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LABORATORY STUDIES OF HYBRIDIZATION IN CALIFORNIA ABALONE (*HALIOTIS*)

Buzz Owen[1] and Richard Meyer[2]

[1] P.O. Box 601, Gualala, California 95445, buzabman@mcn.org
[2] 1615 S.E. 58th Ave., Portland, Oregon 97215, meyer4842@comcast.net

ABSTRACT Interspecific hybridizations involving five species of California *Haliotis* were carried out in the laboratory. The following hybrid progeny were successfully produced, cultured, and positively identified on a morphological basis: *H. corrugata* x *H. rufescens*, *H. corrugata* x *H. walallensis*, *H. kamtschatkana assimilis* x *H. walallensis*, *H. sorenseni* x *H. corrugata*, and *H. sorenseni* x *H. kamtschatkana assimilis*. Additionally, more complex multi-species hybrids and backcrosses were cultured and are listed in the text. The interaction between egg and sperm appears to be the principal mechanism controlling the success of hybrid crosses.

KEY WORDS Cultured abalone, Haliotidae, hybrid cross, multi-species hybrid.

INTRODUCTION

Members of the genus *Haliotis*, commonly known in this country as abalones, are large gastropod mollusks that inhabit rocky shores in many parts of the world, including the coast of California. In an earlier paper (Owen *et al.*, 1971), natural hybridization was reported in California abalones, involving six of the seven local species. This paper describes the results of several attempts to cross California *Haliotis* species in the laboratory. The study was performed at a commercial shellfish hatchery (Pacific Mariculture, Inc.) located on the coast at Pigeon Point (37 degrees north), about 80 kilometers south of San Francisco, California. The senior author, employed at the hatchery since its inception in 1965, has successfully reared many different species and hybrids of *Haliotis* in the hatchery system.

The sexes are separate in this genus, and spawning animals broadcast their gametes directly into the water where fertilization occurs by chance meeting of ova and sperm. This paper presents observations and experiments conducted by Owen on several occasions when "mass spawnings" occurred in the tanks at Pacific Mariculture, Inc. Mass spawnings are those which are apparently spontaneous, generally all-involving, and not artificially induced. Where possible, unfertilized eggs were collected and combined with sperm of homospecific or heterospecific origin. Owen has developed techniques for raising abalone through their planktonic larval stages into adult life. Hybrid progeny were cultured and their development usually compared to the pattern described here for *Haliotis rufescens*.

Identification of the hybrid specimens was made by Owen, and was based largely on the morphology of the shell and epipodium. Detailed morphological characterization of the various hybrid *Haliotis* is described elsewhere (Owen *et al*., 1971).

Table 1 lists the various *Haliotis* species and hybrids used as spawning stock and the locality where they were collected or cultured.

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Table 1. Species and hybrids used as spawning stock with locality of collection:

1. ***Haliotis corrugata*** **(Santa Barbara 34 deg. N)**
2. ***Haliotis cracherodii*** **(Pigeon Point 37 deg. N)**
3. ***Haliotis kamtschatkana assimilis*** **(Point Conception area 34 deg. N)**
4. ***Haliotis kamtschatkana kamtschatkana*** **(Vancouver Island, British Columbia, Canada 49 deg. N)**
5. ***Haliotis rufescens*** **(Pigeon Point 37 deg. N)**
6. ***Haliotis sorenseni*** **(Santa Barbara 34 deg. N)**
7. ***Haliotis walallensis*** **(Point Estero 35 deg. N)**
8. **Hybrid *Haliotis rufescens* x *H. sorenseni*. Male and female. (Santa Barbara 34 deg. N)**
9. **Hybrid *Haliotis rufescens* x *H. kamtschatkana assimilis*. Male. (Point Buchon 35 deg. N)**
10. **Hybrid *Haliotis sorenseni* x *H. kamtschatkana assimilis*. Male. (Point Conception 34 deg. N)**
11. **Hybrid *Haliotis corrugata* x *H. walallensis*. Male. (produced at Pacific Mariculture, Inc. 37 deg. N)**

*12. **Hybrid *Haliotis kamtschatkana assimilis* x *H. walallensis* (produced at Pacific Mariculture, Inc. 37 deg. N)**

(* Female spawned normally but eggs not used in culture experiments.)

MATERIALS AND METHODS

Adult abalone used as spawning stock were maintained in the hatchery in four fiberglass lined plywood tanks of approximately 475 liter capacity. A continuous, nonrecirculated supply of unfiltered seawater was delivered to the tanks at ambient temperature ranging from 8 to 15 deg. C. The abalone were regularly fed a mix of kelp (principally *Nereocystis leutkeana* and *Macrocystis pyrifera*) and the tanks were cleaned about once a week. Precautionary measures were taken to assure control in hybridization experiments. Equipment (plastic pails, etc.) used in the routine manipulation of spawning adults and gametes was first sterilized in a strong hypochlorite solution followed by a rinse in hot, fresh tap water. During spawning and for raising of larvae, running filtered water was obtained by using sea water passed through a one micron pressure filter and sterilized by exposure to ultraviolet light.

To collect unfertilized gametes to be used in hybridization attempts, in almost all cases both males and females were removed from a larger tank that contained stock of multiple species as soon as they were observed to spawn. They were vigorously rinsed in running filtered water, then isolated in a clean plastic pail, and rinsed continuously while they attached to the pail. When attached, the animals were rinsed again for a short time, then the pail was filled with filtered water. Water from the pail into which the male had spawned was used to fertilize eggs. Eggs were collected from the pails on a nylon screen with 48 micron openings (Kressilk Products Inc., Elmsford, N.Y.) and thoroughly rinsed with filtered water. Ordinarily, some eggs of each batch collected were set aside and intentionally not fertilized. Lack of development in eggs of this group was taken as evidence that no unwanted sperm had contacted the eggs. This step is referred to as the "unfertilized control".

Ordinarily, fertilization was carried out in these experiments by adding a small amount of sperm water to eggs that had been rinsed and resuspended in filtered water. Care was taken to limit the amount of sperm added to the eggs. Excessive concentrations of sperm were accompanied by excessive bacterial growth tending to foul incubation water and hamper normal development (*i.e.,* polyspermy). The importance of using limited amounts of sperm has been emphasized by Kikuchi (1964). Water

temperature in egg and larval cultures was maintained in the range of 15 to 18 deg. C., and densities were held between 0.5 and 1.0 per ml. In the case of hybrid crosses, densities were kept well below these levels.

May, 2015: All photography was done by Owen in March through May of 2015, with a Canon A650 IS "PowerShot" digital camera mounted on a tripod. Exposures were usually ½ second, always at f/8. All specimens are in Buzz Owen Collection (BOC).

OBSERVATIONS

Spawning Behavior. In general it was observed that during a mass spawning, all abalones with mature gonads in the hatchery spawned about the same time. On several occasions, as many as five species and three different hybrids were observed spawning simultaneously in the same tank. Without exception, the males spawned first, turning the water milky with sperm. The females released eggs within one to several hours, possibly in response to sperm in the water (though the same stimuli that induces the males to spawn may cause this as well). Release of gametes usually continued for several hours. On several occasions, Owen anticipated a spawning from weather cues, and over two hours before any spawning occurred, removed a female from the tank and isolated it in running one micron filtered water. The isolated females would release eggs a short time after the males in the main tank began spawning, and at the same time as the females in the main tank. As far as could be determined, these isolated females were never exposed to sperm.

The females show a peculiar behavior pattern during spawning. They nearly always become quite active, moving toward the surface where they circle the tank for some 20 minutes before actually releasing eggs. During these periods and for several hours before actual spawning commences, they showed no interest in food. When they begin to spawn, they normally expelled the eggs in intermittent, violent spasms. They may even leave the tank and discharge the eggs into the air. Males seldom become so active; they release sperm in more or less steady streams.

Development of Fertilized Eggs and Larvae of *H. rufescens*. In the experiments reported here, the events surrounding fertilization were often important in judging the relative success of a hybrid combination of gametes. On some occasions, heterospecific sperm were observed to be slow in their approach to unfertilized eggs. Sluggish interaction of sperm at attempted entry into eggs is usually associated with a low percentage of fertilization. Percentage of fertilization was best estimated about 18 hours after adding sperm since, by this time, normally developing embryos have gone through initial cleavage stages and have become ciliated gastrulae or trochophores rotating within the vitelline envelope (Fig. 1). The fraction of eggs that advanced to rotating trochophores was estimated by counting a sample of several hundred embryos under the microscope. Signs of successful fertilization observable at earlier times were the appearance of the polar bodies (about ten minutes after adding sperm), and the appearance of cleavage stages (beginning within the first hour). Normally 90% or more of eggs fertilized with homospecific sperm advance to the rotating trochophore stage. About 24 hours after fertilization, trochophores break out of the membrane and rise actively to the surface where they swim vigorously. Within the next six to twelve hours the embryo assumes the form of the typical molluscan veliger, with larval shell and ciliated velum. Torsion takes place at about 48 hours. After five to eight days of active swimming they settle to the bottom to assume the benthic habit of the adult. The first

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respiratory pore usually appears at 30 to 40 days. These young benthic animals are referred to as "juveniles" until they reach sexual maturity which usually requires one to three or more years, depending on species. Soon after settling, the young abalone begin to feed actively; in these experiments they were given attaching diatoms, primarily *Navicula* sp. and *Nitzschia* sp. **Note:** From the time that the trochophores emerge from the membrane and rise to the surface until the advancement to late veliger stages, there appears to be little mortality, but shortly before or during the transition to the benthic habit there seems to be a substantial die-off. Among the many cultures of *Haliotis* larvae which have been carried through to settling, mortality has often been close to 100% (occasionally100%), while at other times it has been as low as 10%.

The following is a description of the developmental pattern observed when homospecific gametes were used. It is based largely on observations made while working with *H. rufescens*. We will regard these homospecific crosses as "normal" and then use this pattern of development as a reference by which to compare the development of various hybrid combinations.

The freshly released egg is spherical and about 200 microns in diameter. The yolk is opaque and green to dark gray in color. Surrounding the yolk is the perivitelline space, a transparent envelope bound by an outer membrane, the vitelline envelope. This space is 20 to 25 microns thick (Fig. 1). Additionally, there is a gelantinous coat surrounding the egg, which is approximately 100 microns thick.

Haliotis sperm has a recognizable head and tail and swims actively. If sperm is added to unfertilized eggs under a microscope, one can observe highly motile sperm quickly attach to and enter the perivitelline space. Often many sperm penetrate the outer membrane to swim actively about the yolk. Presumably only one enters the egg itself to accomplish fertilization. Reduction division (meiosis) of the egg nucleus occurs after entry of the sperm because the pair of polar bodies can be observed, preceding cleavage stages, in those eggs that become fertilized.

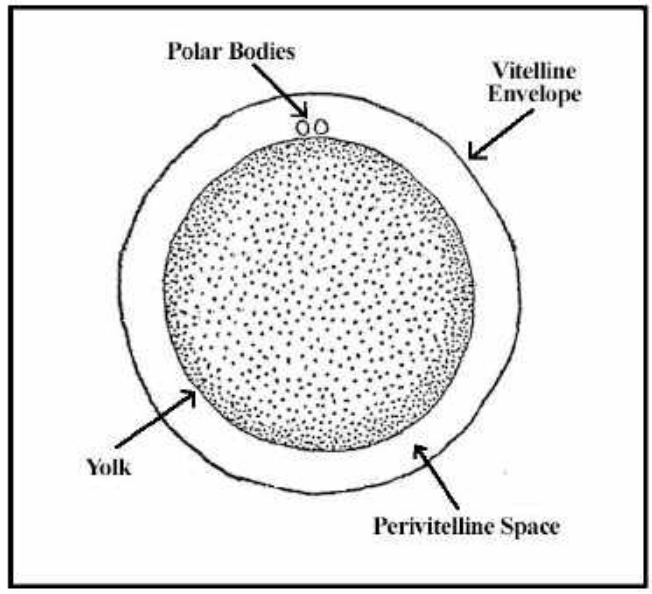

Figure 1. Fertilized egg of *Haliotis*, showing structures mentioned in text (based on Ino, 1952).

Observations of Spawnings. Spontaneous (mass) naturally occurring spawnings appear to be correlated with an increase in temperature (2-3 deg. C.) and pH (to 8.3) of the seawater, conditions that were usually associated with southerly winds, and occur mostly in early to late summer. There was frequently a lunar periodicity about these changes and they often coincided with a full or new moon phase (plus or minus one to three or four days). These changes seemed to initiate an influx of offshore water, the temperature and pH rising as stated above. From cursory observations, this offshore water also seemed to have a different planktonic composition, and is markedly visible: a bold

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line separating the blue-colored incoming water mass from the more turbid color of the inshore seawater which it was displacing is often present. Although others report artificial stimulation of spawning by increasing the temperature and/or pH of the seawater (Ino, 1952; Imai, 1967; Leighton, 1972), we have not attempted to utilize these methods as we had more than sufficient naturally induced spawnings to meet our needs.

The mass spawnings reported in this paper occurred on eight separate occasions: October 6 and December 7, 1965; August 23, 1967; October 31, 1968; July 19 and September 14, 1969; and February 13 and June 24, 1970.

Descriptions of Individual Spawnings.

Mass Spawning of October 6, 1965: This was the first time hybridization experiments had been attempted at Pacific Mariculture, Inc. The technique on this occasion was more exploratory and the exact meaning of the results is not as obvious as in some of the later spawnings. It was clear, however, that viable *H. corrugata* x *H. rufescens* hybrids were produced. This hybrid is the second most common found in natural populations (approximately 70 reported cases).

The single cross carried out was with eggs from a single *H. corrugata* female in a tank with mixed species and sperm water from a different tank housing four spawning male *H. rufescens*. When she spawned, the eggs were collected from the tank rather than from the female in an isolated environment (in later spawnings, isolation of the female before collecting gametes was adopted as standard method). There was very little sperm in the tank and the eggs were collected on a 48 micron nylon screen as they were being released by the female. They were then quickly rinsed while in the screen with running filtered water and then set aside for one hour. After that period, they were examined microscopically and no evidence of fertilization was detected. Later evidence indicated that there probably were some fertilized eggs present, but they went unnoticed. The eggs were then mixed with the *H. rufescens* sperm water and there was prompt microscopic confirmation that fertilization had taken place in the bulk of the eggs.

Development of these "hybrids" appeared to be normal through cleavage, trochophore and veliger stages, judged by comparison with a *H. rufescens* x *H. rufescens* cross made at the same time. However, on the eighth day, when many larvae were beginning to settle, there was a very large mortality, substantially more than the "control" *H. rufescens* batch being cultured at the same time. After 30 days there were approximately 200 survivors of the hybrid cross and after one year they numbered 75. Mortality after the first month was due mostly to human mishandling. As this group of young abalone began to mature, it became clear that most animals were not hybrids as expected, but straight *H. corrugata*. Only about 10% of the group seemed to be the expected *H. corrugata* x *H. rufescens* hybrids, and a single specimen, which didn't seem to fit either category, was later identified as a *H. corrugata* x *H. walallensis* hybrid. At the time of this writing (September, 1971) there are four surviving animals from this spawning: three *H. corrugata* averaging about 35 mm in length, and the one *H. corrugata* x *H. walallensis*, now 59 mm long.

Mass spawning of December 7, 1965: As a result of this spawning, two mature, morphologically identifiable *H. kamtschatkana assimilis* x *H. walallensis* hybrids were produced. Only one such hybrid has been reported from natural populations.

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A mass spawning occurred in a 475 liter tank containing male and female *H. corrugata*, *H. walallensis*, *H. sorensen*i, and *H. kamtschatkana assimilis*. There was no attempt to control fertilization. Fertilized eggs were merely collected from the mixed tank and allowed to develop. Survivorship through the benthic stage appeared normal. After one year there were approximately 200 surviving offspring and all showed a morphology that suggested straight *H. kamtschatkana assimilis*. After two years, there were about 35 survivors, and while most still suggested a pure parentage of that subspecies, two specimens were definitely different from the others and were tentatively identified as *H. kamtschatkana assimilis* x *H. walallensis* hybrids on the basis of shell and epipodial characters (one was temporarily placed in another *Haliotis* aquaculture facility on loan). As of September, 1971, sixteen offspring of this spawning survive: fourteen are obvious homospecific *H. kamtschatkana assimilis*, and the single *H. kamtschatkana* x *H. walallensis* at Pigeon Point is a female measuring 107 mm. This specimen was observed releasing eggs in May, 1970 (4.5 years old). The second specimen was later returned to Pigeon Point and measured 90.4 mm when experiments were terminated in 1972.
May, 2015: A third *H. kamtschatkana assimilis* x *H. walallensis* hybrid was later identified, a smaller specimen measuring 47.2 mm. This specimen is not included on Table 2 since it was not recognized at the time.

Mass Spawning of August 23, 1967: Eggs were collected from three *H. kamtschatkana assimilis* females and fertilized with six different types of sperm, including two hybrids. This allowed the opportunity to determine whether at least some hybrids are fertile.

The spawning animals were isolated and thoroughly rinsed with filtered water before the gametes were collected. In this case, two groups of eggs were collected: one from a single orange banded *H. kamtschatkana assimilis*, and the other from two different specimens of *H. kamtschatkana assimilis* which were also orange banded.

Unfortunately, in this experiment, no eggs were set aside as an unfertilized control. Eggs from the single female were divided among three plastic pails, and each was then fertilized by adding sperm water from a separate, isolated male. One pail was fertilized with sperm from a *H. kamtschatkana assimilis*, another by a *H. walallensis*, and the third by sperm from a *H. rufescens* x *H. sorenseni* hybrid. Eggs from the two females were also divided among three pails, and were fertilized with a *H. sorenseni*, a *H. cracherodii*, and a *H. rufescens* x *H. kamtschatkana assimilis* hybrid respectively. Each of the six combinations of egg and sperm will be discussed separately below.

Haliotis kamtschatkana assimilis* x *H. kamtschatkana assimilis. Fertilization and development progressed normally. About 90% of the eggs became active veligers. There was a high mortality at settling; less than 1% of the veligers underwent successful metamorphosis and became benthic. After 2.5 months, the offspring of this cross were combined in a single aquarium with 75 of the contemporary progeny of the cross to the *H. rufescens* x *H. kamtschatkana assimilis* male. At this time, there were 322 of the suspected straight *H. kamtschatkana assimilis* juveniles. At four years of age, approximately 100 animals remained from this cross, the largest measuring 90 mm.

Haliotis kamtschatkana assimilis* x *H. walallensis. *Haliotis walallensis* sperm did not behave normally in the presence of *H. kamtschatkana assimilis* eggs. Sperm could be

observed attaching to the vitelline envelope, but few penetrated into the perivitelline space. Those that did showed considerably less than normal motility once inside. About 15% of the eggs progressed to rotating trochophores; about one-fourth of these appeared to have developed abnormally, being oddly shaped. A few eggs cleaved abnormally and did not reach the trochophore stage, and the remainder remained unfertilized. Many apparently healthy veligers survived, but all died before becoming benthic.

***Haliotis kamtschatkana assimilis* X (*H. rufescens* x *H. sorenseni*) hybrid.** Sperm from this hybrid were also subdued in their interaction with the *H. kamtschatkana assimilis* eggs, similar to the *H. walallensis* sperm. An estimated 25% of the eggs became rotating trochophores and again, some of these were abnormal. The survivors appeared normal through the larval period, but again, all suddenly died just before becoming benthic.

***Haliotis kamtschatkana assimilis* x *H. sorenseni*.** As with the previous two batches of sperm, that from *H. sorenseni* appeared retarded in its interaction with the *H. kamtschatkana assimilis* eggs. Only about 5% of the eggs appeared fertilized, and the culture was discarded after one day.

***Haliotis kamtschatkana assimilis* x *H. cracherodii*.** This combination showed the most negative interaction of gametes. Sperm did not attach to the vitelline envelope. The *H. kamtschatkana assimilis* eggs seemed to repel *H. cracherodii* sperm: a 30 micron sperm-free halo surrounded the egg. No eggs were observed to be fertilized, although 18 hours later a small number of rotating trochophores were noticed, no more than 2% of the eggs. The identity of these trochophores is not known, but they might represent sperm that did not wash off the female *H. kamtschatkana assimilis* used in this cross. None of the trochophores emerged from the vitelline envelope and the batch was discarded after 24 hours

***Haliotis kamtschatkana assimilis* X (*H. rufescens* x *H. kamtschatkana assimilis*) hybrid.** This backcross combination seemed to develop normally. An estimated 75% of the eggs became rotating trochophores. As with many crosses, a large mortality occurred at metamorphosis, leaving about 100 animals (less than 1% of the veligers) after one month.

For reasons of technical convenience, 75 surviving juveniles of this backcross were combined with 322 suspected *H. kamtschatkana assimilis* (the first mentioned cross of this spawning) at an age of 2.5 months. They were maintained in a lucite aquarium of special design that had proven successful in the rearing of juvenile abalone. At this point, the two groups could not be distinguished morphologically. At one year of age, 227 animals were counted in the aquarium: 203 were recognizable as straight *H. kamtschatkana assimilis*, having lightly pigmented flesh with darker specks; and 24 were obviously different, having solid dark flesh and averaging a larger size. The pigment and the size resembled *H. rufescens*. The shells of the two groups appeared identical except for the size difference. The latter group was assumed to be the backcross progeny. Once two groups were distinguished, they were placed in separate aquaria for continued observation.

When these two groups were 14 months old, another mass spawning occurred (October 31, 1968). Many males of the straight *H. kamtschatkana assimilis* progeny were observed spawning, although the largest of this group was only 30 mm long at this time. None of the suspected backcross group were observed

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spawning. A subsequent visual examination of the gonad area of the young abalone of the two groups revealed that many of the male *H. kamtschatkana assimilis*, some as small as 20 mm, had well developed gonads as judged by the appearance of whitish tissue surrounding the digestive gland of the animal. Only two of the 24 presumed backcrosses showed at most just slight traces of gonad development. Our observations indicate that *H. kamtschatkana assimilis* reaches sexual maturity much younger and at a smaller size than *H. rufescens*, so the later gonad development in the second group (presumed backcrosses) suggests the influence of genes of *H. rufescens*.

Now, at four years of age, 22 of this group of 24 backcrosses survive and are growing well. They range in size from 55 to 103 mm.

May, 2015: The largest specimen measured 114.8 mm when experiments were terminated in mid to late 1972.

Sometimes the shell of *H. kamtschatkana assimilis* has an orange-colored band following inside the row of excurrent apertures (Fig. 2). About 5 to 10% of this subspecies show this trait, but the percentage varies locally. It is not noticeable in other species, except that about one out of four *H. sorenseni* show an orange zone to the right of the aperture row as a juvenile, which fades away after the shell reaches a size of 30 to 35 mm (see Leighton, 1972). Occasionally a similar band may be seen in juvenile *H. rufescens* and *H. walallensis*. Three of the 40 known specimens of *H. rufescens* x *H. kamtschatkana assimilis* have an orange band. The three female *H. kamtschatkana assimilis* that supplied the eggs used in the 1967 experiments were banded, as was the male of that same spawning. The hybrid male *H. rufescens* x *H. kamtschatkana assimilis* was not banded. The frequency of bandedness in the 1967 progeny, then, can give us some information on the mechanism of inheritance of this banded characteristic.

In the progeny of the straight *H. kamtschatkana assimilis* cross (both parents banded) 152 out of 203 progeny (75%) were banded, and of the backcross progeny (only female banded), 11 out of 24 (46%) were banded (see also Discussion).

Figure 2. Shell of *H. kamtschatkana assimilis*, showing orange band.

The important result from the 1967 spawning is the demonstration that the *H. rufescens* x *H. kamtschatkana assimilis* hybrid proved to be a fertile male parent when backcrossed to a *H. kamtschatkana assimilis* female, suggesting a very close genetic relationship between these two species. This is the first demonstration of hybrid fertility in *Haliotis*. Some shells from natural populations have suggested backcrossing (Owen *et al*., 1971).

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Mass Spawning of October 31, 1968: Many of the males in one of the large 475 liter tanks began to release sperm in the early evening. The water quickly became too milky to see well and it was two or three hours before any female was noticed spawning. A female *H. rufescens* x *H. sorenseni* near the top of the tank was the first observed to release eggs. W. W. Budge was the only person present and he made an attempt to collect the eggs by siphoning with rubber tubing. The female then came partly out of the water and began a series of violent contractions, expelling eggs as high as 25 cm into the air. At that point Budge caught the eggs in a plastic pail. He then filtered some of the water of the same tank through nylon screen to remove any eggs and used this water to fertilize the eggs in the pail. Due to the nature of the way this spawning occurred, an unfertilized control would not have been possible and was not attempted. Owen then carried this set of fertilized eggs through a normal larval period. An estimated 95% of the eggs became active trochophores. There was a high mortality, as usual, at the time of settling. An estimated 2000 animals successfully became benthic.

The probability is quite good, but not absolute, that there were no other eggs present in the pail besides those from the *H. rufescens* x *H. sorenseni* female. The eggs were exposed to very little water from the tank before fertilization. No other females were noticed to be spawning, but visibility in the tank was poor. It is not known precisely which males were releasing sperm into the water, but many were. *Haliotis kamtschatkana assimilis*, *H. kamtschatkana kamtschatkana*, *H. cracherodii*, and a hybrid *H. rufescens* x *H. kamtschatkana assimilis* were positively recorded to be releasing sperm. *Haliotis sorenseni* and a hybrid *H. rufescens* x *H. sorenseni* might also have been, and there is less but still some chance that *H. walallensis* and a *H. sorenseni* x *H. kamtschatkana assimilis* hybrid were spawning as well. Many bizarre genetic combinations could have resulted from this cross. The progeny are now about three years old, and approximately 150 are still alive. The most striking point about these animals is their variability. Most cannot be identified morphologically, but at least 20 animals can be positively recognized as the three species hybrid, (*H. rufescens* x *H. sorenseni*) X *H. kamtschatkana assimilis*. And even this group, despite being of similar genetic background, show more variation in shell and epipodial characters than the standard two species hybrids. The largest of the 20 identifiable three species hybrids measured 65 mm and of the other 130 specimens, the largest measured 80 mm.

All the male *H. kamtschatkana assimilis* but none of the *H. kamtschatkana kamtschatkana* that were noticed to be spawning were orange banded, and some of the progeny are showing an orange band. In September of 1969, when the animals were nearly one year old, 225 animals were counted and 16 (7%), of unknown genetic background, were banded.

May, 2015: A more careful examination in 1975 of the shells from the 1968 spawning revealed one outlier that was clearly identifiable as a three species cross of a female *H. cracherodii* with a male *H. corrugata* x *H. walallensis* hybrid, both of which were in the single tank where this spawning occurred (Pl. 8). Given this spawning occurred unexpectedly and after hours with only one person present, it is clear that a few fertilized eggs not of the intended cross got into the mixture of gametes even though attempts were made to prevent this.

Mass Spawning of July 19, 1969: Two crosses of interest were performed during this mass spawning: eggs from a single *H. sorenseni* were fertilized with sperm from a *H. corrugata*; and

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eggs from one *H. corrugata* were fertilized with sperm from a *H. rufescens* x *H. sorenseni* hybrid. Both females were isolated and thoroughly rinsed before they began releasing eggs.

The eggs from the *H. sorenseni* were collected and separated into two cultures; one was set aside and observed as an unfertilized control, while the other was mixed with sperm water derived from an isolated *H. corrugata*. The following day, the unfertilized batch showed no evidence of development, and about 25% of the eggs fertilized with *H. corrugata* sperm had developed to rotating trochophores. Subsequent development appeared normal, about 10% surviving to the benthic stage. At the age of 60 days there were 943 individuals of this cross, but because of space limitations, most were released. At this writing (September, 1971), 86 of these remain, clearly recognizable as *H. corrugata* x *H. sorenseni* hybrids. The largest of this group are about 50-53 mm in length.

The eggs collected from the isolated *H. corrugata* were mixed with sperm water from an isolated *H. rufescens* x *H. sorenseni* hybrid. None were set aside as unfertilized controls in this case, but the same isolation techniques were employed as in the above *H. corrugata* x *H. sorenseni* cross, which effectively restricted unwanted fertilization. Eighty percent of the eggs developed to rotating trochophores and continued through a normal larval period. Over 90% of the veligers completed metamorphosis by the 10th day. This rate of survival is strikingly much higher than is normally observed, even with homospecific crosses done during these experiments. Many thousands of these suspected three species crosses were carried through the first several months of growth. Some were lost due to mishandling, many were planted on an experimental area of ocean bottom, and a few were kept in the hatchery for observation. At this time, 16 individuals remain alive from this cross ranging up to 72 mm in length. It is not yet possible to positively identify the *H. corrugata* parentage, but *H. rufescens* and *H. sorenseni* morphology are evident. Since the *H. rufescens* and *H. sorenseni* characters are those of the intended male parent, contaminating sperm was apparently avoided in this cross.

May, 2015: One shell illustrating *H. corrugata* morphology was later identified (Pl. 6).

Mass Spawning of September 14, 1969: During this spawning, eggs were collected from an isolated *H. sorenseni* and fertilized with the sperm water of a *H. kamtschatkana assimilis* male. Some eggs were set aside as an unfertilized control and showed no development, indicating no contaminating sperm were present. Seventy-five percent of the eggs to which sperm were added developed to rotating trochophores. The larvae appeared to do well, and more than 50% advanced to the benthic stage. There was a small, post-benthic mortality, leaving an estimated 20,000 survivors. At about 10 months of age, 8-10 thousand remained. Most of these were transplanted to ocean bottom off Turtle Bay, Baja California, Mexico, leaving about 100 in the hatchery. These measured up to 42 mm in length as of September, 1971. This same batch of *H. sorenseni* eggs was also fertilized with homospecific *H. sorenseni* sperm, and provided an interesting comparison with the above cross. Virtually 100% fertilization was observed in this case and development was normal, very similar in rate to the *H. sorenseni* x *H. kamtschatkana assimilis* culture. However, the straight *H. sorenseni* progeny showed complete pre-benthic mortality on the seventh day. One earlier attempt to produce homospecific *H. sorenseni* also ended in total pre-benthic die-off. This total failure seemed odd as *Haliotis sorenseni* adults feed actively, grow well, seem to spawn normally in the

hatchery, and produced at least one extremely successful hybrid: the *H. sorenseni* x *H. kamtschatkana assimilis* culture, which developed successfully through settling and produced fast-growing juveniles.

In the hybrid combination, the male *H. kamtschatkana assimilis* was orange banded. The *H. sorenseni* female showed no band in the early whorl of the shell. Very close to 50% of the surviving hybrid progeny were orange banded (a random sample of 200 individuals showed 103 banded and 97 unbanded). Sixteen juvenile specimens are illustrated on Pl. 3.

Mass Spawning of February 13, 1970: During this unseasonal, but still very vigorous spawning, a *H. rufescens* female was successfully crossed with a *H. sorenseni* x *H. kamtschatkana assimilis* hybrid, producing another three species hybrid (Pl. 5).

Haliotis rufescens eggs were collected from an isolated female and fertilized with sperm water from an isolated *H. sorenseni* x *H. kamtschatkana assimilis* male. The usual unfertilized control was set aside and showed no development. Fertilization was quite slow; after 45 minutes, an estimated 20% of the eggs showed polar bodies, but after 18 hours, over 90% or the batch had progressed to rotating trochophores. Fertilization thus took place relatively slowly, but at high frequency. This culture of larvae developed especially well, and an estimated 90% of the veligers completed metamorphosis to the benthic stage by the 10th day. An estimated 6,000 progeny from this cross remained at eight months and all but approximately 100 were transplanted to Mexico (along with the *H. sorenseni* x *H. kamtschatkana assimilis* progeny of September, 1969). Of the remainder in the hatchery, the largest measured 56 mm in September, 1971. By that date, morphological characteristics of all three species involved in the parental cross were easily noticeable. *Haliotis rufescens* is evident from the dark pigmentation; the upper margin of the epipodium shows regularly spaced protuberances typical of *H. sorenseni*; and *H. kamtschatkana assimilis* is evident from the "threaded" pattern of the spiral sculpture in the shell.

May, 2015: The two largest specimens measured 88.6 mm and 95.0 mm when experiments were terminated in mid to late 1972. This was exceedingly rapid growth.

Mass Spawning of June 24, 1970: On this occasion, two hybrids of entirely different ancestry were crossed producing a batch of suspected four species hybrids.

Owen correctly anticipated this spawning from weather cues, and had isolated the prospective parents well in advance of the actual spawning. The male parent was a hatchery-raised *H. corrugata* x *H. walallensis* hybrid (born October 6, 1965), and the female was a *H. rufescens* x *H. sorenseni* collected from natural populations. The female was exposed only to filtered water, and the fact that she spawned suggests that sperm is not always necessary to stimulate the female spawning response (this had been observed on a number of occasions in straight *H. rufescens* spawnings done for commercial purposes as well). As usual, the male parent spawned before the female parent. The eggs were divided into two batches, and one was set aside as an unfertilized control. It showed no subsequent activity. The second batch was mixed with *H. corrugata* x *H. walallensis* sperm. Fertilization was quite feeble. Microscopically, sperm appeared to become immobile after contact with the vitelline envelope. After 45 minutes, no polar bodies were seen, and no sperm were observed within the vitelline space. After 24 hours, about 1% of

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the eggs had advanced to rotating trochophores, and at five days there were about 3,000 swimming veligers. About 5% of these successfully became benthic. Currently (September, 1971), at 15 months of age, about 100 of this group remain. The largest are about 41 mm long and beginning to show recognizable characteristics. A great deal of variability is evident in shell details and epipodial and body pigmentation. Many shells strongly suggest *H. corrugata*, and since there were no sexually mature *H. corrugata* males within 300 kilometers of the hatchery at the time of this spawning, this tends to further verify the presumed paternity of the *H. corrugata* x *H. walallensis* hybrid. (**Note:** The hatcheries single male *H. corrugata* of the July 19, 1969 spawning had died months earlier). Other shells of this group look very much like young *H. rufescens*, and still others have a very mixed appearance (Pl. 7). At least two different epipodial pigmentation patterns seem to be developing, and these differences do not seem to correlate with variations in the shell.

May, 2015: The two largest specimens measured 74.2 and 71.5 mm when experiments were terminated in mid to late 1972. This was exceedingly rapid growth.

It seems consistently true that when hybrid abalone are used as parents in these artificial crosses, the morphological variability in the progeny is greatly increased. In this particular case, where both parents are hybrids of different background, the variability in the progeny appeared even greater than in previous cases (October, 1968; July, 1969; and February, 1970) where only one of the parents was a hybrid.

Table 2 is a summary of the different crosses performed and the results, assessed as number and size of survivors as of September, 1971. (No information provided within various May, 2015, text sections is incorporated into this historical table.)

Table 2. Summary of crosses performed and results

Date of Mass Spawning	Cross performed Female x Male	Egg-Sperm Interaction	% of eggs becoming "rotating trochophores"	% of veligers becoming benthic	Number and maximum size (in mm) of survivors (Sept., 1971)		Progeny Positively Identified?
October 6, 1965	Co x Co	n.o.	n.o.	n.o.	3	37	yes
	Co x R	n.o.	n.o.	n.o.	0	N/A	yes
	Co x W	n.o.	n.o.	n.o.	1	59	yes
December 7, 1965	Ka x Ka	n.o.	n.o.	n.o.	14	100	yes
	Ka* x W*	n.o.	n.o.	n.o.	2	107	yes
August 23, 1967	Ka x Ka	normal	90	1	~100	90	yes
	Ka x W	subnormal	15	0	0	N/A	N/A
	Ka x Cr	none	2	0	0	N/A	N/A
	Ka x S	subnormal	5	0	0	N/A	N/A
	Ka X (R x S)	subnormal	25	0	0	N/A	N/A
	Ka X (R x Ka)	normal	75	1	22	103	yes
October 31, 1968	(R x S) X Ka	n.o.	n.o.	n.o.	20	65	yes
	(R x S) X **	n.o.	95	n.o.	130	80	no
July 19, 1969	S x Co	subnormal	25	10	86	53	yes
	Co X (R x S)	n.o.	80	90	16	72	R and S (and 1 Co)
September 14, 1969	S x Ka	normal	75	50	~100	42	yes
	S x S	normal	over 95	0	0	N/A	N/A
February 13, 1970	R X (S x Ka)	subnormal	90	90	~100	56	yes
June 24, 1970	(R x S) X (Co x W)	subnormal	1	5	~100	41	yes

KEY

Co = *H. corrugata*
R = *H. rufescens*
W = *H. walallensis*
Ka = *H. kamtschatkana assimilis*
Cr = *H. cracherodii*
S = *H. sorenseni*

(R x S) = *H. rufescens* x *H. sorenseni*
(R x Ka) = *H. rufescens* x *H. kamtschatkana assimilis*
(S x Ka) = *H. sorenseni* x *H. kamtschatkana assimilis*
(Co x W) = *H. corrugata* x *H. walallensis*
n.o. = not observed
* = sexes of parent species uncertain
** = parent species uncertain

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EXPLANATION OF PLATES

Eight plates are included which illustrate the various crosses cultivated. They are arranged in an ascending order of complexity based on information gleaned from all of the spawning events between 1965 and 1970. Dates of specific spawnings are listed in "Conclusions" on pg. 183. All specimens are hatchery cultured unless noted otherwise (on plates or text). Specimens listed being from natural populations are from California, unless otherwise recorded.

May, 2015: A ninth plate illustrating a pair of two species hybrids cultured at other laboratories (after 1972) is included, one which may not have been cited in literature; additionally, some specimens of several crosses were kept alive after these experiments were concluded and reached sizes larger than those listed in Table 2.

Pl. 1 – Illustrates the six species used in the experiments (both *H. kamtschatkana assimilis* and *H. kamtschatkana kamtschatkana* are included). All specimens are from natural populations.

Pl. 2 – Illustrates the five two species hybrids cultivated at Pacific Mariculture, Inc. These mass spawnings occurred on October 6 and December 7, 1965, and July 19 and September 14, 1969.

Pl. 3 – The four bottom rows illustrate sixteen hatchery cultured 7-11 mm juvenile *H. sorenseni* (non-banded female) x *H. kamtschatkana assimilis* (male with genetic orange band). Eight show the orange band, and eight are without band. This mass spawning occurred on September 14, 1969.

Pl. 4 – Illustrates four orange banded backcrosses of a female orange banded *H. kamtschatkana assimilis* crossed with a male *H. rufescens* x *H. kamtschatkana assimilis* hybrid. This mass spawning occurred on August 23, 1967.

Pl. 5 – Illustrates four three species hybrids of a female *H. rufescens* crossed with a male *H. sorenseni* x *H. kamtschatkana assimilis* hybrid. This mass spawning occurred on February 13, 1970.

Pl. 6 – The bottom four rows illustrate fourteen extremely confusing specimens resulting from the eggs of a *H. rufescens* x *H. sorenseni* hybrid being fertilized with sperm water from a tank containing spawning males of at least four or five species and two or three different hybrids. Additionally, there were a few fertilized eggs that came from this tank. Many bizarre genetic combinations resulted from this spawning as this plate demonstrates, including a number of confirmed-with-animal three species crosses of this hybrid female with a male *H. kamtschatkana assimilis*. These latter crosses cannot be differentiated from the remainder of this group by shell morphology and thus cannot be positively identified. The mass spawning that yielded these specimens occurred on October 31, 1968. The top row of this plate illustrates a three species cross of a *H. corrugata* female and a male *H. rufescens* x *H. sorenseni* hybrid. This mass spawning occurred on July 19, 1969.

Pl. 7 – Illustrates sixteen specimens of a four species cross of a female *H. rufescens* x *H. sorenseni* hybrid with a hatchery cultured male hybrid *H. corrugata* x *H. walallensis*. The morphology of all four species is visible throughout this group. This mass spawning occurred on June 24, 1970.

Pl. 8 – Illustrates a bizarre three species cross between a female *H. cracherodii* and a hatchery cultured male *H. corrugata* x *H. walallensis* (from the mass spawning of October 31, 1968). Also compared are a typical southern *H. kamtschatkana assimilis* and a specimen exhibiting the northern *H. kamtschatkana kamtschatkana* morphology of its obvious Canadian male parent. These two animals came from the spawning of December 7, 1965.

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Pl. 9 – Includes hatchery cultured hybrids beyond the experiments discussed thus far. More discussion to follow.

DISCUSSION

Culture Techniques. By improving techniques during the first several years that these experiments were carried out, gradually more control was gained over the fertilization step. In the first mass spawning (October, 1965) the eventual results showed that 90% of the surviving progeny represented fertilizations by sperm that were thought not to be present. This indicated that avoiding fertilization by contaminating sperm was more difficult than expected, and led to the adoption of techniques where desired parents were removed from the main tank, rinsed, and held in isolation in filtered water (see Materials & Methods). By 1969, contamination by unwanted sperm could be almost entirely avoided.

Since these larvae had been cultured under hatchery conditions, human factors may be responsible for part or all of the mortality. With more experience the human factors are becoming more controlled, but it still appears that there is a natural variability in survival at settling. This may depend on the genetic makeup of the parents, the nutritional condition of the parents when they spawn, or both. Since a single pair of spawning adults may produce more than a million developing embryos, a low rate of survival through metamorphosis will still allow many progeny to survive under hatchery conditions. In nature, mortality is undoubtedly high due to such additional factors as predation and the chance of settling in an unfavorable environment. Perhaps a high level of genetic variability in abalone offspring may result in high mortality of less well-adapted variants during the larval stage; the unpredictability of this factor complicates the evaluation of relative success of hybrid combinations. We were unable to determine if the mortality of a group of hybrid larvae was due to a lethal combination of inherited factors or typical mortality rates seen with homospecific crosses.

Egg-Sperm Interaction. One of the results that best seemed to reflect the success of any particular hybrid combination was the relative speed of successful penetration of the egg by sperm. Homospecific gametes show high affinity for each other, since sperm rapidly penetrate the vitelline envelope and swim actively within the perivitelline space of the egg (Fig. 1). Sperm from different species tend to be repelled or blocked at the envelope surface, or suppressed in activity once inside. In the August, 1967, spawning, *H. walallensis*, *H. sorenseni*, and hybrid *H. rufescens* x *H. sorenseni* sperm were all sluggish in their interaction with *H. kamtschatkana assimilis* eggs, although effective fertilization occurred in a small number of eggs. The cross attempted in February, 1970, between a *H. rufescens* female and a *H. sorenseni* x *H. kamtschatkana assimilis* male, showed very slow rates of penetration, but apparently a large fraction of the eggs successfully became fertilized. In the June, 1970, spawning where two hybrids of entirely different genetic background were used as parents, fertilization was slower yet, but about 1% of the eggs eventually developed.

This relative speed of fertilization could be an important factor preventing wholesale hybridization in nature. During a natural mass spawning an egg might be exposed to sperm of a number of different species, and if homospecific sperm in the water could penetrate the eggs faster than others, a small amount of homospecific sperm in the water could virtually ensure homospecific fertilization of all the eggs. Only in circumstances where active males of the

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species are absent would hybrids be produced. A possible example of an area of this sort is the coastline three to six miles east of Point Conception (Fig. 3). At least two and perhaps three of the four known *H. sorenseni* x *H. kamtschatkana assimilis* hybrids have been found in this area. It is approximately the northern limit of the distribution of *H. sorenseni*, and a number of years diving in the area show the species to be quite rare. *Haliotis kamtschatkana assimilis* is locally very common and a large spawning of that species in the area of a lone *H. sorenseni* female might provide conditions that would give the slow penetrating *H. kamtschatkana assimilis* sperm sufficient time to penetrate the *H. sorenseni* eggs, free of competition from homospecific sperm.

Figure 3 illustrates Point Conception area.

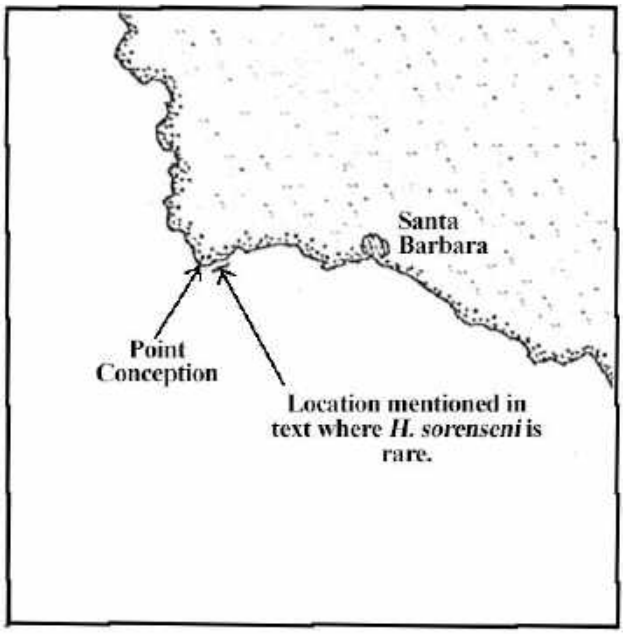

Figure 3. Map of Point Conception area, showing where two of the four known *H. sorenseni* x *H. kamtschatkana assimilis* hybrids have been found.

Among the combinations performed in August 1967, was an attempt to fertilize *H. kamtschatkana assimilis* eggs with *H. sorenseni* sperm. Fertilization percentage was recorded at less than 5% and no progeny survived past the larval stage. During the September, 1969 spawning, *H. sorenseni* eggs were fertilized with *H. kamtschatkana assimilis* sperm, and good fertilization and larval survival were observed. This represents the only case among all the spawnings reported that could be called a "reciprocal cross", *i.e.*, the same kinds of parents with the sexes reversed. In this case, the results were quite different in the two reciprocal combinations. Imai and Sakai (1961) performed reciprocal fertilizations with different species of the oyster genus *Crassostrea*. They also observed different rates of fertilization when the sexes were reversed.

Failure of *H. cracherodii*. As of September, 1971, *Haliotis cracherodii* is the only species of Eastern Pacific *Haliotis* for which no hybrids have been reported in nature, and in this light, the behavior of *H. cracherodii* sperm in the August, 1967, spawning is of interest. While other heterospecific sperm penetrated slowly, *H. cracherodii* sperm seemed to be actually repelled from *H. kamtschatkana assimilis* eggs, leaving a sperm-free zone around the vitelline envelope. We suspect that there was no fertilization taking place, although a few trochophores were observed in this batch the following day. Considering the methods used during this spawning and what has been learned since, it seems likely to us that there was a low level contamination of this culture by some *H. kamtschatkana assimilis* sperm. Future spawnings are being planned to test more thoroughly whether *H. cracherodii* gametes can successfully combine with those of other Eastern Pacific abalone species.

The one species of Eastern Pacific abalone not used in these experiments is *Haliotis fulgens*. It would have been interesting to use gametes from this species because only *H. cracherodii* is less apt to hybridize than *H. fulgens*. *Haliotis*

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fulgens is known to cross only with *H. corrugata* (25 known cases), and *H. rufescens* (2 known cases). Excepting *H. cracherodii*, the three unknown hybrids all involve *H. fulgens*: *H. sorenseni* x *H. fulgens*, *H. kamtschatkana assimilis* x *H. fulgens*, and *H. walallensis* x *H. fulgens*. (Owen *et al*., 1971).

May, 2015: It is now known that *H. cracherodii* hybridizes with *H. corrugata* (unique) and *H. fulgens* (six known currently) and both hybrids have also been cultured in hatcheries (Owen & Leighton, 2002). Additionally, *H. fulgens* has been successfully crossed with *H. sorenseni* in a marine laboratory (Leighton, 2000). The two existing specimens of the latter are illustrated herein (Pl. 9).

Comparison with Hemocyanin Relationships. Meyer has studied the hemocyanins (oxygen-carrying blood proteins) of the California abalone, comparing immunological properties of these proteins in the different species (Meyer, 1967). Since the hemocyanins might also be taken as a measure of genetic relatedness, it would be interesting to compare genetic interpretations of the hemocyanin results with the laboratory results. Broadly, the hemocyanin data suggest relationships as depicted in Figure 4. First, *H. cracherodii* is the most different from the others. Apart from *H. cracherodii*, there are three more or less equally distinct stems: *H. walallensis* alone, a *H. corrugata-fulgens* line, and a *H. rufescens-sorenseni-kamtschatkana assimilis* line. The latter three all have closely related hemocyanins, thus are judged to be a closely related trio. *Haliotis corrugata* and *H. fulgens* are less close to one another, but still closer to each other than they are to other species of California abalone.

Figure 4 illustrates the relationships among the seven species of California *Haliotis*, based on immunochemical comparisons of their hemocyanins.

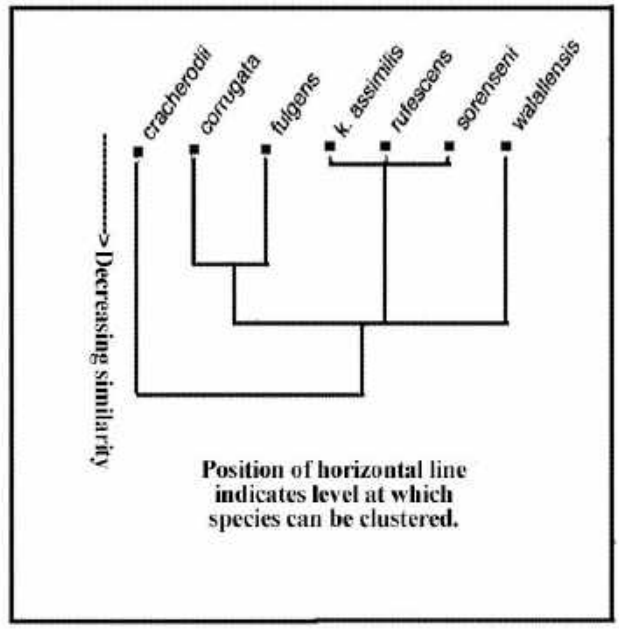

Figure 4. Relationships among the several species of California *Haliotis* as suggested by immunochemical comparisons of hemocyanins.

The very different hemocyanin of *H. cracherodii* points to a genetic difference that is consistent with the behavior of *H. cracherodii* sperm in the 1967 experiments. The success of the backcross in those same experiments indicates close genetic affinity between *H. rufescens* and *H. kamtschatkana assimilis*, consistent with the hemocyanin results. However, hemocyanins also point to a close relationship between *H. sorenseni* and *H. kamtschatkana assimilis*, yet both *H. sorenseni* and *H. rufescens* x *H. sorenseni* hybrid sperm were classed as "subnormal" in their interaction with *H. kamtschatkana assimilis* eggs, similar to *H. walallensis*, which has a hemocyanin quite distinct from *H. kamtschatkana assimilis*. The reciprocal combination of *H. kamtschatkana assimilis* x *H. sorenseni* performed in September, 1969, however, showed good survival of progeny.

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The Orange Band Variant in *H. kamtschatkana assimilis*. The orange banded shell characteristic sometimes found in *H. kamtschatkana assimilis* appears to be an inherited trait and some of the spawnings give information that allows speculation on the pattern of inheritance of the band. The data so far collected fit well with the supposition that the banded character is determined by a single dominant gene, but it should be emphasized that other explanations might also be consistent with the data collected, so that apparent fit with the single gene explanation is not necessarily proof of that interpretation.

In the spawning of August, 1967, a straight *H. kamtschatkana assimilis* cross was performed and both parents were banded. Out of 203 progeny, 153 (75%) were banded and 50 (25%) were unbanded. If both parents were heterozygous for the trait and it were due to a single dominant gene, one would expect three-fourths of the progeny to show the dominant character (banded) and one-fourth the recessive character (unbanded), as occurred here.

In another cross with the above female in the same 1967 spawning, the backcross parent (*H. rufescens* x *H. kamtschatkana assimilis* male) was not banded and 11 of the 24 backcross progeny (46%) were banded. According to the single dominant gene hypothesis, with the banded parent being heterozygous, a cross with an unbanded parent should give half banded and half unbanded progeny, a result not far from the observed one.

In the September, 1969 spawning, a banded male *H. kamtschatkana assimilis* was crossed with a *H. sorenseni* female. The female did not show a band in the early whorls of the shell. Almost exactly one-half (103/200) of the progeny of this cross came out to have banded shells. Assuming the banded male parent here was heterozygous, the cross would be similar to the backcross combination of 1967, and in accord with the single dominant gene interpretation, half of the progeny would be expected to have a band, as occurred here.

CONCLUSIONS

1. Hybridization of California *Haliotis* can be performed under hatchery conditions.

2. The following two species hybrids have been produced in the hatchery. The offspring have matured sufficiently to be positively identified on a morphological basis (Pl. 2).
Haliotis corrugata x *H. rufescens* (October, 1965)
Haliotis corrugata x *H. walallensis* (October, 1965)
Haliotis kamtschatkana assimilis x *H. walallensis* (December, 1965)
Haliotis sorenseni x *H. corrugata* (July, 1969)
Haliotis sorenseni x *H. kamtschatkana assimilis* (September, 1969)

3. At least some hybrid abalone of both sexes have been shown to be fertile. A *H. kamtschatkana assimilis* female was successfully backcrossed to a *H. rufescens* x *H. kamtschatkana assimilis* hybrid male (August, 1967; Pl. 4). A female *H. rufescens* x *H. sorenseni* hybrid successfully produced three species hybrid progeny with a male *H. kamtschatkana assimilis*, 20 specimens being confirmed by animal morphology (October, 1968; Pl. 6). (**Note:** A large number of specimens of exceedingly confused identity resulted from this spawning due to an unknown number of different species and hybrid male parents being involved. From shell alone, it is not possible to identify individual specimens of this group). One *H. rufescens* x *H. sorenseni* hybrid proved a successful male parent, combining with *H. corrugata* eggs (July, 1969;

Pl. 6, top row). Another *H. rufescens* x *H. sorenseni* female successfully crossed with a male hatchery-raised hybrid *H. corrugata* x *H. walallensis* (June, 1970).

4. A *H. rufescens* x *H. sorenseni* hybrid female was crossed with a *H. kamtschatkana assimilis* male (October, 1968, Pl. 6) and the progeny have been positively identified as the first known case of a three species hybrid in *Haliotis*. A second attempt at a three species cross, *H. corrugata* X (*H. rufescens* x *H. sorenseni*) was also successful (July, 1969, Pl. 6 top row), and identification was confirmed on at least one of the larger animals when the *Haliotis* experiments were terminated in late 1972. A third example of a three species hybrid has been confirmed, the cross of a *H. rufescens* female with a hybrid *H. sorenseni* x *H. kamtschatkana assimilis* male (February 1970, Pl. 5). Finally, a fourth three species cross was later confirmed with the discovery of a single specimen of *H. cracherodii* X (*H. corrugata* x *H. walallensis*). (**Note:** This specimen was a byproduct of the October, 1968 spawning. Pl. 8, 2nd row).

5. Viable four species hybrid progeny (*H. rufescens* x *H. sorenseni*) X (*H. corrugata* x *H. walallensis*) were produced during the June, 1970, spawning (Pl. 7).

6. The pattern of inheritance of the orange band in *H. kamtschatkana assimilis* is consistent with the banded character being determined by a single dominant gene.

7. Whatever the factor that induced spawning, it was carried through water that was filtered through a one micron filter and UV sterilized (spawning of June, 1970). During these spawnings, other instances occurred where isolated females spawned copiously in non-UV treated water as well.

8. Behavior of sperm during fertilization is apparently quite important to the fate of a hybrid combination. Many sperm seem to be retarded in activity near eggs from other species.

9. One reciprocal cross was performed. *Haliotis sorenseni* sperm with *H. kamtschatkana assimilis* eggs (August, 1967), was an unsuccessful combination, but *H. kamtschatkana assimilis* sperm with *H. sorenseni* eggs resulted in many thousands of healthy progeny.

10. The morphological variability in a group of progeny was observed to be much greater when hybrid abalone served as parents. The most variable batch of offspring was the presumed four species hybrids, where both parents were hybrids of different genetic background.

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Taxonomic Note - new species:
***Haliotis geigeri* Owen, 2014**

This small species is endemic to São Tomé and Príncipe Islands off the coast of Gabon, West Africa. It is the smallest member of the *Haliotis tuberculata tuberculata* Linnaeus, 1758, group, seldom exceeding 40 mm in length. Little is known of its preferred habitat or biology, and it is uncommon in collections. Figured specimen measures 38.4 mm, and was taken at Lagoa Azul Beach, São Tomé Island, in 3-5 m. (Owen, B. 2014. A new species of *Haliotis* (Gastropoda) from São Tomé & Príncipe Islands, Gulf of Guinea, with comparisons to other *Haliotis* found in the Eastern Atlantic and Mediterranean. Zootaxa 3838 (1): 113-119. 2 pls.) (Photo credit: Buzz Owen)

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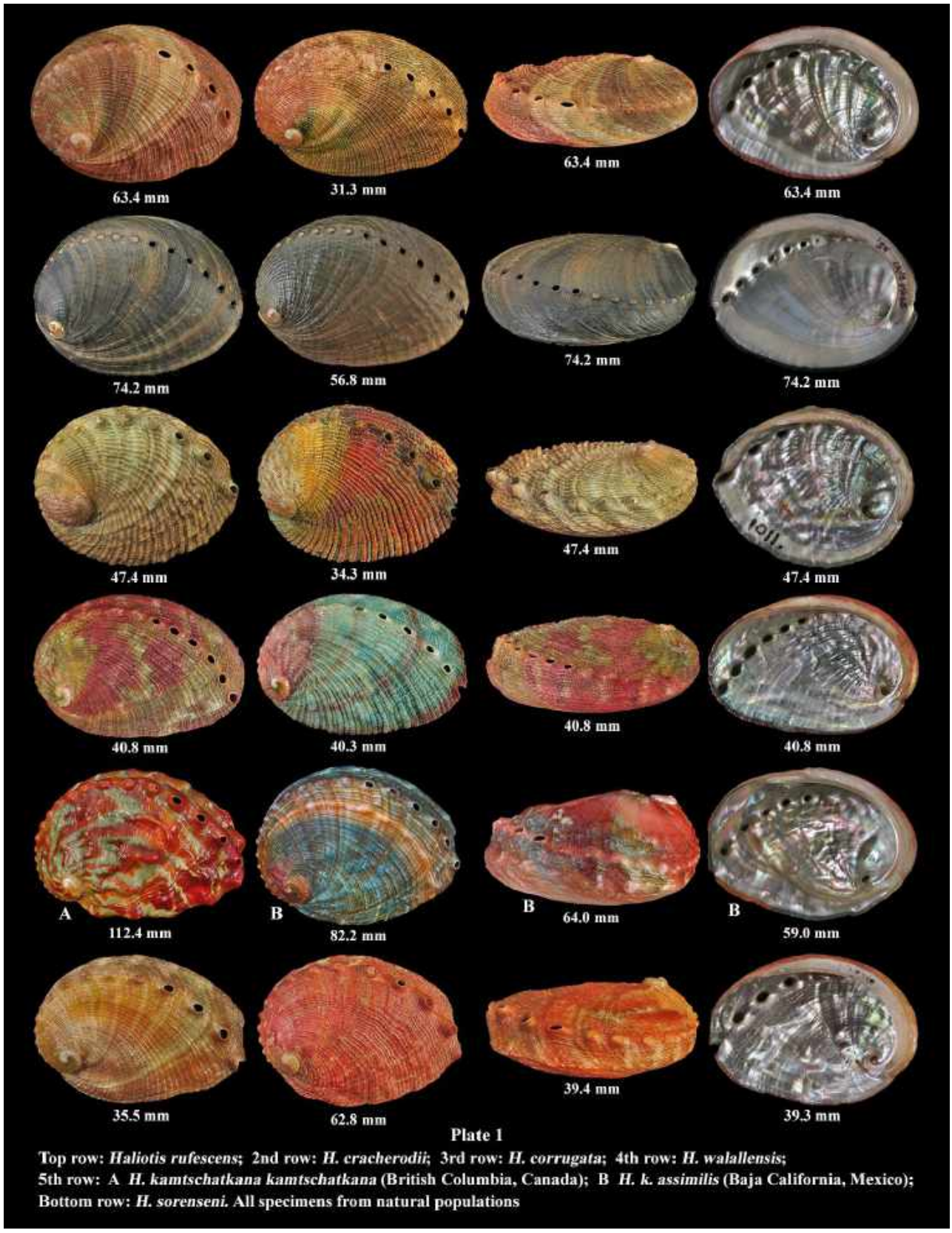

Plate 1

Top row: *Haliotis rufescens*; 2nd row: *H. cracherodii*; 3rd row: *H. corrugata*; 4th row: *H. walallensis*; 5th row: A *H. kamtschatkana kamtschatkana* (British Columbia, Canada); B *H. k. assimilis* (Baja California, Mexico); Bottom row: *H. sorenseni*. All specimens from natural populations

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Plate 2

Top row: ***Haliotis corrugata*** x ***H. rufescens***; 2nd row: ***H. corrugata*** x ***H. walallensis***
3rd row: ***H. kamtschatkana assimilis*** x ***H. walallensis***; 4th row: ***H. sorenseni*** x ***H. corrugata***
Bottom row: ***H. sorenseni*** x ***H. kamtschatkana assimilis***. All specimens hatchery cultured

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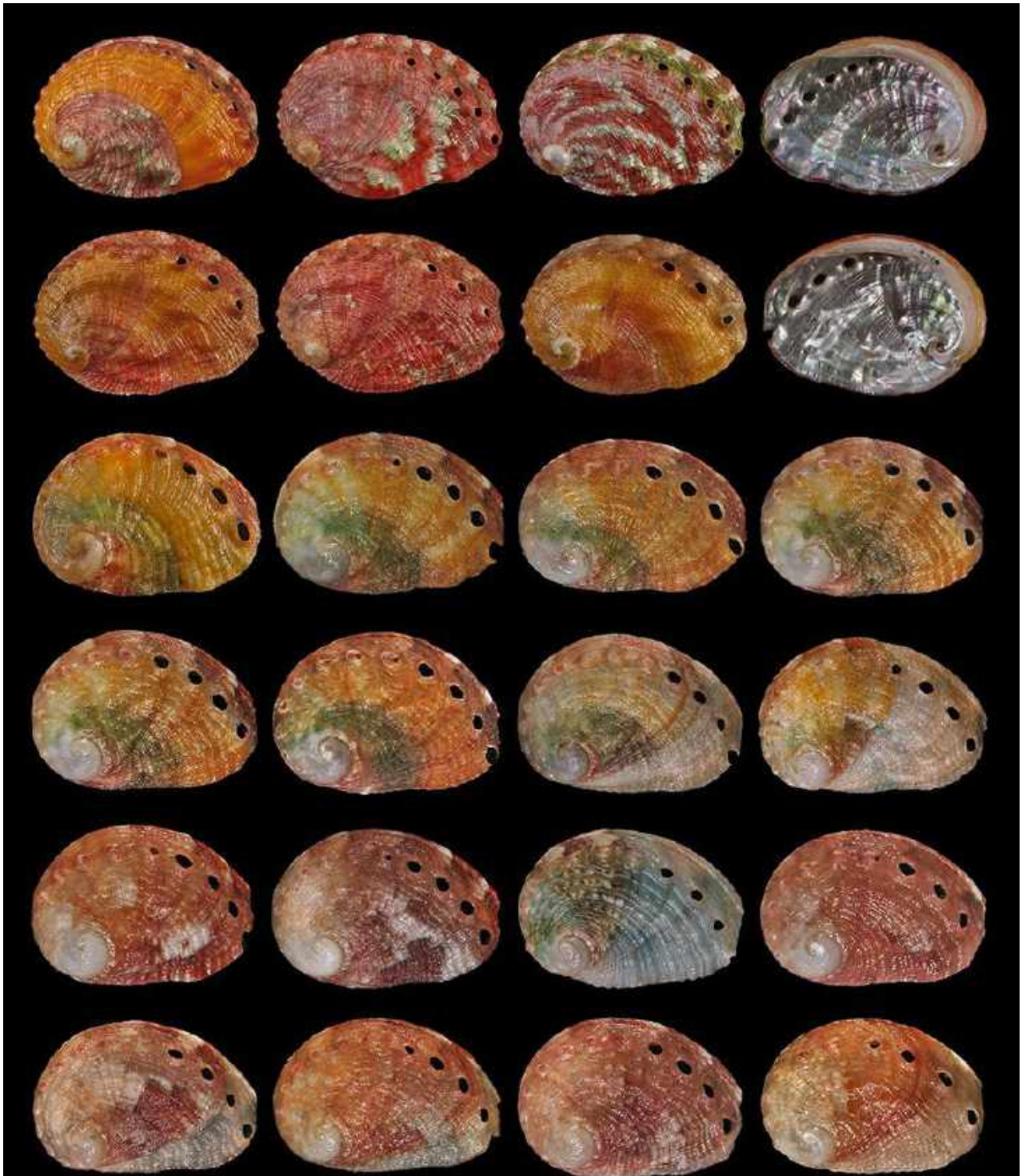

Plate 3

Top row: *Haliotis kamtschatkana assimilis*. 35–40 mm. Natural populations
2nd row: *H. sorenseni*. 26-34 mm. Natural populations
Bottom 4 rows: *H. sorenseni* x *H. kamtschatkana assimilis*. 7.5-11.5 mm. Hatchery cultured

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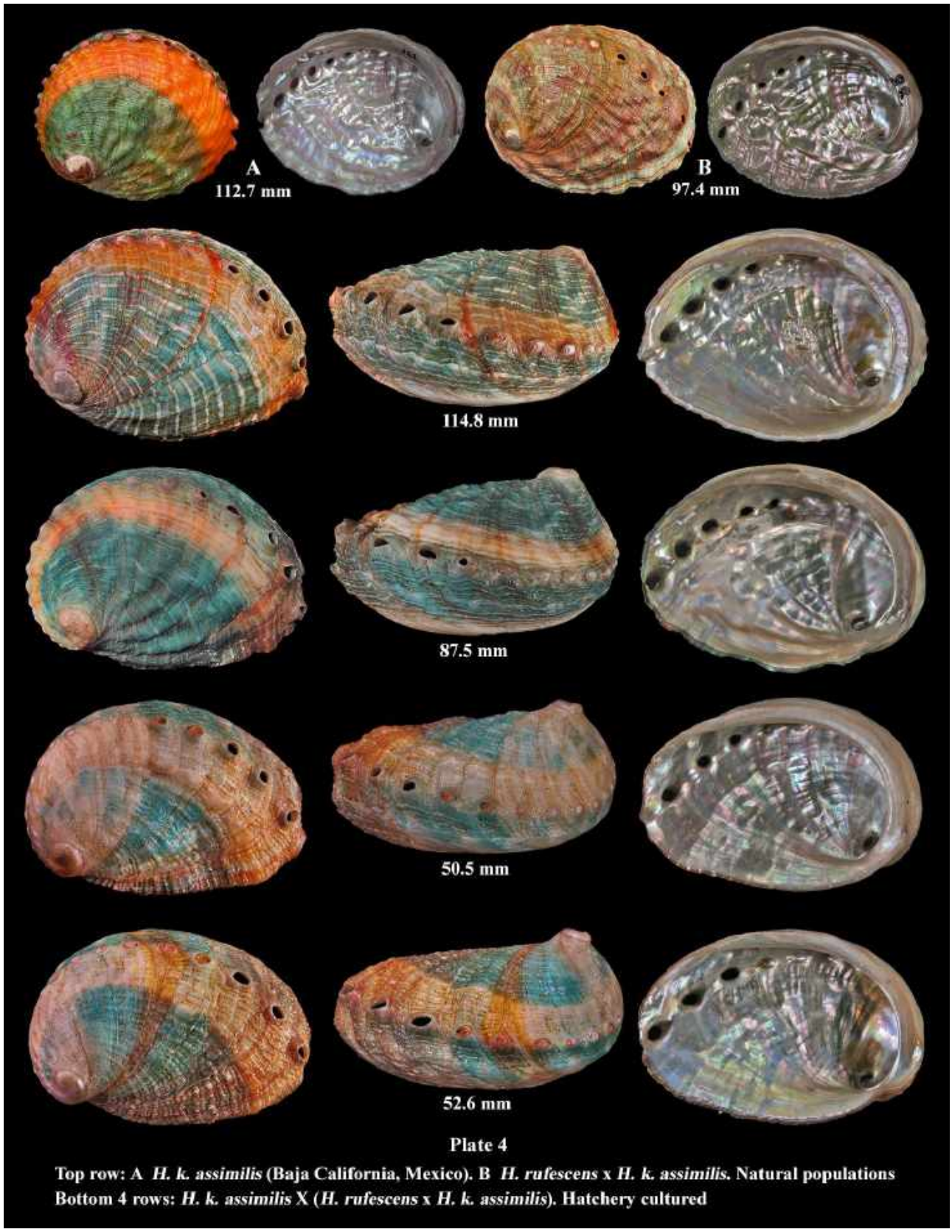

Plate 4

Top row: A *H. k. assimilis* (Baja California, Mexico). B *H. rufescens* x *H. k. assimilis*. Natural populations
Bottom 4 rows: *H. k. assimilis* X (*H. rufescens* x *H. k. assimilis*). Hatchery cultured

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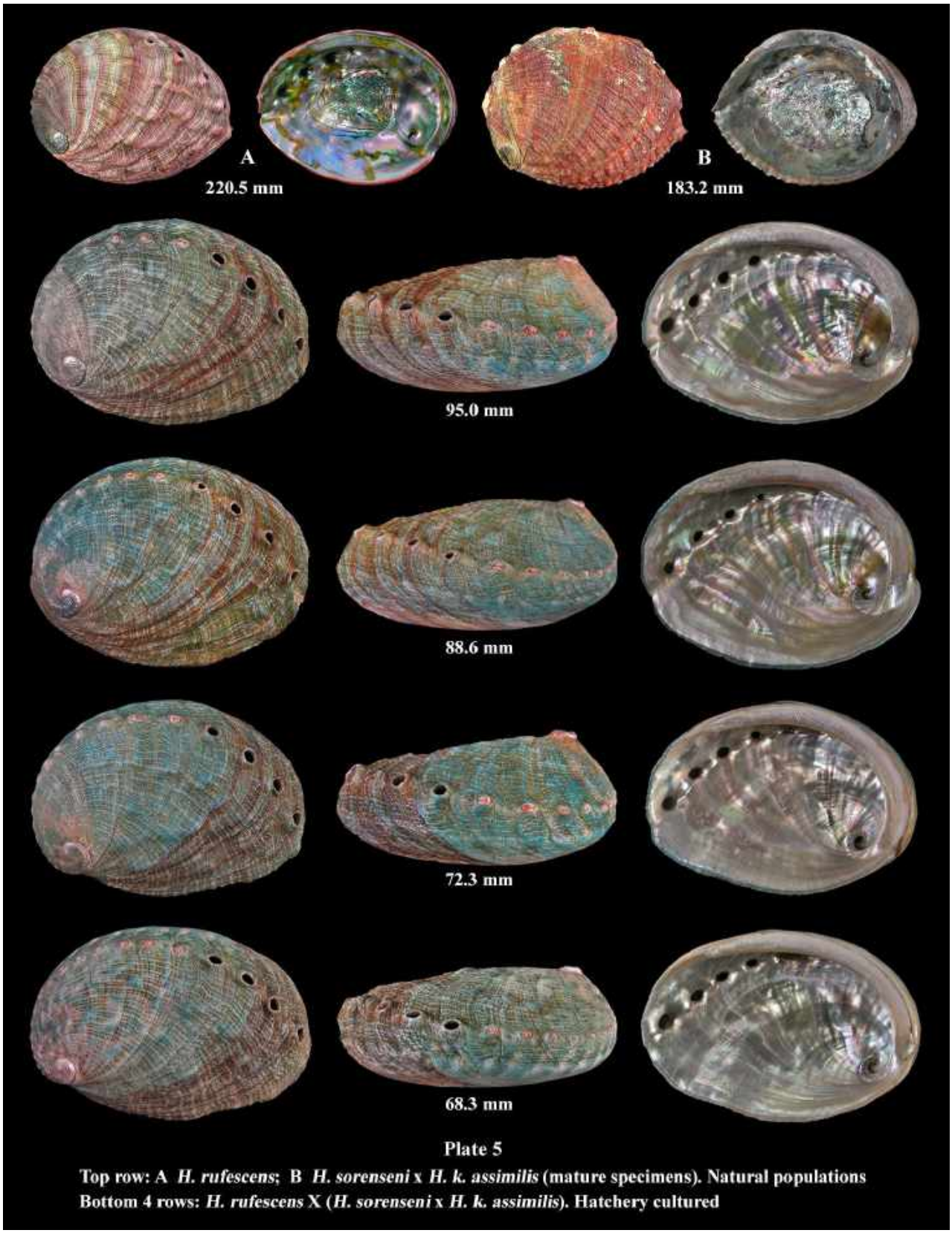

Plate 5

Top row: A *H. rufescens*; B *H. sorenseni* x *H. k. assimilis* (mature specimens). Natural populations
Bottom 4 rows: *H. rufescens* X (*H. sorenseni* x *H. k. assimilis*). Hatchery cultured

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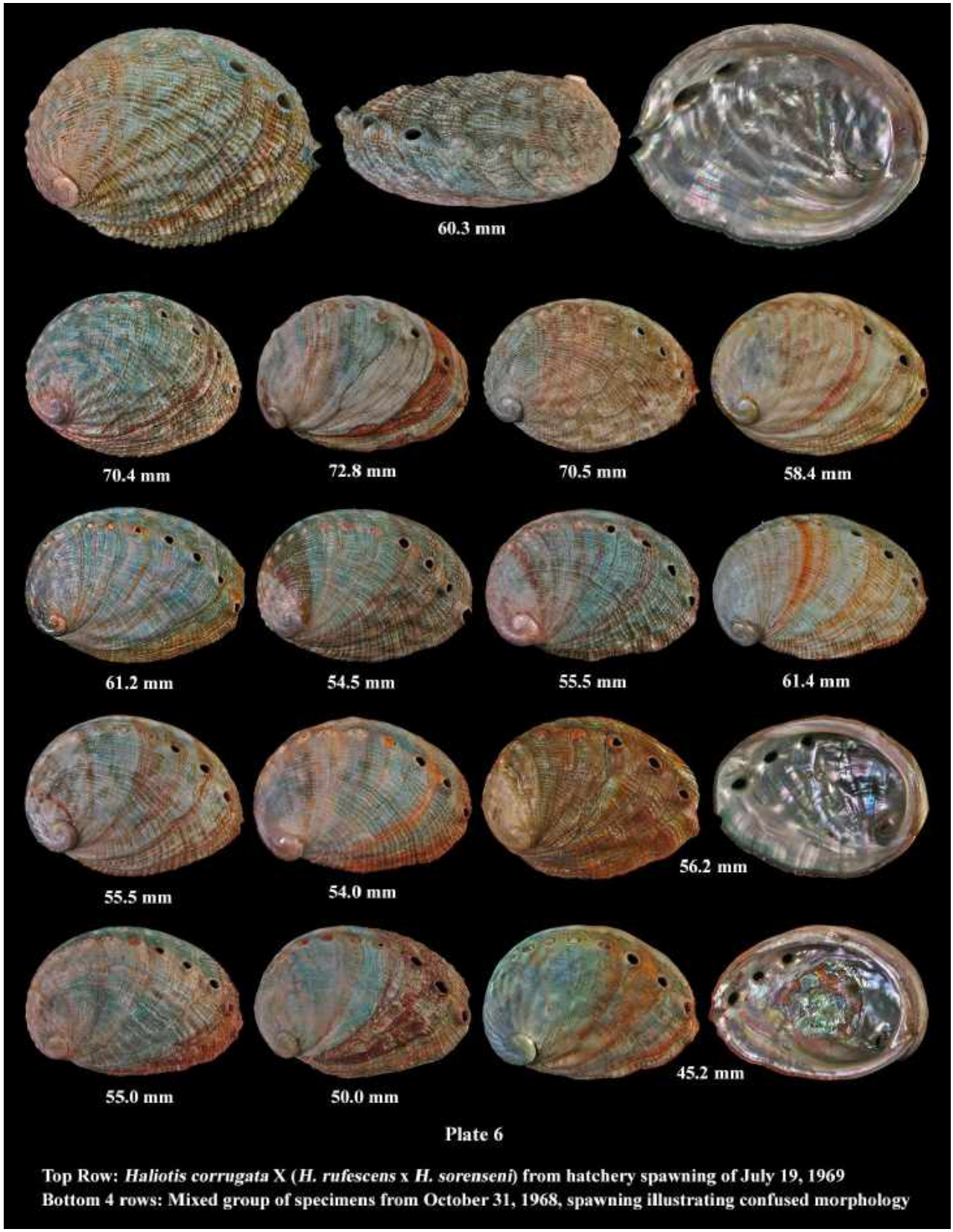

Plate 6

Top Row: *Haliotis corrugata* X (*H. rufescens* x *H. sorenseni*) from hatchery spawning of July 19, 1969
Bottom 4 rows: Mixed group of specimens from October 31, 1968, spawning illustrating confused morphology

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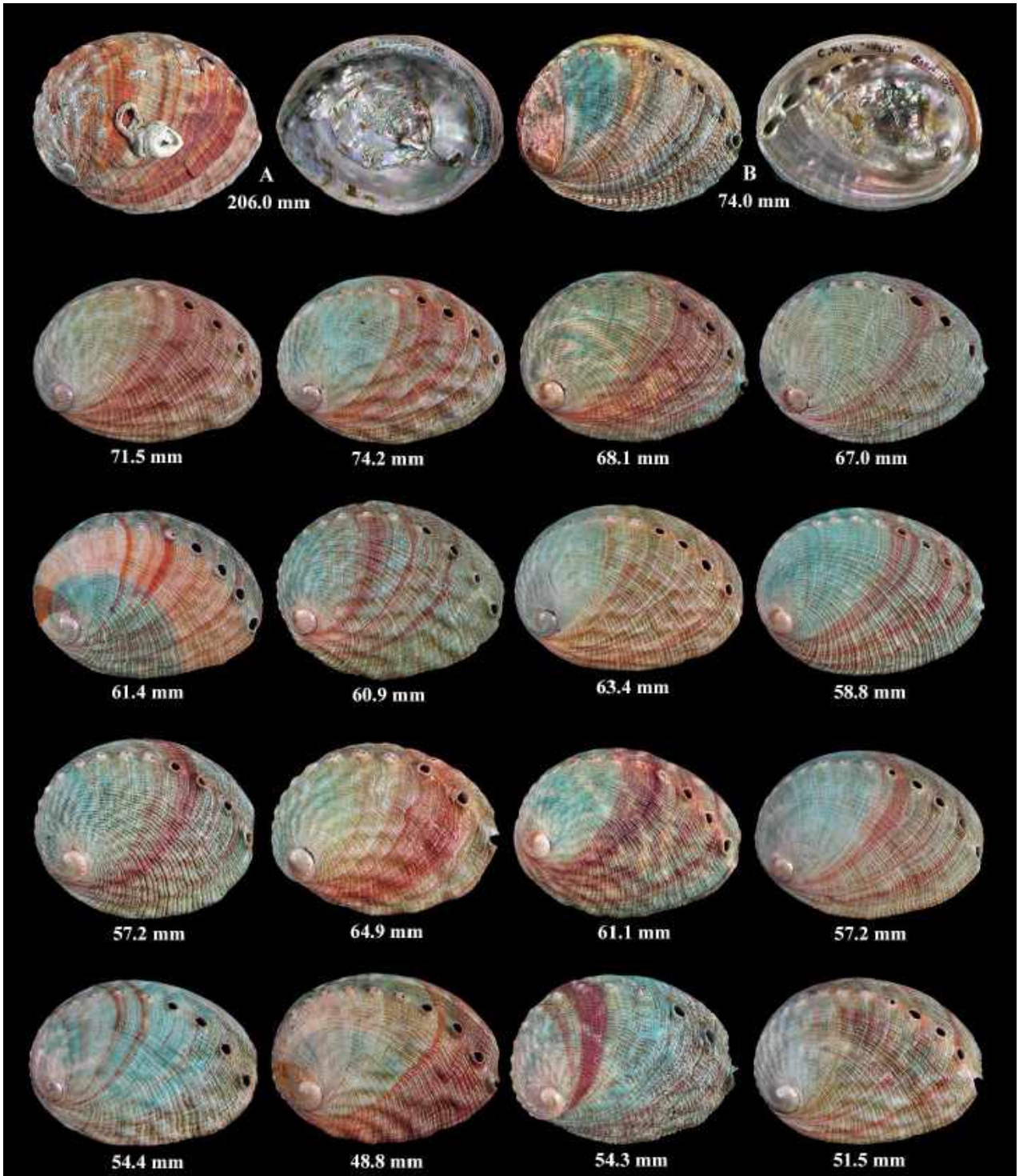

Plate 7

Top Row: A *H. rufescens* x *H. sorenseni* (female-natural). B *H. corrugata* x *H. walallensis* (male-hatchery)
Rows 2-5: (*H. rufescens* x *H. sorenseni*) X (*H. corrugata* x *H. walallensis*). Hatchery cultured

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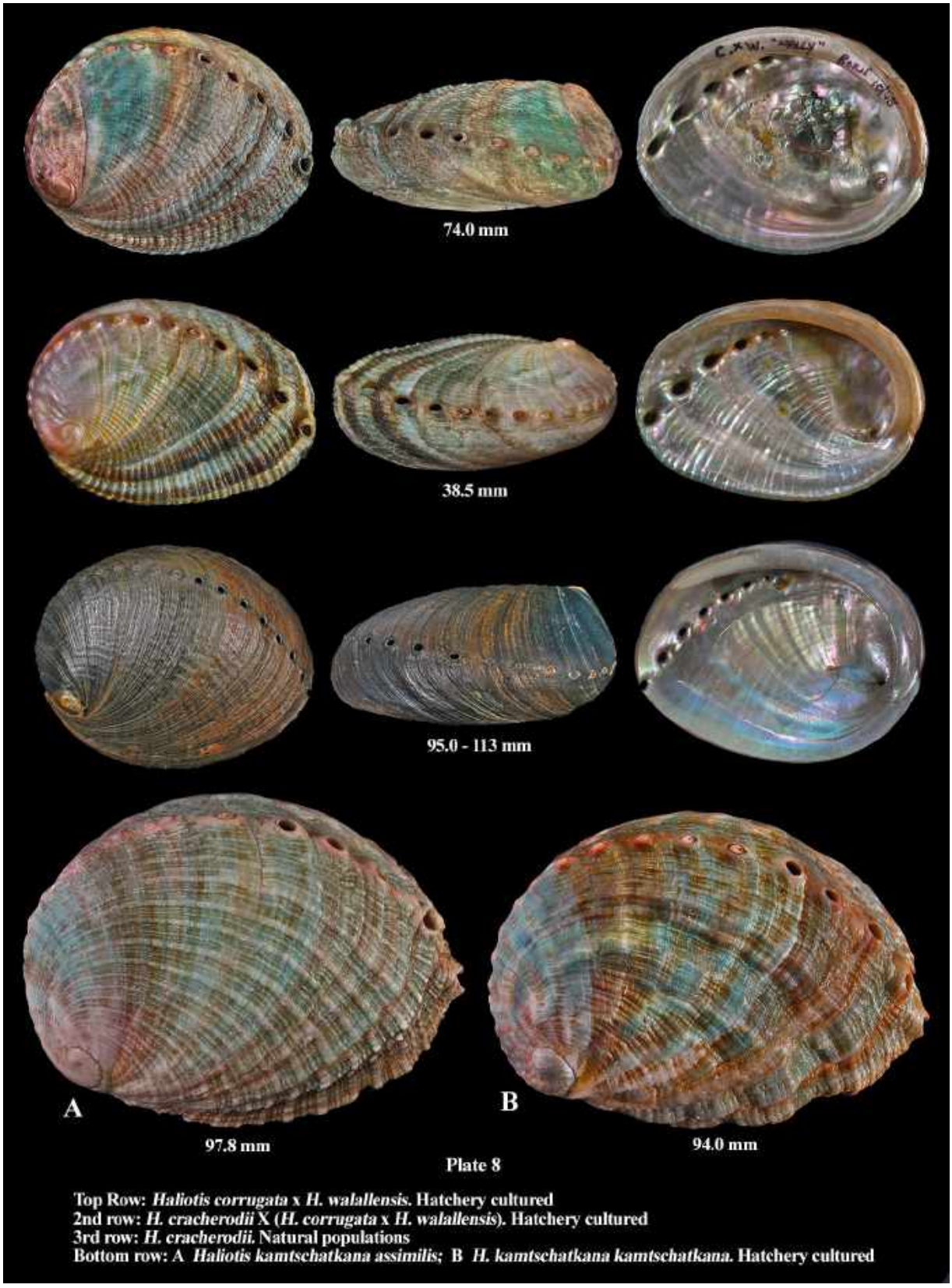

Plate 8

Top Row: *Haliotis corrugata* x *H. walallensis*. Hatchery cultured
2nd row: *H. cracherodii* X (*H. corrugata* x *H. walallensis*). Hatchery cultured
3rd row: *H. cracherodii*. Natural populations
Bottom row: A *Haliotis kamtschatkana assimilis*; B *H. kamtschatkana kamtschatkana*. Hatchery cultured

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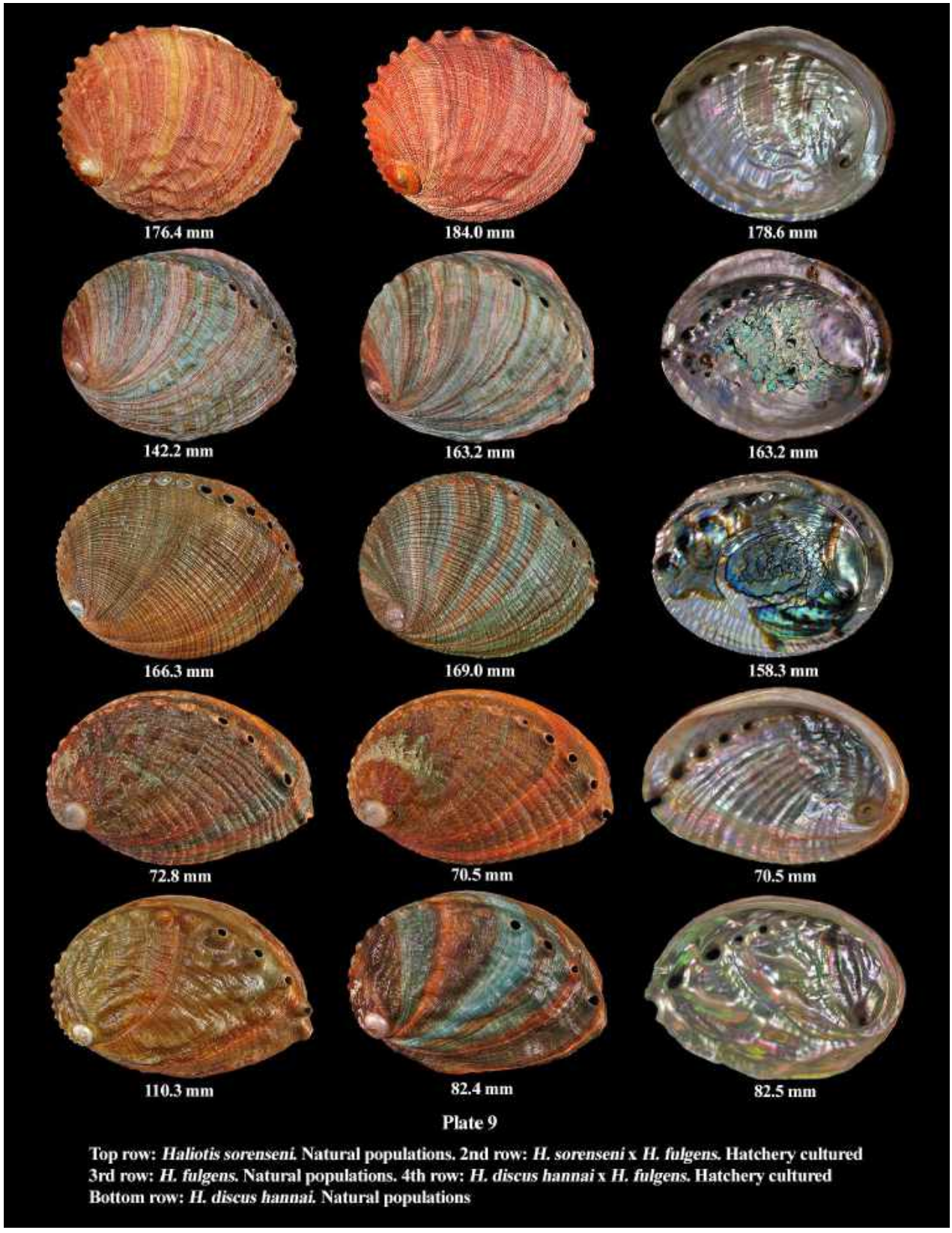

Plate 9

Top row: *Haliotis sorenseni.* Natural populations. 2nd row: *H. sorenseni* x *H. fulgens.* Hatchery cultured
3rd row: *H. fulgens.* Natural populations. 4th row: *H. discus hannai* x *H. fulgens.* Hatchery cultured
Bottom row: *H. discus hannai.* Natural populations