

RESPONSE OF RED ABALONE REPRODUCTION TO WARM WATER, STARVATION, AND DISEASE STRESSORS: IMPLICATIONS OF OCEAN WARMING

LAURA ROGERS-BENNETT,^{1,2*} RICHARD F. DONDANVILLE,³ JAMES D. MOORE^{1,2} AND L. IGNACIO VILCHIS⁴

¹California Department of Fish and Game; ²U.C. Bodega Marine Laboratory, PO Box 247, Bodega Bay, CA 94923; ³5342 Winding View Trail, Santa Rosa, CA 95404; ⁴Scripps Institution of Oceanography, University of California San Diego, La Jolla, CA 92093

ABSTRACT Changes in ocean temperature can have direct and indirect effects on the population dynamics of marine invertebrates. We examined the impacts of warm water, starvation, and disease on reproduction in red abalone (*Haliotis rufescens*). We found that sperm production was highly sensitive to warm water and starvation, suggesting there may be a dramatic temperature threshold above which sperm production fails. Wild males from northern (72%) and southern (81%) California had sperm. In contrast, only 30% of the males exposed to warm water (18°C) for 6 mo or starvation for 13 mo had sperm, with spermatogenesis dropping dramatically from 300,000 presperm cells/mm³ (wild) to 46,000 presperm cells/mm³ (warm water) and 84,000 presperm cells/mm³ (starvation). In a longer warm-water experiment (12 mo), males had total reproductive failure in temperatures greater than 16°C, irrespective of food treatment. Egg production was less sensitive to warm water, but was impacted more by starvation, especially food quantity relative to quality. Wild females from northern (97%) and southern (100%) California had mature oocytes averaging 3 million eggs and 21 million eggs, respectively. Females exposed to 18°C water for 6 mo had diminished fecundity, averaging only 400,000 mature eggs whereas females in the starvation experiment did not produce any mature eggs. Normal sperm and egg production was found in abalone testing positive for Rickettsiales-like-prokaryote (RLP), the agent of Withering Syndrome in cool water. However, abalone with RLP also exposed to warm water developed the disease withering syndrome and did not produce any mature gametes. The temperature-mediated lethal and sublethal effects on red abalone reproduction described here, combined with temperature's known impacts on abalone growth, kelp abundance, and disease status, clearly demonstrate population-level consequences. We suggest that temperature needs to be explicitly incorporated into red abalone recovery and management planning, because California's ocean has warmed and is predicted to warm in the future.

KEY WORDS: global warming, temperature, abiotic factors, *Haliotis rufescens*, sublethal effects, withering syndrome

INTRODUCTION

There is a growing awareness of the impact of ocean temperature on the productivity of marine populations (Roemmich & McGowan 1995, Francis et al. 1998), including abalone (Hobday & Tegner 2002). Ocean warming has occurred (Field et al. 2006) and is predicted to continue in the next 100 y (IPCC 2001, IPCC 2007, Solomon et al. 2007). Despite this trend, we have little mechanistic understanding of the potential impacts of warm water on marine populations. Shifts in species ranges have been observed in intertidal (Barry et al. 1995, Connolly & Roughgarden 1998) and subtidal marine populations (Zacherl et al. 2003, Rogers-Bennett 2007). Many species, in marine and terrestrial systems appear to be moving poleward in response to warming (Parmesan & Yohe 2003, Zeidberg & Robison 2007, Ling 2008). The mechanism for this shift, however, may not be the movement of adults, many of whom may be fixed to the benthos, such as barnacles (Wetthey 1983). Instead, warmer ocean temperatures can have lethal and sublethal impacts that take place over time, producing local extinctions and differential productivity.

Warmer ocean temperatures directly and indirectly affect abalone populations by impacting abalone themselves and the availability of kelp food resources (Tegner et al. 1996, Tegner et al. 2001, Vilchis et al. 2005). Storms associated with some El Niño events can cause direct mortality of benthic invertebrates and reduce kelp resources (Dayton et al. 1992). Warm ocean conditions are linked with slower abalone growth rates (Haaker et al. 1998) and decreased abalone reproduction (Vilchis et al.

2005). Major disease events have been associated with warm ocean conditions such as coral bleaching (Harvell et al. 1999), sea urchin death (Scheibling & Stephenson 1984) and withering syndrome (WS) in abalone (Steinbeck et al. 1992, Moore et al. 2002). Because of the links between water temperature, food shortages, and disease events, it can be difficult to distinguish between interacting factors in the wild.

California abalone populations have been impacted by a number of factors in addition to ocean temperature. The abalone complex *Haliotis* spp. has been serially depleted by human fishing (Karpov et al. 2000). Sea otter predation has had a large impact on abalone populations in central California, where abalone population densities are now low and individuals are small (Micheli et al. 2008). In southern California, the decline in white abalone, *H. sorenseni*, (Bartsch, 1940) from human fishing has been so dramatic (Hobday et al. 2001) that this species is the first marine invertebrate on the endangered species list (Federal Register 66:103, June 2001). Black abalone, *H. cracherodii* (Leach, 1814), have been devastated by fishing and the disease WS, and is now also on the endangered species list (Federal Register 74:9, January 2009). The bacteria that causes WS in abalone is a rickettsialeslike prokaryote (RLP) that has been identified as *Candidatus Xenohaliotis californiensis* (Friedman et al. 2000, Moore et al. 2001). Both the bacteria and warm water are needed to trigger WS, which causes abalone mortality. A federal White Abalone Recovery Plan (2008) has been drafted and the state of California has written an Abalone Recovery and Management Plan for the *Haliotis* complex (California Department of Fish and Game [CDFG] Abalone Recovery and Management Plan [ARMP] 2005). These plans need explicit information on whether temperature plays a large enough

*Corresponding author. E-mail: rogersbennett@ucdavis.edu

role on abalone productivity to warrant consideration in recovery and management and, if so, what the critical thermal limits are.

We examined potential stressors of red abalone, *H. rufescens* (Swainson, 1822), independently: warm water, starvation, and disease on the reproductive condition. In the wild, these factors often co-occur, and so distinguishing their individual impacts is not possible. To do this we examined (1) wild abalone from cold-water habitats, (2) abalone kept in warm water with abundant food, (3) abalone starved to mimic kelp shortages during warm-water events, (4) abalone exposed to RLP and maintained in cool and warm water (Moore et al. 2001), and (5) abalone maintained in 3 water temperatures in combination with 6 food treatments (see Vilchis et al. 2005). Red abalone within these experiments were examined for (1) body condition, (2) gamete production, and (3) the presence of RLP and WS. We discuss the lethal and sublethal impacts of temperature, food, and disease on reproduction in red abalone, and the implications of ocean warming for abalone management and conservation.

METHODS

Abalone Collections and Treatments

Experiment 1: Wild Collection

Healthy wild red abalone ($n = 62$) were collected from Van Damme State Park, northern California, on February 5, 2007 (Fig. 1). Abalone ranged in shell length from 161–222 mm and were sacrificed for histological examination. Monthly temperatures at Van Damme ranged from 8.5–16.0°C (Fig. 2). The monthly average sea temperature at a depth of 10 m at Van Damme for the 12 mo prior to collection was 10.8°C, ranging from 9.5°C for June 2006–12.6°C for November 2006.

Experiment 2: Warm-Water Experiment

In the warm-water experiment, 52 red abalone were collected from Timber Cove, northern California, on August 8, 2006, and maintained at the Bodega Marine Laboratory (BML). They ranged in shell length from 137–226 mm. Water temperature in the aquaria was gradually increased over 2 wk from 13.5–18°C. Red abalone were maintained in warm water at 18°C for 26 wk and liberally fed giant kelp, *Macrocystis pyrifera*. In comparison, the temperature in the natural environment, ambient BML seawater temperature averaged 12.4°C, ranging from 15°C in October 2006–9.9°C in January 2007. During the experiment, 3 abalone (6%) died. On February 28, 2007, the surviving abalone were sacrificed for histological examination. The abalone were somewhat lethargic at the time of sacrifice, and 39 had no growing lip around the perimeter of the shell.

Experiment 3: Starvation Experiment

In the starvation experiment, red abalone ($n = 54$; shell length, 181–222 mm) were collected from the wild at Van Damme State Park on August 22, 2002, and maintained at the BML. These abalone were starved with no kelp provided for 13 mo. They were maintained at ambient BML seawater temperatures that averaged 12.8°C, ranging from 17.9°C in October 2002–8.9°C in June 2003. During the course of the experiment, 15 animals (28%) died. On September 26, 2003, the starvation experiment was terminated and the remaining 39 animals were sacrificed for histological examination. The abalone were RLP free.

Experiment 4: Disease Experiments

Two disease experiments were conducted that examined both wild RLP-infected abalone and abalone exposed to RLP bacterial infection in the laboratory. We examined wild red abalone ($n = 39$; shell length, 123–217 mm) collected on September 6, 2006, from San Miguel Island in southern California, where the pathogen is known to be endemic (Fig. 1). Prior to collection from October 2005 through September 2006, the monthly average water temperature at San Miguel Island, at a depth of 13 m at Wyckoff Ledge, was 12.4°C, ranging from 11.0°C for June 2005–12.9°C for November 2005 (temperature data courtesy of D. Kushner Kelp Forest Monitoring Program). At collection, 14 were RLP negative (Tables 1 and 3, column 4a) and 25 (64%) were RLP positive (Tables 1 and 3, column 4b), but none showed signs of WS, presumably because of cool water temperatures.

In the second disease experiment, RLP-free abalone from northern California (where the pathogen is known to be absent), were intentionally exposed to the bacteria in the laboratory. Exposure occurred by either intradigestive gland injection of infected postesophageal homogenate (IPEH) or IPEH filtrate, bath exposure, or cohabitation of infected and uninfected animals (for more information see Moore et al. 2001). The experiment started with 100 red abalone, and lasted 14.5 mo. Abalone in the exposure experiment were maintained in 18°C water, giving us a combination of warm water and disease. At the termination of the experiment, there were 39 WS-free survivors, of which 33 were suitable for histological examination (Tables 1 and 3, column 4c). During the experiment, 61 abalone developed WS, and 54 died (Tables 1 and 3, column 4d). Of these, 34 were dissected after death, and there were 7 survivors (Tables 1 and 3, column 4d). Only information regarding presence and absence of gametes was obtained because gonad measures were not made.

Experiment 5: Temperature–Food Combinations

We examined abalone tissues from a previous experiment (Vilchis et al. 2005) using hatchery-reared small abalone with an initial shell length of 80–97 mm. The experiment simulated cool, normal, and warm-water conditions found in southern California. Five hundred sixty-seven abalone were placed into aquaria (21 abalone each) with 27 different water temperature (warm, ambient, and cold), food quantity, and food quality (high, medium, and low) combinations lasting 49 wk at Scripps Institution of Oceanography. Ambient temperatures were those found in La Jolla, CA; warm temperatures were 2.5°C warmer than ambient; cool temperatures were 2.5°C colder than ambient. During the experiment, the ambient temperature ranged from 14–21°C, with an average of about 16°C. The average cool-water temperature was about 13.5°C, almost 3°C warmer than ambient in northern California (Fig. 2). During the experiment, mortality was 2% in cold and ambient water and 22% in warm water (Vilchis et al. 2005). At the end of the experiment, 9 abalone per treatment, a total of 243 animals, were randomly selected and sacrificed for histology. One hundred abalone were male (determined by gonad histological examination) with an average shell length of 98 ± 6.1 mm (SD) and 135 were females with an average length of 99 ± 6.9 mm. Another 8 animals could not be sexed because of a lack of gonad tissue on the section. These abalone were within the endemic zone for the RLP bacteria, and postmortem histology revealed that 94% of the males and 92% of the females were infected.

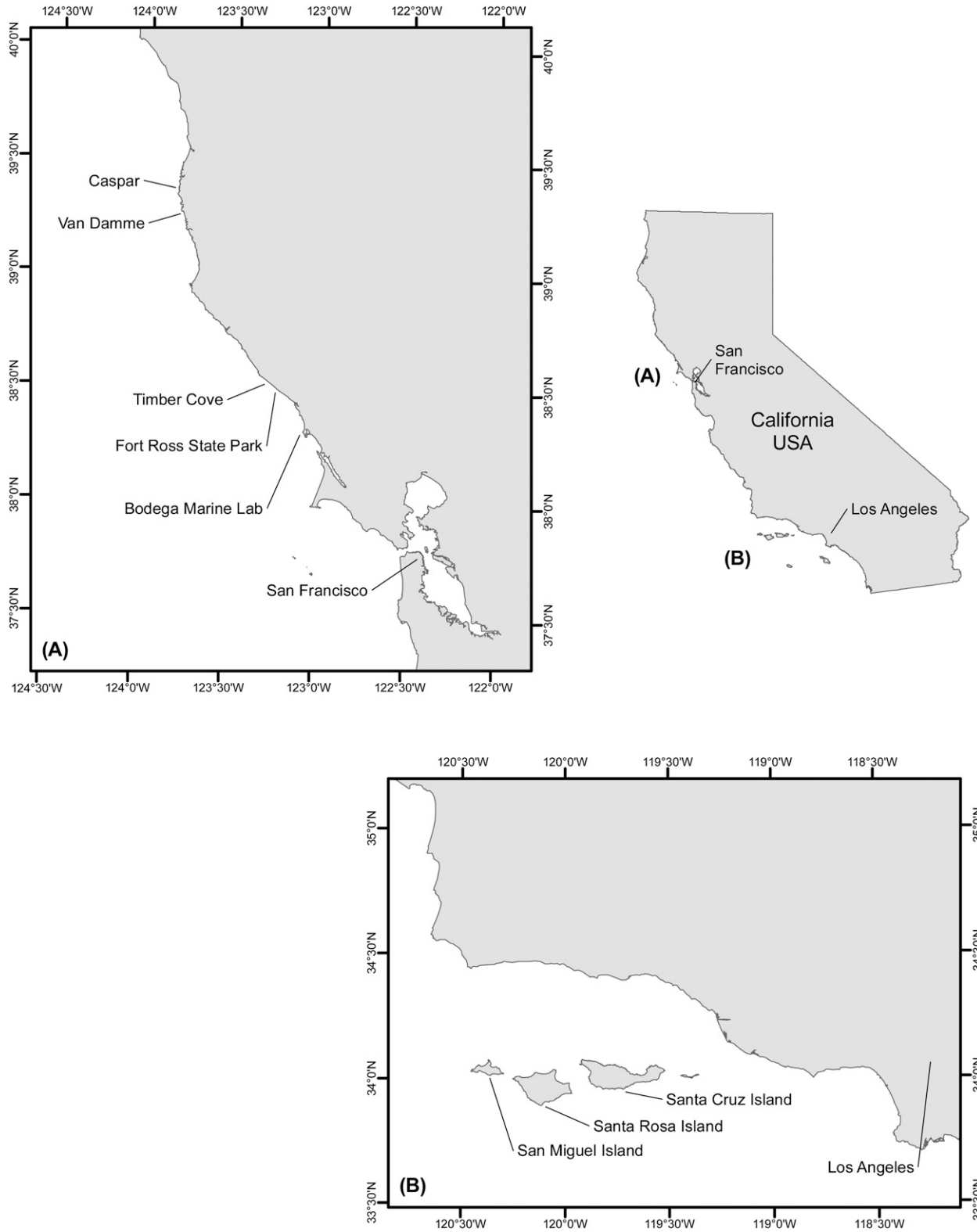


Figure 1. Map of sites in northern and southern California where wild red abalone were collected for inclusion in experiments and dissection to examine reproductive condition.

Measurements, Tissue Sampling, and Histological Examination

Abalone from each of the 5 experiments were measured and their gonad tissues sampled. Abalone measures included shell

length, total weight, shell weight, and body weight. The condition index, a nonlethal measurement, defined as total animal weight/(shell length/10)³ was determined for all treatments. Dimensions of the gonad and digestive gland were used

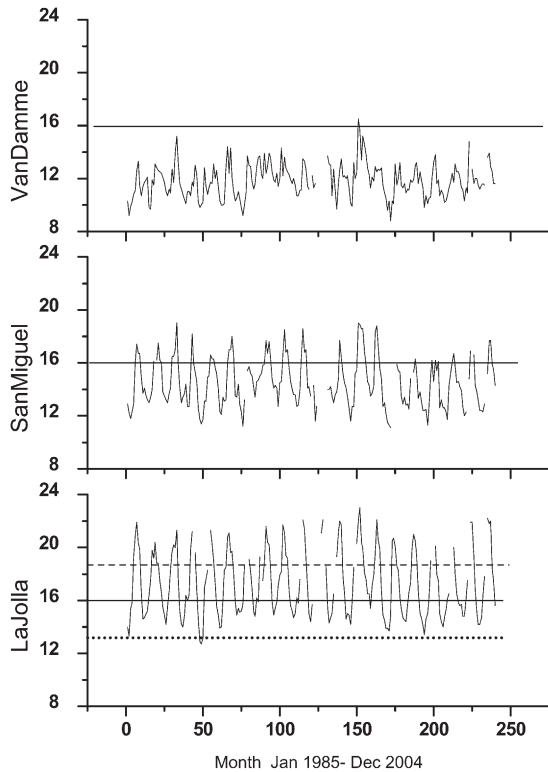


Figure 2. Temperature record of monthly means for sea surface temperatures from January 1985 to December 2004 for 3 locations in California: Scripps in La Jolla, San Miguel Island, and Van Damme State Park in Mendocino County, northern California. Advanced Very High Resolution Radiometer Pathfinder SST data were obtained from the Physical Oceanography Distributed Active Archive Center (PO.DAAC) at the NASA Jet Propulsion Laboratory, Pasadena, CA. Data from <http://podaac.jpl.nasa.gov/>. The solid black lines in the 3 temperature graphs is at 16°C, where sperm production was impacted in the Scripps Institution of Oceanography experiment. The dashed and dotted lines are 2.5°C higher and lower, respectively, than the ambient temperature at Scripps Institution of Oceanography, representing the colder and warmer water treatments, respectively.

to determine the volume of the gonad. The gonad–digestive gland assemblage was assumed to be a cone (gonad)–within-a-cone (gonad + digestive gland). The gonad and digestive gland volumes were determined using the method described by Tutschulte (1976) and Tutschulte and Connell (1981). The gonad index and the digestive gland index were determined relative to body weight as gonad or digestive gland volume \times 100/body weight. Because total digestive gland dimensions could not be determined as a result of shape, digestive gland index was a relative measure.

Samples of gonad tissue were taken from the gonad mid-section for histological preparation. Gonad samples were fixed in invertebrate Davidson’s solution, a formalin-based fixative (Shaw & Battle 1957) for 24 h, then transferred to 70% ethanol and processed for paraffin histology. “Deparaffinized” 5- μ m sections were stained with hematoxylin and eosin (Luna 1968), and mounted on slides for examination using light microscopy.

Gamete production was determined for abalone in all 5 experiments. In females, 3 classes of oocytes were identified—immature, mature, and necrotic (degenerating)—following the

descriptions of Young and DeMartini (1970) and Giorgi and DeMartini (1977). Fecundity, the total number of mature oocytes, was enumerated for each female in which gonad dimensions were quantified. All mature oocytes in 4 microscope fields (200 \times) were counted, then divided by the volume of the 4 fields for a measure of eggs per unit volume and then multiplied by the gonad volume (see Rogers-Bennett et al. 2004). Four fields were used because the oocytes are closely packed in the gonad and the number counted in each field is fairly constant. Gonad volume, on the other hand, can change dramatically among females, yielding different fecundities. The volume of a microscope field is equal to the area of the field multiplied by the thickness of the gonad section examined. Area was calculated using an ocular micrometer. Thickness was defined as the average oocyte diameter, including the jelly layer. All the mature oocytes present in the field are counted, even though some are partial views within the plane of the microscope section. Similarly, the number of immature and necrotic oocytes was enumerated. Oocyte diameter was determined by taking the average diameter, with and without the jelly layer, of 100 of the largest, roundest oocytes on slides from at least 5 female abalone within each experiment. Lipid content was estimated examining the nonnuclear portion of the egg and scoring random points as either a white lipid material, generally less than 6 μ m, or magenta yolk. We used 100 points per egg, 5 eggs per abalone, at least 5 abalone per experiment, for a minimum of 2,500 points per experiment.

In males, because sperm abundance makes sperm counts impractical, sperm production was qualitatively ranked. The ranking was based on the abundance of sperm in a 200 \times magnification field. Ranks were assigned as follows: 6, densely packed sperm along all trabeculae; 5, sperm along all trabeculae, but clearly room for more sperm; 4, sperm more dispersed along less than 100%, but greater than 50% of trabeculae; 3, dispersed sperm along less than 50% of trabeculae; 2, 2–4 patches of dispersed sperm; 1, fewer than 100 sperm in field; 0, no sperm. The sperm score for each abalone was the average score from 12 random fields. In contrast to oocytes, sperm are unevenly distributed in the testes, necessitating observations in more fields. Abundant sperm was defined as a score of 4 or more. It was possible to see healthy wild males with sperm scores of 0 or 1, indicating the males had recently spawned. For example, in multiple collections of wild red abalone from Van Damme State Park, of 251 males, 73 (29%) were spawned out. These males, however, had abundant presperm germ cells.

The abundance of presperm germ cells, spermatogonia, and spermatocytes was determined and reported as a combined number per cubic millimeter. These germ cells appear evenly distributed in testes and ranged in diameter from 2–8 μ m. Their number was counted in one 400 \times magnification field. That number was then divided by the volume investigated, similar to the method used for oocytes. The average diameter of these germ cells was defined as 5 μ m. No attempt was made to enumerate the spermatids, which are smaller than the spermatogonia and spermatocytes.

All abalone were examined for the presence of the RLP bacteria causing WS. We examined histological sections of the postesophagus and the digestive gland for the presence of the bacterial inclusions in a 200 \times magnification field. Abalone were defined as RLP bacteria positive if they had at least 1–10 bacterial

TABLE 1.
Comparison of wild male abalone in warm water, starved, and disease exposure treatments.

Experiment	Experiment 1 Wild Collection, RLP Negative, 10.8°C Water	Experiment 2 Warm Water, 18°C	Experiment 3 Starved, 12.8°C Water	Experiment 4a SMI Wild RLP Negative, 12.4°C Water	Experiment 4b SMI Wild RLP Positive, 12.4°C Water	Experiment 4c RLP Exposure, Survivors WS Negative, 18°C Water	Experiment 4d RLP Exposure Moribund + 4 Survivors WS Positive, 18°C Water
No. males	32	21	24	8	13	16	25
Size range, mm	161–218	137–226	181–222	184–214, <i>n</i> = 5	174–217, <i>n</i> = 4	119–190	115–199
Mean size, mm ± 1 SD	197 ± 13	180 ± 24	203 ± 10	204 ± 12, <i>n</i> = 5	203 ± 15, <i>n</i> = 4	153 ± 20	161 ± 24
Mean final condition index	0.207 ± 0.029	0.165 ± 0.020	0.184 ± 0.020	0.222 ± 0.031, <i>n</i> = 5	0.232 ± 0.030, <i>n</i> = 4	0.171 ± 0.028	0.108 ± 0.018
Mean digestive gland index	3,327 ± 734	1,289 ± 613	1,152 ± 418	1,702 ± 629, <i>n</i> = 5	2,438 ± 725, <i>n</i> = 4	ND	ND
No. with RLP	0	0	0	0	13	8 (50%)	25 (100%)
No. with WS	0	0	0	0	0	0	25(100%)
Mean gonad index	1,326 ± 527	301 ± 314	321 ± 120	1,248 ± 342, <i>n</i> = 5	1,151 ± 367, <i>n</i> = 9	ND	ND
No. with sperm	23 (72%)	6 (29%)	7 (29%)	5 (63%)	12 (92%)	5 (31%)	0
No. with abundant sperm: score ≥ 4	8 (25%)	1 (5%)	0	3 (38%)	5 (38%)	1(6%)	0
Mean sperm score	2.0 ± 2.0	0.44 ± 1.3	0.17 ± 0.71	2.5 ± 2.8	3.0 ± 1.9	0.79 ± 1.7	NA
Mean spermatogonia + spermatozoa/mm ³ in testis	302,000 ± 139,000	45,600 ± 33,800	83,700 ± 27,000	346,000 ± 142,000	432,000 ± 172,000	226,000 ± 188,000	5,200 ± 4,600
Yellow granular masses, % of testis	5% ± 8%	30% ± 20%	27% ± 14%	0%	4% ± 6%	6% ± 7%	19 ± 17%

NA, not applicable; ND, no data; SMI, San Miguel Island.

inclusions, with many having 11–100 inclusions or more within the tissues in a single field of view (Friedman et al. 1997).

RESULTS

Male Response to Stressors

Reproduction in male red abalone was highly sensitive to the stressors examined, resulting in dramatic decreases in the production of sperm and presperm germ cells (Table 1).

Experiment 1: Wild Collection

The majority of wild males from northern and southern California had sperm, 72% and 81% respectively; with 25% and 38% having abundant sperm, respectively (Fig. 3A). Of the northern abalone, 28% had spawned recently, as indicated by few sperm with abundant spermatogonia/spermatocytes.

Experiment 2: Warm Water

Male red abalone maintained in warm water (18°C) with no other stressors (RLP free and food) had a dramatic decrease in sperm production, with 71% of the males failing to produce sperm and only 5% having abundant sperm (Figs. 3B and 4). Males maintained in warm water had only 46,000 cells/mm³ presperm germ cell concentrations in comparison with 300,000 cells/mm³ for wild males. Therefore, warm water alone appears responsible for negatively impacting spermatogenesis.

Experiment 3: Starvation

Starvation also severely impacted male reproduction, with the majority (71%) of males having no sperm and none having abundant sperm (Fig. 3C). Again, these animals were RLP free during and after the experiment. In both the warm-water and starvation experiments, the male gonad index declined to about 25% that of the wild northern California abalone. In the testes

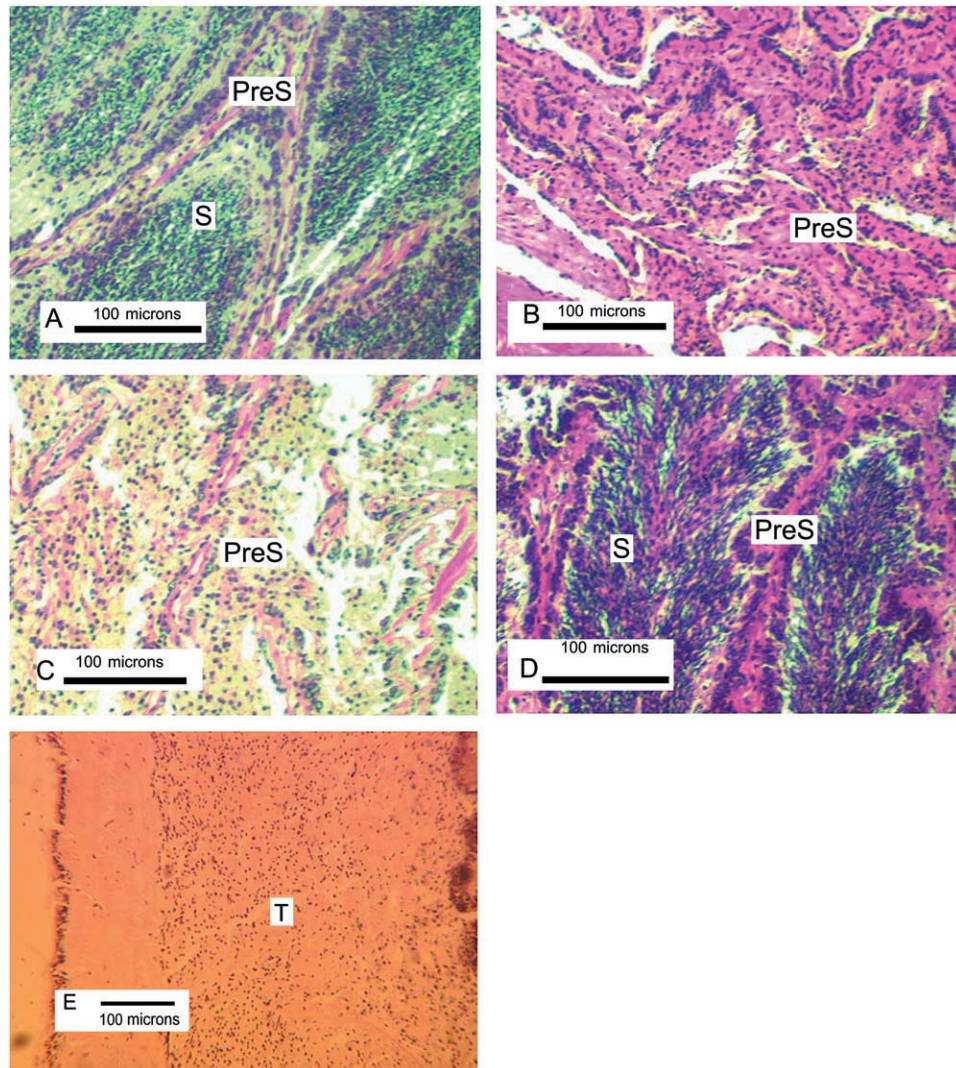


Figure 3. Male red abalone. (A) Testes section of wild male red abalone from northern California. Normal testes with abundant sperm (S) and presperm germ (PreS) cells. (B) Testes section of male red abalone maintained in warm seawater (18°C) for 6 mo. Testes lacking sperm having only a few PreS germ cells. (C) Testes section of male red abalone starved for 13 mo while maintained in cold water. Testes lacking sperm with only a few PreS germ cells. (D) Testes section of male red abalone from San Miguel Island infected with WS-RLP. Testes has abundant sperm and PreS germ cells aligned along the trabeculae, and abalone shows no clinical signs of withering. (E) Testes section of male red abalone showing clinical signs of WS with no sperm, few PreS germ cells, and contorted and compressed trabeculae (T).

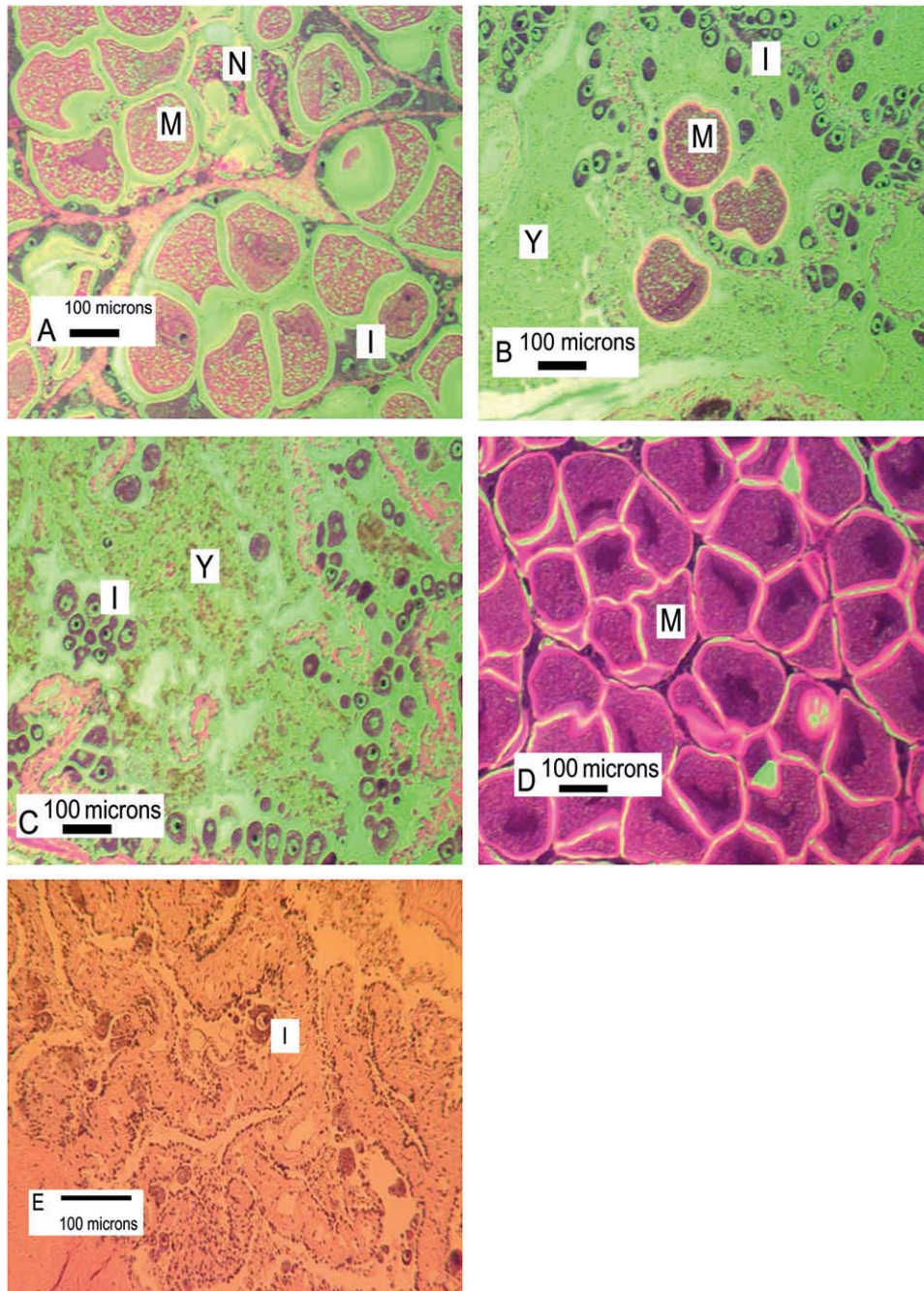


Figure 4. Female red abalone. (A) Ovary section of wild female red abalone from northern California. The M indicates mature oocytes and the N indicates necrotic degenerating oocytes. (B) Ovary section of female red abalone maintained in warm seawater (18°C) for 6 mo. There are few mature oocytes as indicated by M, immature oocytes as indicated by I, and yellow masses indicated by Y. (C) Ovary section of female red abalone starved for 13 mo while maintained in cold water. There are no mature oocytes. I indicates immature oocytes and Y indicates yellow masses. (D) Ovary section of female red abalone exposed to WS RLP disease from San Miguel Island, but showing no clinical signs of withering. M indicates mature, healthy oocytes. (E) Ovary section of female red abalone showing clinical signs of WS with no mature oocytes, few small immature oocytes, and contorted trabeculae.

of males in the starvation and warm-water experiments, the trabeculae were usually misshapen and contorted, indicating functional abnormalities.

Experiment 4: Disease

In the wild collection of RLP-infected abalone from San Miguel Island in cool water, the males had extremely high quantities of sperm despite 62% testing positive for RLP (Fig. 3D).

Sperm quantities in the RLP-positive males were comparable with the RLP-negative males also from San Miguel Island. However, in the second disease experiment, when abalone were exposed to RLP and warm water (18°C) for 14.5 mo, only 31% of the males had sperm, similar to the results of experiment 2, the warm-water experiment. If the disease progressed and the males began to wither, we then found total reproductive failure. In the testes of males that succumbed to WS, extreme contortion

and compaction of the trabeculae gave the testes a massive, unstructured dysfunctional appearance (Fig. 3E). Interestingly, we found a higher percent of males infected with the bacteria in this laboratory experiment (50%) compared with females (29%), suggesting further work could explore the potential for susceptibility differences between the sexes. These results show that the presence of RLP infection alone (without warm water) does not impact sperm production.

Experiment 5: Temperature–Food Combinations

No sperm were present in the males exposed to ambient (16°C) or warm (18°C) water and only 28% of the males had sperm in the coldwater combination (Table 2). The males lacked evidence of new sperm production in the form of spermatogonia/spermatocytes. Presperm germ cell concentrations averaged 16,100/mm³ in cold water, 9,100/mm³ in ambient water, and 5,400/mm³ in warm water, indicating very low spermatogenesis in all water treatments. In comparison, the abalone collected in the wild in northern California had an average presperm germ cell concentration of 300,000/mm³, more than 18 times that of the coldwater males in this experiment.

In the temperature–food combinations experiment, most of the red abalone tested positive for RLP, because this bacteria is endemic in the waters in southern California where the experiment took place. Of the 100 males, 94% were RLP positive. No males in cold water developed clinical signs of WS, and only 1 in ambient water developed clinical signs of WS. In warm water, all 38 males were positive and 19 (50%) had withered and shrunken bodies, expressing the onset of WS. All males with WS had no sperm.

Female Response to Stressors

Females were less sensitive than males to temperature stress, although they were highly sensitive to food deprivation. Females continued to produce mature oocytes at warmer temper-

atures in all experiments, but were sensitive to reductions in food quantity both in the starvation and the temperature–food experiments (Tables 2 and 3).

Experiment 1: Wild Collection

Ninety-seven percent of the females had mature eggs, with an average fecundity of more than 2.8 million eggs per female (Fig. 4A). The mean condition (0.184) and digestive gland indices (4,744) were greater in the wild females in comparison with females in the warm and starved experiments.

Experiment 2: Warm Water:

The females had reduced oocyte production with an average of 400,000 eggs per female (83% of the females had mature eggs). In both the warm-water and starvation experiments, the female gonad index declined to less than 25% that of the wild females. The condition (0.161) and digestive gland (1,787) indices were lower in the warm-water females than the wild females in cold water.

Experiment 3: Starvation

The females in the starved and warm-water experiments (2 and 3) had high numbers of immature oocytes (Fig. 4B, C), in contrast to the low presperm germ cell concentrations in the males, suggesting oogenesis was still possible. In experiment 3, the females were dramatically impacted by starvation, with almost no mature eggs being produced. Thirteen of the 15 females had no mature eggs, and the other 2 had only 1 mature egg each in the ovary section (Fig. 4C). The condition (0.153) and digestive gland (1,243) indices were lower in the starved females than the wild females in cold water.

Experiment 4: Disease

The reproductive potential of RLP-infected females from San Miguel Island differed from those in northern California in

TABLE 2.

Experiment 5: Reproductive condition of small, hatchery-reared male and female red abalone exposed to WS-RLP and variable temperature, food quality, and food quantity treatments for 13 mo.

Temperature Treatment	RLP/WS Status	Males, <i>n</i>	No. with Sperm	Mean Presperm germ cells/mm ³	Females, <i>n</i>	No. with Mature Oocytes	Fecundity, Mean Mature Oocytes	Mean Mature Oocytes/mm ³	Mean Immature Oocytes/mm ³
Cold, ambient –2.5°C Average, 13.5°C	RLP –/WS –	4	3 (75%)	13,800 ± 6,850	8	3 (38%)	548,000 ± 438,000	168 ± 37	276 ± 44 <i>n</i> = 4
	RLP +/WS –	25	5 (20%)	16,400 ± 8,100	34	17 (50%)	459,000 ± 378,000 <i>n</i> = 15	166 ± 56	388 ± 454 <i>n</i> = 21
	RLP +/WS +	0	NA	NA	0	NA		NA	NA
Ambient Average, 16.0°C	RLP –/WS –	2	0	15,500 ± 13,700	2	0		NA	ND
	RLP +/WS –	27	0	8,625 ± 6,500 <i>n</i> = 25	43	21 (49%)	163,000 ± 252,000 <i>n</i> = 20	93 ± 56	999 ± 985
Warm, Ambient + 2.5°C Average, 18.5°C	RLP +/WS +	1	0	1,170	2	0		NA	ND
	RLP –/WS –	0	NA	NA	0	NA	NA	NA	NA
	RLP +/WS –	19	0	9,048 ± 9,341	27	11 (41%)	112,000 ± 117,000 <i>n</i> = 9	97 ± 25	260 ± 238 <i>n</i> = 12
	RLP +/WS +	19	0	1,809 ± 3,393	7	0		NA	ND

Data are irrespective of food treatments.

NA, not applicable; ND, no data, usually because of scant gonad tissue.

TABLE 3.
Comparison of wild female abalone in warm water, starved, and disease exposure treatments.

Experiment	Experiment 1: Wild Collection RLP Negative, 10.8°C Water	Experiment 2: Warm Water, 18°C	Experiment 3: Starved, 12.8°C Water	Experiment 4a: SMI Wild RLP Negative, 12.4°C Water	Experiment 4b: SMI Wild RLP Positive, 12.4°C Water	Experiment 4c: RLP Exposure, Survivors WS Negative 18°C Water	Experiment 4d: RLP Exposure, Moribund + 3 Survivors, WS Positive 18°C Water
No. females	30	29	15	6	12	17	16
Size range, mm	172–222	139–207	170–212	123–215	174–210	113–188	122–185
Mean size ± 1 SD	193 ± 13	181 ± 14	196 ± 13	174 ± 40	193 ± 12	155 ± 20	154 ± 19
Mean final condition index	0.184 ± 0.021	0.161 ± 0.019	0.153 ± 0.021	0.174 ± 0.020	0.220 ± 0.024	0.166 ± 0.022	0.102 ± 0.009
Mean digestive gland index	4,744 ± 2,272	1,787 ± 675	1,243 ± 381	3,802 ± 2,649	5,935 ± 2,837	ND	ND
No. with RLP	0	0	0	0	12	5 (29%)	14
No. with WS	0	0	0	0	0	0	16
Mean gonad index	2,707 ± 1,647	642 ± 461	548 ± 219	8,555 ± 5,458 <i>n</i> = 3	10,533 ± 6,355	ND	ND
No. with mature oocytes	29 (97%)	24 (83%)	2 (15%), <i>n</i> = 13	6 (100%)	12 (100%)	9 (53%)	0
Mean diameter mature oocytes, without jelly layer, mm	0.182 ± 0.013	0.171 ± 0.012	Only 2 mature oocytes	0.174 ± 0.013	0.177 ± 0.010	0.184 ± 0.012	NA
Lipids, % of oocyte	32.6 ± 5.2	35.9 ± 14.9	Only 2 mature oocytes	34.5 ± 5.0	33.5 ± 4.3	39.8 ± 6.0	NA
Fecundity, mean total mature oocytes	2.8 × 10 ⁶ ± 2.8 × 10 ⁶	0.4 × 10 ⁶ ± 0.6 × 10 ⁶	476 ± 1,217	15.6 × 10 ⁶ ± 14.3 × 10 ⁶ , <i>n</i> = 4	20.1 × 10 ⁶ ± 14.6 × 10 ⁶	NA	NA
Mean total large, immature oocytes	5.7 × 10 ⁶ ± 3.5 × 10 ⁶	4.3 × 10 ⁶ ± 3.7 × 10 ⁶	5.6 × 10 ⁶ ± 5.4 × 10 ⁶	20.6 × 10 ⁶ ± 15.6 × 10 ⁶ , <i>n</i> = 3	21.6 × 10 ⁶ ± 18.8 × 10 ⁶	ND	ND*
Mean total necrotic oocytes	0.85 × 10 ⁶ ± 1.1 × 10 ⁶	0.02 × 10 ⁶ ± 0.05 × 10 ⁶	0	0.48 × 10 ⁶ ± 0.38 × 10 ⁶	1.3 × 10 ⁶ ± 2.0 × 10 ⁶	ND	0
Yellow granular masses, % of ovary	2% ± 8%	21% ± 15%	23% ± 14%, <i>n</i> = 14	0	0	2.5% ± 4.3%	9.4% ± 8.7%

* Immature oocytes present in all, but no gonad size data for gonad volume. SMI, San Miguel Island.

a number ways. In southern California, at San Miguel Island, despite 73% of females testing RLP positive, still produced 7 times more eggs (21 million eggs) than females from the north that were RLP free (Fig. 4D). Wild females from northern California also had fewer immature oocytes (6 million) than those from the south (21 million). Females in the south had higher condition (0.204) and digestive gland (5224) indices and much higher gonad indices (nearly 4 times) compared with wild females from the north. Where abalone were exposed to both the RLP and warm water for 14.5 mo, some females (53% of the 17 female survivors) remained RLP free. Of these females, only 47% had mature oocytes compared with 83% of the RLP-free females in the shorter 6-mo warm-water experiment (experiment 2). This suggests the longer the warm-water exposure, the more of an impact on egg production. Females, like the males, that had begun to wither in experiment 4 had no egg production. Furthermore, trabeculae in the shrunken ovaries were extremely contorted (Fig. 4E), suggesting they, too, were dysfunctional.

Experiment 5: Temperature–Food Combinations

The females had depressed oocyte production in the low-quantity food treatments (Fig. 5). The percent of females with oocytes dropped from 81% for the high-food quantity treatment to 28% and 12% in the medium- and low-food quantity treatments. This result was more dramatic than decreases resulting from food quality and water temperature treatments. Females were able to produce mature oocytes in all temperature–food treatments with the exception of those females developing WS and starting to wither. Of the 123 females examined in the temperature–food experiment, 113 (92%) were RLP positive, including 49 with mature oocytes. No females in cold water had WS, only 2 did in ambient water, and 7 (20%) in the warm water had WS.

Females did not adjust the size or quality of the eggs as measured by lipid score in response to warm water or poor food conditions. There were significant differences in oocyte diameter among treatments or between the north and south, with an average egg diameter of 182 μ m for females from northern

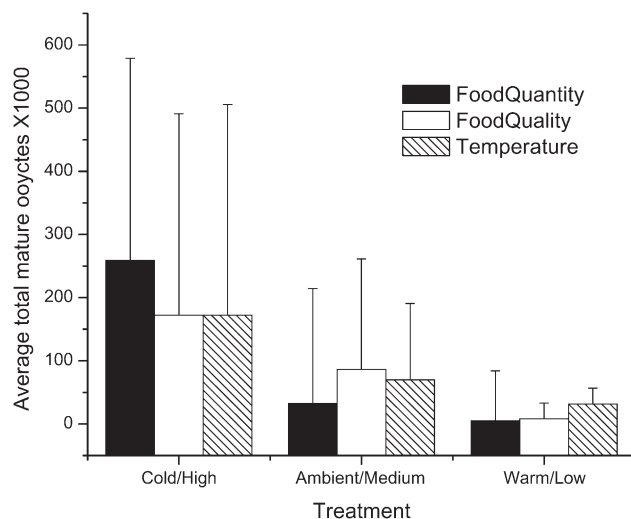


Figure 5. Total number of mature oocytes averaged (with SDs) for small females in experiment 5 maintained in each food quantity (high, medium, low), food quality (high, medium, low), and temperature (warm, ambient, and cold) treatment combination.

California (Table 3). There was generally no significant difference in lipid score of mature oocytes, which averaged about 35% in all treatments. The one exception was females exposed to warm water for 14.5 mo (t -test, $df = 29$, $P = <0.001$). In experiment 4, the disease experiment, the females averaged about 40% lipids in the oocytes, more than in any other treatment. Wild females had more necrotic eggs than females in both the warm and starved treatments because many of these females did not have mature eggs. There were more necrotic or degenerating eggs in females from the north compared with the south for the dates sampled.

We noted yellowish stained granular masses in the lumen of ovaries and testes of abalone when stressed. These granules were similar in appearance to the description presented by Giorgi and DeMartini (1977). They associated the granules with necrotic oocytes; however, we did not observe this association. We are not aware of any references that define what these granules are; however, they appear to be phagocytes (S. Teh, pers. comm.). The masses are composed of discrete ovoid granules ranging in size from submicroscopic to about 15 μ m. They were speckled with finer, dark material. The masses occurred in the intertrabecular spaces usually occupied by gametes. In the warm and starved experiments, abalone had numerous yellow masses occupying 20–30% of the gonad, whereas wild abalone in northern and southern California had only 2–5%. Therefore, we associate the presence of these granules with stress.

DISCUSSION

Abalone Reproduction and Potential Stressors

Warm water alone negatively impacted red abalone sperm production. In the warm-water experiment (experiment 2), 71% of the males maintained at 18°C had no sperm after just 6 mo of exposure. The reduction in sperm production was confirmed by a sharp decrease in presperm germ cells. Total reproductive failure also occurred in small male red abalone after 1 y of exposure to warm water at 16°C and 18°C (Fig. 6). This result suggests that longer exposures to 16°C may be just as deleterious as shorter exposures to 18°C. Sperm production was also negatively impacted by 1 y of starvation in cool water (12°C), after which 71% of the males lacked sperm. Males testing RLP positive in cool water had normal sperm production whereas those exposed to warm water and RLP developed WS had no sperm and few presperm germ cells. This supports earlier work in which RLP-positive animals in warm water developed WS (Moore et al. 2000) and showed reproductive failure (Braid et al. 2005). Clearly, testes damage in withering animals is extreme and irreversible (and the abalone is destined to die); however, it is unknown whether the impacts of warm water and starvation can be reversed with the restoration of optimal temperature conditions.

Female reproduction, in contrast, appeared to be more affected by food limitation than warm water. In the starvation experiment (experiment 3), nearly all the females lacked mature oocytes. Food quantity had a significant effect on the percent of females with mature eggs and the gonad index (Table 3), as did food quality, but less so (Fig. 6). Females did not compensate for reduced food by making smaller mature eggs or lowering lipid content; they simply stopped producing mature eggs. Females

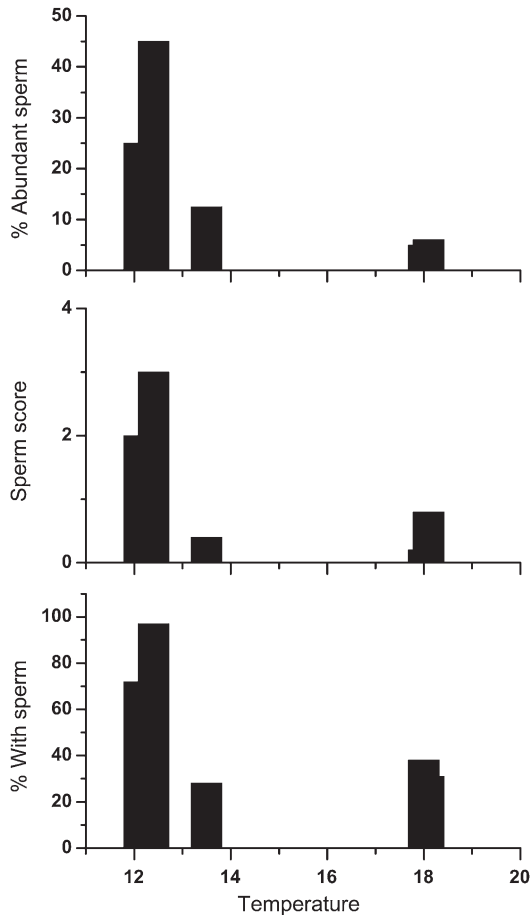


Figure 6. Bar graph showing 3 measures of male sperm production (percent of males with sperm, sperm score, percent of males with abundant sperm) compared with the temperature at which the male red abalone were maintained during experiments 1–4.

produced eggs in all water temperature treatments, including 18°C, although females maintained in warm water produced fewer eggs than females maintained at cooler temperatures. As with the males, female egg production was not affected by the presence of RLP infection alone. When this infection was coupled with warm water and abalone developed clinical signs of the disease WS, females—like the males—experienced total reproductive failure, lacking mature oocytes. Whether warm water and starvation simply arrest egg development or irreparably damage egg production is unknown.

The reproductive failure described here for males at warm temperatures (>16–18°C) may act to set an upper thermal limit for optimal reproduction. The production of gametes, however, is just one step toward successful reproduction. Fertilization experiments show that 100% of eggs are fertilized at temperatures from 12–15°C, but this drops to 80% at 18°C (Ebert & Hamilton 1983)—further evidence that successful fertilization is negatively impacted by warm temperatures. The next step in reproduction is larval survival. Larvae cultured in the laboratory survived over a range of temperatures from 10–19.5°C, but only reached advanced larval stages after 3 wk at temperatures between 14°C and 18°C (Leighton 1974). Lecithotrophic larvae, such as red abalone, that do not settle within 2–3 wk would presumably have poor survival rates after exhausting their yolk

reserves. These data taken in combination suggest that the window for male spawning success, fertilization success, and optimal larval development may be quite narrow. This critical temperature window may be an important factor in limiting successful recruitment for red abalone. Red abalone recruitment has been shown to be highly variable in central and southern California (Hines & Pearse 1982, Tegner et al. 1989, Tegner et al. 1992). Abalone recruitment variability in many cases has been linked to environmental factors rather than stock size (McShane 1995). Given this relationship between sperm production and temperature, future work may consider examining links between abalone recruitment and sea surface temperatures to develop environmental proxies for management.

Ocean Warming and Abalone Restoration

Sea surface temperatures have increased by 0.8°C in the past 100 y (IPCC 2007). This increase in temperature has impacted marine organisms in pelagic (Field et al. 2006), subtidal (Zacherl et al. 2003), and intertidal habitats (Southward et al. 1995). Many marine animals have responded negatively to the ocean warming either by dropping out of the system (Roemmich & McGowan 1995), exhibiting a lower body condition index (Wells et al. 2006), dying from disease (Harvell et al. 1999), or failing to reproduce (Sydeman et al. 2001). In the shallow subtidal, flat and northern abalones are at lower densities in the southern warmer portions of their ranges in central California compared with a cooler period 25 y ago (Rogers-Bennett 2007). Endangered white abalone from deep, coldwater habitats may be uniquely vulnerable to warm water, whereas green abalone, *H. fulgens*, may respond favorably, as indicated by previous temperature–food experiments (Vilchis et al. 2005).

Ocean warming can impact abalone populations via multiple pathways. Growth in red abalone is affected during El Niño events when kelp is scarce in southern California (Haaker et al. 1998). Warmer water temperatures are correlated with reductions in nitrate availability, depressing kelp growth, damaging tissue, and reducing nitrogen content (food quality), which can lead to massive loss of kelp biomass (food quantity) (Tegner & Dayton 1987, Tegner et al. 1996). Previous work has shown that warm-water conditions coupled with reductions in food quantity and quality leads to lower abalone gonad indices, especially in females (Vilchis et al. 2005). Ocean warming may also act synergistically with fishing to impact abalone populations, particularly if fishing removes individuals with resistance to disease (Harley & Rogers-Bennett 2004).

In many areas in southern California, abalone populations are not recovering despite 10 y of a fishing moratorium. Recent abalone surveys (2007 to 2008) in the Santa Rosa and Santa Cruz islands found only 1 red, 2 pink, and 2 green abalones in 960 min of search time (I. Taniguchi and C. Juhasz, pers. comm.). Sea surface temperatures at these islands and neighboring San Miguel Island can reach 16°C for parts of each year (Fig. 2). Our results suggest that poor red abalone recovery in the south may be exacerbated by ocean warming. In population models of red abalone, warm-water conditions alone were enough to drive populations lower even in the absence of predation and fishing pressure (Hobday & Tegner 2002).

Increasing atmospheric CO₂ emissions are projected not only to increase sea surface temperatures, but also to reduce ocean pH (Orr et al. 2005, Fabry et al. 2008), as well as influence ocean circulation patterns (Barth et al. 2007). In northern

California, upwelling has been shown to bring deep acidic water into shallow nearshore habitats (Feely et al. 2008). Shallow kelp forest communities where abalone and other marine invertebrates reside may be negatively impacted by caustic waters, particularly during shell calcification. Early life history stages may be especially sensitive to ocean acidification because many gastropod and bivalve veligers contain aragonite crystalline ultrastructures in the developing shell (Weiss et al. 2002). Ocean acidification is known to impact calcification and shell growth in marine invertebrates such as mussels (Michaelidis et al. 2005). At the same time, upwelling ocean circulation patterns are predicted to change, and the impacts of these changes on the transport of larval abalone are unknown. Furthermore, sea surface temperatures are projected to continue to increase up to 5–7°C under the most extreme CO₂ emissions model scenarios by the year 2100 (IPCC 2007, Pope et al. 2008). This will increase the chances of red abalone being exposed to temperature thresholds that can effectively castrate male red abalone.

Therefore, abalone fishery management and conservation planning will need to take into consideration the impacts of increasing sea surface temperatures by including known thermal limits for successful reproduction on a species-by-species basis. More work needs to be done on the potential impacts of ocean acidification and changes in local oceanographic dispersal patterns and how they may impact abalone recruitment success in California. Fisheries for red abalone may need to be ratcheted back during warming trends if temperatures exceed known thresholds, taking an adaptive management approach by closely monitoring temperature or incorporating temperature into harvest control rules (Conser et al. 2001). Abalone restoration actions will need to recognize that temperature may impact

restoration efforts (adult translocations and stocking), necessitating sampling for early life history stages as indicators of reproductive success. As a result of ocean temperature's sublethal effects on reproduction, it may not be enough to conduct restoration actions in regions that historically had abundant abalone (Rogers-Bennett et al. 2002), because adults may survive, yet not reproduce. Restoration may need to occur in new habitats that may be north of or deeper than traditional abalone grounds in recognition of the potential impacts of ocean warming that has occurred and that is predicted to continue.

ACKNOWLEDGMENTS

This paper is dedicated to the memory of Dr. M. J. Tegner, who hypothesized that environmental variability adversely impacted abalone reproduction, leading her to design laboratory experiments to tease apart the role of water temperature from food quantity and quality; we are simply carrying on with the program she outlined. We thank P. Dayton for support of this project and words of encouragement. We thank T. Robbins and C. Juhasz for their help in the laboratory, and C. Vines for help with the photomicrographs. The Advanced Very High Resolution Radiometer Pathfinder SST data were obtained from the Physical Oceanography Distributed Active Archive Center (PO.DAAC) at the NASA Jet Propulsion Laboratory, Pasadena, CA (<http://pdaac.jpl.nasa.gov>) and we thank them for these data. We thank the California Department of Fish and Game Marine Region for supporting this work, especially M. Vojkovich. We thank the reviewers for helpful comments and suggestions. This publication is a contribution of the Bodega Marine Laboratory, University of California, Davis.

LITERATURE CITED

- Barry, J. P., C. H. Baxter, R. D. Sagarin & S. E. Gilman. 1995. Climate-related, long-term faunal changes in a California rocky intertidal community. *Science* 267:672–675.
- Barth, J. A., B. A. Menge, J. Lubchenko, F. Chan, J. M. Bane, A. Kirincich, M. A. McManus, K. J. Nielsen, S. D. Pierce & L. Washburn. 2007. Delayed upwelling alters nearshore coastal ocean ecosystems in the northern California current. *Proc. Natl. Acad. Sci. USA* 104:3719–3724.
- Braid, B., J. D. Moore, T. T. Robbins, R. P. Hedrick, R. S. Tjeerdema & C. S. Friedman. 2005. Health and survival of red abalone, *Haliotis rufescens*, under varying temperature, food supply and exposure to the agent of withering syndrome. *J. Invertebr. Pathol.* 89:219–231.
- Connolly, S. R. & J. Roughgarden. 1998. A range extension for the volcano barnacle, *Tetraclita rubescens*. *Calif. Fish Game* 84:182–183.
- Conser, R. J., Hill, K. T., Crone, P. R., Bergen, D., 2001. Stock assessment of Pacific sardine with management recommendations for 2002: executive summary. Portland, OR: Pacific States Fishery Management Council. pp. 1–9.
- Dayton, P. K., M. J. Tegner, P. E. Parnell & P. B. Edwards. 1992. Temporal and spatial patterns of disturbance and recovery in a kelp community. *Ecol. Monogr.* 62:421–445.
- Ebert, E. E. & R. M. Hamilton. 1983. Ova fertility relative to temperature and to the time of gamete mixing in the red abalone, *Haliotis rufescens*. *Calif. Fish Game* 69:115–120.
- Fabry, V. J., B. A. Seibel, R. A. Feely & J. C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.* 65:414–432.
- Feely, R. A., C. L. Sabine, J. M. Hernandez-Ayon, D. Ianson & B. Hales. 2008. Evidence for upwelling of corrosive “acidified” water onto the continental shelf. *Science* 320:1490–1492.
- Field, D. B., T. B. Baumgartner, C. Charles, V. Ferriera-Bartrina & M. D. Ohman. 2006. Planktonic foraminifera of the California Current reflect 20th-century warming. *Science* 311:63–66.
- Francis, R. C., S. R. Hare, A. B. Hollowed & W. S. Wooster. 1998. Effects of interdecadal climate variability on the oceanic ecosystems of the NE Pacific. *Fish. Oceanogr.* 7:1–21.
- Friedman, C. S., K. B. Andree, A. Beauchamp, J. D. Moore, T. T. Robbins, J. D. Shields & R. P. Hedrick. 2000. *Candidatus Xenohaliotis californiensis*, a newly described pathogen of abalone, *Haliotis* spp., along the west coast of North America. *Int. J. Syst. Evol. Microbiol.* 50:847–855.
- Friedman, C. S., M. Thompson, C. Chun, P. L. Haaker & R. P. Hedrick. 1997. Withering syndrome of the black abalone *Haliotis cracherodii* (Leach): water temperature, food availability, and parasites as possible causes. *J. Shellfish Res.* 16:403–411.
- Giorgi, A. E. & J. D. DeMartini. 1977. A study of the reproductive biology of the red abalone, *Haliotis rufescens*, Swainson, near Mendocino, California. *Calif. Fish Game* 63:80–94.
- Haaker, P. L., D. O. Parker, K. C. Barsky & C. S. Y. Chun. 1998. Growth of red abalone, *Haliotis rufescens*, (Swainson.), at Johnsons Lee, Santa Rosa Island, California. *J. Shellfish Res.* 17:747–753.
- Harley, C. D. G. & L. Rogers-Bennett. 2004. The potential synergistic effects of climate change and fishing pressure on exploited invertebrates on rocky intertidal shores. *Calif. Coop. Ocean Fish. Invest. Rep.* 45:98–110.
- Harvell, C. D., K. Kim, J. M. Burkholder, R. R. Colwell, P. R. Epstein, D. J. Grimes, E. K. Hofmann, A. D. M. E. Osterhaus, R. M. Overstreet, J. W. Porter, G. W. Smith & G. R. Vista. 1999. Emerging marine diseases: climate links and anthropogenic factors. *Science* 285:1505–1510.
- Hines, A. H. & J. S. Pearse. 1982. Abalones, shells and sea otters: dynamics of prey populations in central California. *Ecology* 63:1547–1560.

- Hobday, A. J. & M. J. Tegner. 2002. The warm and the cold: influence of temperature and fishing on local population dynamics of red abalone. *Calif. Coop. Ocean Fish. Invest. Rep.* 43:74–96.
- Hobday, A. J., M. J. Tegner & P. L. Haaker. 2001. Over-exploitation of a broadcast spawning marine invertebrate: decline of the white abalone. *Rev. Fish Biol. Fish.* 10:493–514.
- IPCC. 2001. Climate change 2001: synthesis report. A contribution of working groups I, II, and III to the Third Assessment Report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press. 881 pp.
- IPCC. 2007. In: S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, & H. L. Miller, editors. Climate change 2007: the physical science basis. Contribution of working group I to the Fourth Assessment. Report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press. 996 pp.
- Karpov, K. A., P. L. Haaker, J. K. Taniguchi & L. Rogers-Bennett. 2000. Serial depletion and collapse of the California abalone fishery. In: A. Campbell, ed. Workshop on rebuilding abalone stocks in British Columbia. *Can. Spec. Pub. Fish. and Aquat. Sci.* 130:11–24.
- Leighton, D. L. 1974. The influence of temperature on larval and juvenile growth in three species of southern California abalones. *Fish Bull.* 72:1137–1145.
- Ling, S.D., 2008. Range expansion of a habitat-modifying species leads to loss of taxonomic diversity: a new and impoverished reef state. *Oecologia* 156:883–894.
- Luna, L. G. 1968. Manual of Histological Staining Methods of the Armed Forces Institute of Pathology: Am. Reg. Pathol., 3rd edition. New York: McGraw-Hill. 258 pp.
- McShane, P. E. 1995. Recruitment variation in abalone: its importance to fisheries management. *Mar. Freshw. Res.* 46:555–570.
- Michaelidis, B., C. Ouzounis, A. Palaras & H. O. Poertner. 2005. Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Mar. Ecol. Prog. Ser.* 293:109–118.
- Micheli, F., A. O. Shelton, S. M. Bushinsky, A. L. Chiu, A. J. Haupt, K. W. Heiman, C. V. Kappel, M. C. Lynch, R. G. Martone, R. B. Dunbar & J. Watanabe. 2008. Persistence of depleted abalones in marine reserves of central California. *Biol. Conserv.* 141:1078–1090.
- Moore, J. D., C. A. Finley, C. S. Friedman & T. T. Robbins. 2002. Withering syndrome and restoration of southern California abalone populations. *CCOFI Rep.* 43:112–119.
- Moore, J. D., T. T. Robbins & C. S. Friedman. 2000. Withering syndrome in farmed red abalone *Haliotis rufescens*: thermal induction and association with a gastrointestinal rickettsiales-like prokaryote. *J. Aquat. Anim. Health* 12:26–34.
- Moore, J. D., T. T. Robbins, R. P. Hedrick & C. S. Friedman. 2001. Transmission of the rickettsiales-like prokaryote *Candidatus Xenohaliotis californiensis* and its role in withering syndrome of California abalone, *Haliotis* spp. *J. Shellfish Res.* 20:867–874.
- Orr, J. C., L. G. Anderson, N. R. Bates, L. Bopp, V. J. Fabry, E. Jones & D. Swingdow. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437:681–686.
- Parmesan, C. & G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37–42.
- Pope, V., F. Carroll, D. Britton & J. Lowe, editors. 2008. Avoiding dangerous climate change. Cambridge: Met Office Hadley Centre.
- Roemmich, D. & J. A. McGowan. 1995. Climatic warming and the decline of zooplankton in the California current. *Science* 267:1324–1326.
- Rogers-Bennett, L. 2007. Is climate change contributing to range reductions and localized extinctions in northern, (*Haliotis kamtschatkana*), and flat, (*Haliotis walallensis*), abalones? *Bull. Mar. Sci.* 81:283–296.
- Rogers-Bennett, L., R. F. Dondanville & J. Kashiwada. 2004. Size specific fecundity of red abalone *Haliotis rufescens*: evidence for reproductive senescence? *J. Shellfish Res.* 23:553–560.
- Rogers-Bennett, L., P. K. Haaker, K. A. Karpov & D. J. Kushner. 2002. Using spatially explicit data to evaluate marine protected areas for abalone in southern California. *Conserv. Biol.* 16:1308–1317.
- Scheibling, R. E. & R. L. Stephenson. 1984. Mass mortality of *Strongylocentrotus droebachiensis* (Echinodermata: Echinoidea) off Nova Scotia, Canada. *Mar. Biol.* 78:153–164.
- Shaw, B. L. & H. I. Battle. 1957. The gross and microscopic anatomy of the digestive tract of the oyster, *Crassostrea virginica* (Gmelin). *Can. J. Zool.* 35:325–347.
- Solomon, S., D. Qin, M. Manning, M. Marquis, K. Averyt, M. M. B. Tignor, H. L. Miller, Jr., & Z. Chen, editors. 2007. Climate change 2007: the physical science basis. Cambridge: Cambridge University Press. 944 pp.
- Southward, A. J., S. J. Hawkins & M. T. Burrows. 1995. Seventy years observations of changes in distribution and abundance of zooplankton and intertidal organisms in the western English Channel in relation to rising sea temperature. *J. Therm. Biol.* 20:127–155.
- Steinbeck, J. R., J. M. Groff, C. S. Friedman, T. McDowell & R. P. Hedrick. 1992. Investigations into a mortality among populations of the California black abalone, *Haliotis cracherodii*, on the central coast of California, USA. In: S. A. Shepard, M. J. Tegner, S. A. Guzmán del Prío, editors. Abalone of the world: biology, fisheries and culture: Proceedings of the 1st International Symposium on Abalone. Cambridge: Fishing News Books. pp. 203–213.
- Sydeman, W. J., M. M. Hester, J. A. Thayer, P. Martin & J. Buffa. 2001. Climate change, reproductive performance and diet composition of marine birds in the southern California Current system, 1969–1997. *Oceanography* 49:309–329.
- Tegner, M. J., P. A. Breen & C. J. Lennert. 1989. Population biology of red abalones (*Haliotis rufescens*) in southern California and management of the red and pink (*H. corrugata*) abalone fisheries. *Fish Bull.* 87:313–339.
- Tegner, M. J. & P. K. Dayton. 1987. El Niño effects on southern California kelp forest communities. *Adv. Ecol. Res.* 17:243–279.
- Tegner, M. J., P. K. Dayton, P. B. Edwards & K. L. Riser. 1996. Is there evidence for long-term climatic change in southern California kelp forests? *Calif. Coop. Oceanic Fish. Invest. Rep.* 37:111–126.
- Tegner, M. J., P. L. Haaker, K. L. Riser & L. I. Vilchis. 2001. Climate variability, kelp forests, and the southern California red abalone fishery. *J. Shellfish Res.* 20:755–763.
- Tegner, M. J., J. D. Martini & K. A. Karpov. 1992. The California red abalone fishery: a case study in complexity. In: S. A. Shepherd, M. J. Tegner, & S. A. Guzmán del Prío, editors. Abalone of the world: biology, fisheries and culture. Oxford: Blackwell. pp. 370–383.
- Tutschulte, T. C. 1976. The comparative ecology of three sympatric abalones. PhD diss., University of California at San Diego.
- Tutschulte, T. C. & J. H. Connell. 1981. Reproductive biology of three species of abalone (*Haliotis*) in Southern California. *Veliger* 23:195–206.
- Vilchis, L. I., M. J. Tegner, J. D. Moore, C. S. Friedman, K. L. Riser, T. T. Robbins & P. K. Dayton. 2005. Ocean warming effects on growth, reproduction, and survivorship of Southern California abalone. *Ecol. Appl.* 15:469–480.
- Vogel, G. 2000. Migrating otters push law to limit. *Science* 289:1271–1273.
- Weiss, I. M., N. Tuross, L. Addadi & S. Weiner. 2002. Mollusc larval shell formation: amorphous calcium carbonate is the precursor phase for aragonite. *J. Exp. Mar. Biol. Ecol.* 293:478–491.
- Wells, G. K., C. B. Grimes, J. C. Field & C. S. Reiss. 2006. Covariation between average lengths of mature coho (*Oncorhynchus kisutch*) and Chinook salmon (*O. tshawytscha*) and the ocean environment. *Fish. Oceanogr.* 15:67–79.
- Wetthey, D. S. 1983. Geographic limits and local zonation: the barnacles *Semibalanus (Balanus)* and *Chthamalus*. *N. Engl. Biol. Bull.* 165:330–341.
- Young, J. S. & J. D. DeMartini. 1970. The reproductive cycle, gonadal history, and gametogenesis of the red abalone *Haliotis rufescens* (Swainson). *Calif. Fish Game* 56:298–309.
- Zacherl, D., S. D. Gaines, & S. I. Lonhart. 2003. The limits to biogeographical distributions: insights from the northward range extension of the marine snail, *Kelletia kelletia* (Forbes 1852) *J. Biogeogr.* 30:913–924.
- Zeidberg, L. D. & B. D. Robison. 2007. Invasive range expansion by the Humboldt squid, *Dosidicus gigas*, in the eastern North Pacific. *Proc. Natl. Acad. Sci. USA* 104:12948–12950.