and development took place at 15 psu; and (2) the combined sperm from three males at 25 psu were crossed with eggs from the one female and development took place at 25 psu. In all experiments fertilization success was measured by normal development to straight-hinge larvae. Fertilization and subsequent embryo and larval development were successful at both 15 and 25 psu (Table 3).

The second salinity tolerance experiment (16 July 1997) was conducted with gametes produced by clams collected in March 1997. Clams produced viable sperm at 5 and 15 psu and viable oocytes at 15 and 25 psu (Table

TABLE 3

FERTILIZATION CROSSES: EGGS AND SPERM WERE TAKEN FROM THE SALINITIES INDICATED IN THE COLUMN OF FERTILIZATION CROSSES, AND FERTILIZATION WAS THEN ATTEMPTED AT THE "FERTILIZATION SALINITY"; FERTILIZATION SUCCESS WAS ASSESSED BY NORMAL DEVELOPMENT TO STRAIGHT-HINGE LARVAE

DATE OF SPAWN INDUCTION	FERTILIZATION CROSSES	FERTILIZATION SALINITY (psu)	FERTILIZATION SUCCESS
11 March 1997	Eggs from 25 psu × sperm from 25 psu	25	Yes
28 May 1997	Eggs from 25 psu × sperm from 25 psu	25	Yes
	Eggs from 25 psu × sperm from 15 psu	15	Yes
11 June 1997	Eggs from 5 psu × sperm from 15 psu	5	Yes
	Eggs from 15 psu × sperm from 15 psu	15	Yes
	Eggs from 25 psu × sperm from 25 psu	25	No
16 July 1997	Eggs from 15 psu × sperm from 5 psu	15	Yes
	Eggs from 15 psu × sperm from 5 psu	10	Yes
	Eggs from 15 psu × sperm from 5 psu	5	Yes; marginal
	Eggs from 25 psu × sperm from 5 psu	25	No
	Eggs from 25 psu × sperm from 5 psu	15	Yes
	Eggs from 25 psu × sperm from 5 psu	5	Yes; marginal
9 June 1998	Eggs from 15 psu × sperm from 15 psu	15	Yes
30 June 1998	Eggs from 15 psu × sperm from 15 psu	15	Yes; marginal

TABLE 4

PERCENTAGE FERTILIZATION SUCCESS FOR 16 JULY 1997 FERTILIZATION CROSSES
(FERTILIZATION SUCCESS WAS MEASURED BY NORMAL DEVELOPMENT TO STRAIGHT-HINGE LARVAE)

FERTILIZATION CROSSES	FERTILIZATION SALINITY (psu)	% FERTILIZATION (Rep. A)	% FERTILIZATION (Rep. B)	AVERAGE % FERTILIZATION
Eggs from 15 psu × sperm from 5 psu	15	93	94	94
Eggs from 15 psu × sperm from 5 psu	10	71	73	72
Eggs from 15 psu × sperm from 5 psu	5	8	9	9
Eggs from 25 psu × sperm from 5 psu	25	0	0	0
Eggs from 25 psu × sperm from 5 psu	15	67	76	72
Eggs from 25 psu × sperm from 5 psu	5	0	1	1

TABLE 5

SALINITY TOLERANCE EXPERIMENT WITH GAMETES AND EMBRYOS (JUNE 1998)

DEVELOPMENTAL STAGE	SALINITY (psu)					
	2	10	15	20	25	30
Fertilization (gamete)			×	×	×	
Early embryos (two to three cleavages, 2 hr postfertilization)		×	×	×	×	×
Ciliated blastulas (12 hr postfertilization)	×	×	×	×	×	×

Note: Spawning was induced at 15 psu, and then gametes, early embryos, and ciliated blastulas were transferred from 15 psu to the indicated salinities. Xs indicate salinities at which normal development to straight-hinge veliger larvae was observed after the transfer.

2). Six crosses were performed (Table 3). Percentage of successful fertilization (based on an average of two replicates) was highest at 10 and 15 psu and declined substantially at 5 and 25 psu (Table 4).

A third salinity tolerance experiment was conducted using gametes produced in June 1998 (Table 3). Of the 30 clams selected for spawn induction, four females and two males released gametes. One of the four females produced nonviable oocytes with large vacuoles within the yolk, and most were misshapen without complete outer membranes. The other three females produced viable oocytes, and the two males produced active sperm. Oocytes from the three females and sperm from the two males were pooled to produce one stock of oocytes and one stock of sperm.

Gametes spawned at 15 psu were able to undergo fertilization and develop to straight-hinge larvae at 15, 20, and 25 psu (Table 5). Embryos that had undergone only two to three cell cleavages (about 2 hr old) were transferred from 15 psu to 2, 10, 15, 20, 25, and 30 psu and developed successfully to straight-hinge larvae in salinities ranging from 10 to 30 psu. Ciliated blastulas (12 hr old) transferred from 15 psu to 2, 10, 15, 20, 25, and 30 psu developed normally throughout the entire range.

DISCUSSION

Aspects of Larval Development and Dispersal

Although the timing of early larval development in *P. amurensis* is similar to that of other bivalves with planktonic larvae (Rattenbury and Berg 1954, Loosanoff and Davis 1963, Strathman 1992), comparison of *P.*

amurensis with its congener *P. laevis* (Hinds, 1843) reveals some interesting specific differences. The eggs of *P. amurensis* are larger than those of *P. laevis* (68 μm for *P. amurensis* versus 45 μm for *P. laevis* [Wei 1984, Wei and Guan 1985*a,b*, 1986, cited in Carlton et al. 1990]). The time for development to the straight-hinge stage is longer in *P. amurensis* than in *P. laevis* (48 hr for *P. amurensis* versus 20 to 22 hr for *P. laevis*), but temperature may be a confounding factor. We studied development of *P. amurensis* at 15°C in our laboratory. Wei and Guan (1985*a,b*, 1986, cited in Carlton et al. 1990), however, probably followed development of *P. laevis* at somewhat higher temperatures because they reported that *P. laevis* spawns when water temperatures are between 16 and 20°C. We found that *P. amurensis* settled at a shell length of approximately 135 μm 17 to 19 days after fertilization, whereas Wei and Guan found that *P. laevis* settled at a shell length of 370 μm after 30 to 40 days. *Potamocorbula amurensis* reached a shell length of approximately 380 μm by 27 days (10 days postsettlement). The shorter planktonic stage in *P. amurensis* is consistent with its larger egg size. *Potamocorbula amurensis* may invest more energy in each egg with the result that its larvae spend less time feeding in the plankton.

Our observations of the timing of larval development in *P. amurensis* are consistent with the hypothesis that its route of initial introduction to San Francisco Bay was as veliger larvae transported in ballast water by trans-Pacific cargo ships (Carlton et al. 1990, Carlton and Geller 1993). We do not know the maximum time that *P. amurensis* larvae can spend in the plankton, but current shipping traffic transits the Pacific Ocean in about 10 to 14 days (Carlton and Geller 1993, Kelly 1993), and thus even the minimum time to settlement of 17 to 19 days that we observed is sufficient for transport of larvae across the Pacific by cargo vessels.

We can further speculate that *P. amurensis,* with relatively more energy invested in each larva and a shorter larval period than its congener, *P. laevis,* might be more likely to be introduced successfully via ballast water than *P. laevis.* Planktonic food resources could be limiting in ballast water because consumption can continue while primary production cannot, and food availability might be further reduced by dilution after incomplete open-ocean ballast water exchanges. Larvae of *P. amurensis* might be more likely to survive food limitation during transit in ballast water, and they might be more likely to be able to settle quickly upon release in a recipient estuary. Larvae of *P. laevis,* in contrast, might experience higher attrition due to food limitation during ballast water transport. In addition, if they do survive transport, they might have to spend some period of time feeding in the plankton in the recipient estuary before settling and thus might have a greater probability of being washed out of the estuary.

Duda (1994) found that genetic differentiation among populations of *P. amurensis* in San Francisco Bay is low and suggested that, if the length of the larval period in *P. amurensis* were similar to that of *P. laevis,* gene flow could be extensive and low genetic differentiation could indicate that the bay population is highly panmictic. Our results show that the length of the larval period in *P. amurensis* may, in fact, be about one-half that in *P. laevis* (about 2.5 weeks for *P. amurensis* versus about 5 weeks for *P. laevis*), but we suggest that, given circulation patterns and water mass residence times in San Francisco Bay, this shorter larval period is probably still sufficient to result in substantial gene flow.

The direction of larval dispersal and gene flow is most likely from North Bay to South Bay populations. Hydraulic replacement time in the North Bay (the time it takes to replace the water volume in the North Bay) ranges from about 2 days under high-flow (winter/spring) conditions to about 160 days under low-flow (summer/fall) conditions (Walters et al. 1985). Under high-flow conditions water from the North Bay can intrude into the South Bay (Cloern 1996). North Bay populations of *P. amurensis* spawn in the spring and in the fall (Parchaso 1995). Larvae produced by North Bay populations in the spring might be likely to be washed out of

the North Bay and possibly into the South Bay before they settle, whereas larvae produced by North Bay populations in the fall might be more likely to be retained in the North Bay and to recruit to North Bay populations.

Hydraulic residence time in the South Bay ranges from 220 days under high-flow conditions to 2700 days under low-flow conditions (Walters et al. 1985), and the residual, tidally averaged circulation is slow (Cheng and Gartner 1985). South Bay populations are reproductive all year round (Parchaso 1995), but larvae produced in the South Bay are most likely to be retained in the South Bay and to recruit to South Bay populations, although occasionally strong summer winds can intensify South Bay circulation (Huzzey et al. 1990) and may result in transport of larvae from the South Bay to the Central Bay.

Tolerance of Salinity Fluctuations during Development

Marine and estuarine bivalves are osmoconformers that can exhibit substantial capacity for euryhalinity (Berger and Kharazova 1997), and *P. amurensis* is no exception. *Potamocorbula amurensis* can spawn successfully in salinities ranging from at least 5 to 25 psu, and eggs and sperm can each tolerate at least a 10-psu step increase or decrease in salinity. Fertilization occurred at salinities from 5 to 25 psu with a maximum at about 10 to 15 psu. The eggs of *P. amurensis* are negatively buoyant, so fertilization and initial embryonic development occur in the more saline bottom waters of the estuary. Embryos that are only 2 hr old can tolerate salinities that range from 10 to 30 psu, and ciliated blastulas (12 hr old) can tolerate basically the same range of salinities that adult clams can (2 to 30 psu). Thus, by the time that embryos are actively swimming in the water column (i.e., at 2 days old), they are able to tolerate salinity variations that they might encounter vertically in the water column and that they might encounter over time with variations in freshwater input to San Francisco Bay.

Rapid drops in salinity can affect larval growth rates, and the earlier in development the drop in salinity occurs, the slower larval growth is and the smaller the larvae are (Richmond and Woodin 1996). Our results suggest that any rapid change in salinity, either an increase or a decrease, may affect larval growth in *P. amurensis*. Five days after fertilization, *P. amurensis* larvae that developed from gametes that were stepped up or down in salinity by 5 psu or more before fertilization were smaller (mean shell length = 96.5 μm, SD = 2.2 μm) than larvae that developed from embryos that were stepped up or down in salinity by 5 psu or more just 2 hr after fertilization (mean shell length = 107.5 μm, SD = 4.3 μm). These size differences persisted in 10-day-old larvae (mean shell length = 120.9 μm, SD = 16.5 μm for the group stepped up or down in salinity before fertilization versus mean shell length = 134.2 μm, SD = 25.2 μm for the group stepped up or down in salinity 2 hr after fertilization).

Salinities in San Francisco Bay can vary dramatically between the winter/spring wet season and summer/fall dry season and between relatively wet and relatively dry years. For example, in May 1994, a critically dry year in which runoff was 40% of normal, surface and bottom salinities were 28 psu in the southern arm of the bay (USGS station 27) and 12 and 22 psu, respectively, in the North Bay (San Pablo Bay, USGS station 12). In October 1994 surface and bottom salinities reached 32 psu in the South Bay (USGS station 27) and 24 and 28 psu, respectively, in the North Bay (USGS station 12) (Cloern et al. 1996, U.S. Geological Survey 1999). In contrast, in May 1995, an extremely wet year in which runoff was 180% of normal, surface and bottom salinities were 18 psu in the South Bay (USGS station 27) and 2 and 9 psu, respectively, in the North Bay (San Pablo Bay, USGS station 12.5). By October 1995 surface and bottom salinities were 27 psu in the South Bay (USGS station 27) and 18 and 22 psu, respectively, in the North Bay (USGS station 12.5) (Cloern et al. 1996, U.S. Geological Survey 1999).

In general, except in the most extreme

conditions, bottom salinities in San Francisco Bay, at least in the spring, are within the range where *P. amurensis* gamete survival is greatest and fertilization and development are most likely to be successful. In contrast, for example, larvae of the soft-shelled clam *Mya arenaria* Linnaeus, 1758, also an introduced species in San Francisco Bay, do not appear to be able to tolerate to the same degree the salinity fluctuations that can occur in the bay. Larvae of *M. arenaria* can tolerate salinities from 8 to 32 psu, but at 8 psu only 1% of the larvae survived to the straight-hinge stage; survival was highest at 23 psu (Stickney 1964). Bottom salinities, particularly in the spring in the northern arm of San Francisco Bay, often decrease to levels that could negatively impact reproduction and recruitment of *M. arenaria.*

Since the introduction of *P. amurensis,* populations of *M. arenaria* in the North Bay have declined (Nichols et al. 1990), and differences in larval tolerance of salinity fluctuations and resulting differences in larval survival could be one factor contributing to this pattern. *Potamocorbula amurensis* may be able to recruit to North Bay communities in years when bottom salinities are relatively low. Recruitment of *M. arenaria,* in contrast, may be reduced or absent at similarly low salinities. This potential for differences in recruitment as a function of salinity may allow *P. amurensis* populations to increase and be maintained at levels where *P. amurensis* may negatively affect *M. arenaria* through competition for food or other resources.

Gametes, embryos, and larvae of *P. amurensis* can tolerate step increases and decreases of at least 10 psu, with the ability to tolerate larger step changes increasing in older developmental stages. The step changes in salinity that we used to test salinity tolerance by *P. amurensis* have environmental analogs. Estuarine salinities can vary on relatively short time scales (e.g., hours to days) as a result of pulses of storm runoff. Salinity changes associated with storm runoff would be more gradual than the step changes we used but can still be relatively rapid (Richmond and Woodin 1996). The ability of *P. amurensis* to tolerate relatively large step changes in salinity suggests that its abilities to tolerate rapid, gradual changes in salinity associated with storm runoff would be at least as great, if not greater.

The ability to tolerate step changes in salinity can also be important in determining survival during transoceanic transport in ballast water and survival in the estuary receiving the ballast water. Organisms like *P. amurensis* that can tolerate wide and relatively abrupt changes in salinity would be likely to survive incomplete oceanic exchanges of ballast water (Carlton and Geller 1993, Locke et al. 1993). Tolerance of step changes in salinity could also have been important in the initial survival and recruitment of *P. amurensis* to San Francisco Bay, particularly if the salinity of the ballast water in which the larvae were transported differed from that of the receiving waters in San Francisco Bay.

It is likely that *P. amurensis* will eventually spread from San Francisco Bay to other estuaries along the Pacific coast of North America (Carlton 1992). The broad salinity tolerances of larvae and adults indicate that salinity may not be a barrier to longshore dispersal of *P. amurensis* from San Francisco Bay. However, natural (as opposed to human-mediated) dispersal of *P. amurensis* from San Francisco Bay may be limited instead by the relatively short time *P. amurensis* larvae spend in the plankton and the absence of other suitable muddy-bottom estuarine habitats in close proximity to San Francisco Bay. If *P. amurensis* is introduced to other eastern Pacific estuaries, the route of introduction will probably be transport in ballast water, either from San Francisco Bay or from estuaries in eastern Asia.

CONCLUSIONS

1. The diameter of eggs of *P. amurensis* is about 50% larger than the diameter of eggs of its congener *P. laevis,* and the length of the larval period of *P. amurensis* (about 20 days at 15°C) is about half as long.

2. The length of the larval period in *P. amurensis* is sufficient for the transport of veliger larvae in ballast water of cargo ships

to have been the initial route of introduction from Asia to San Francisco Bay.

3. The length of the larval period of *P. amurensis* relative to water mass residence times in San Francisco Bay is sufficient to account for the relatively rapid dispersal of *P. amurensis* throughout San Francisco Bay following its initial introduction (Carlton et al. 1990) and to account for observations (Duda 1994) that genetic differentiation among populations of *P. amurensis* in San Francisco Bay is low.

4. All life history stages of *P. amurensis* appear to be markedly euryhaline: fertilization occurred in a range of salinities (5 to 25 psu) that is only slightly narrower than the range of salinities (2 to 30 psu) that is tolerated by developmental stages from 12-hr-old ciliated blastulas to adults.

5. Gametes, embryos, and larvae of *P. amurensis* can tolerate step increases and decreases in salinity of at least 10 psu, although rapid changes in salinity appear to reduce larval growth.

6. Information about all life history stages, particularly larval stages, is necessary to understand why human-mediated introductions of some species may be more likely than others from the same geographic area or evolutionary lineage. Species like *P. amurensis* with larval periods of intermediate length and with larvae that can tolerate relatively large ranges of salinity and relatively large step changes in salinity may be more likely to survive transoceanic transport in ballast water and recruit successfully as new introductions in recipient estuaries.

LITERATURE CITED

ALPINE, A. E., and J. E. CLOERN. 1992. Trophic interactions and direct physical effects control phytoplankton biomass and productivity in an estuary. Limnol. Oceangr. 37:946–955.

BEADLE, L. C. 1972. Physiological problems for animal life in estuaries. Pages 51–60 *in* R. S. K. Barnes and J. Green, eds. The estuarine environment. Applied Science Publishers, London.

BERGER, V. J., and A. D. KHARAZOVA. 1997. Mechanisms of salinity adaptations in marine molluscs. Hydrobiologia 355:115–126.

CARLTON, J. T. 1992. Introduced marine and estuarine mollusks of North America: An end-of-the-20th-century perspective. J. Shellfish Res. 11:489–505.

CARLTON, J. T., and J. B. GELLER. 1993. Ecological roulette: The global transport of nonindigenous marine organisms. Science (Washington, D.C.) 261:78–82.

CARLTON, J. T., J. K. THOMPSON, L. E. SCHEMEL, and F. H. NICHOLS. 1990. Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam, *Potamocorbula amurensis*. Introduction and dispersal. Mar. Ecol. Prog. Ser. 66:81–94.

CHENG, R. T., and J. W. GARTNER. 1985. Harmonic analysis of tides and tidal currents in south San Francisco Bay, California. Estuarine Coastal Shelf Sci. 21:57–74.

CLOERN, J. E. 1996. Phytoplankton bloom dynamics in coastal ecosystems: A review with some general lessons from sustained investigation of San Francisco Bay, California. Rev. Geophys. 34:127–168.

CLOERN, J. E., B. E. COLE, and J. L. EDMUNDS. 1996. Seasonal fluctuations of water quality in San Francisco Bay during the first three years of the Regional Monitoring Program, 1993–1995. Pages 31–43 *in* 1995 Annual Report, San Francisco Estuary Regional Monitoring Program for Trace Substances. San Francisco Estuary Institute, San Francisco.

DECHO, A. W., and S. N. LUOMA. 1991.

Time-courses in the retention of food material in the bivalves *Potamocorbula amurensis* and *Macoma balthica:* Significance to the absorption of carbon to chromium. Mar. Ecol. Prog. Ser. 78:303–314.

DUDA, F. 1994. Genetic population structure of the recently introduced Asian clam, *Potamocorbula amurensis,* in San Francisco Bay. Mar. Biol. (Berl.) 119:235–241.

HOLLIBAUGH, J. T., and I. WERNER. 1991. *Potamocorbula amurensis:* Comparison of clearance rates and assimilation efficiencies for phytoplankton and bacterioplankton. Interagency Ecological Studies Program for the Sacramento–San Joaquin Estuary, Technical Report 29.

HUZZEY, L. M., J. E. CLOERN, and T. M. POWELL. 1990. Episodic changes in lateral transport and phytoplankton distribution in south San Francisco Bay. Limnol. Oceanogr. 35:472–478.

KELLY, J. M. 1993. Ballast water and sediments as mechanisms for unwanted species introductions into Washington State. J. Shellfish Res. 12:405–410.

KIMMERER, W. J., E. GARTSIDE, and J. J. ORSI. 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton of San Francisco Bay. Mar. Ecol. Prog. Ser. 113:81–93.

LOCKE, A., D. M. REID, H. C. VAN LEEUWEN, W. G. SPRULES, and J. T. CARLTON. 1993. Ballast water exchange as a means of controling dispersal of freshwater organisms by ships. Can. J. Fish. Aquat. Sci. 50:2086–2093.

LOOSANOFF, V. L., and H. C. DAVIS. 1963. Rearing of bivalve mollusks. Adv. Mar. Biol. 1:1–136.

NICHOLS, F. H., J. K. THOMPSON, and L. E. SCHEMEL. 1990. Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam *Potamocorbula amurensis.* II. Displacement of a former community. Mar. Ecol. Prog. Ser. 66:95–101.

PARCHASO, F. 1995. Seasonal reproduction of *Potamocorbula amurensis* in San Francisco Bay, California. M.S. thesis, San Francisco State University, San Francisco.

RATTENBURY, J. C., and W. E. BERG. 1954. Embryonic segregation during early development of *Mytilus edulis.* J. Morphol. 95 (3): 393–414.

RICHMOND, C. E., and S. A. WOODIN. 1996. Short-term fluctuations in salinity: Effects on planktonic invertebrate larvae. Mar. Ecol. Prog. Ser. 133:167–177.

STICKNEY, A. P. 1964. Salinity, temperature, and food requirements of soft-shelled clam larvae in laboratory culture. Ecology 45:283–291.

STRATHMAN, M. F. 1992. Reproduction and development of marine invertebrates of the northern Pacific coast. Data and methods for the study of eggs, embryos, and larvae. University of Washington Press, Seattle.

U.S. GEOLOGICAL SURVEY. 1999. Water quality of San Francisco Bay. Long term data set. http://sfbay.wr.usgs.gov/access/wqdata/archive/longterm.html.

WALTERS, R. A., R. T. CHENG, and T. J. CONOMOS. 1985. Time scales of circulation and mixing processes of San Francisco Bay waters. Hydrobiologia 129:13–36.

WEI, L. 1984. A preliminary experiment on the artificial rearing of *Aloidis laevis* (Hinds) and its habits. Mar. Sci. (Quingdao) 6:32–35 (in Chinese, English abstract).

WEI, L.-P., and F.-T. GUAN. 1985a. The growth of *Aloidis laevis.* Chin. J. Zool. 20 (2): 3–7 (in Chinese).

———. 1985b. The breeding of *Aloidis laevis.* Chin. J. Zool. 20 (3): 4–7 (in Chinese).

———. 1986. Growing and breeding habits of *Potamocorbula laevis.* Trans. Chin. Soc. Malacol. 2:94–101 (in Chinese, English abstract).

WERNER, I., and J. T. HOLLIBAUGH. 1993. *Potamocorbula amurensis:* Comparison of clearance rates and assimilation efficiencies for phytoplankton and bacterioplankton. Limnol. Oceanogr. 38:949–964.