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LARVAL BIOLOGY AND ENVIRONMENTAL TOLERANCES OF THE KING CRAB PARASITE *BRIAROSACCUS REGALIS*

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ABSTRACT: Rhizocephalan barnacles in the genus *Briarosaccus* parasitize and castrate king crab hosts, thereby preventing host reproduction and potentially altering host abundance. To better understand how environmental factors in Alaska may influence *Briarosaccus* prevalence, we studied the effects of temperature and salinity on the larvae of *Briarosaccus regalis* (previously *Briarosaccus callosus*). Nauplius larvae were reared at 7 temperatures (2 to 16 C) and 8 salinities (19 to 40) to determine larval survival and development rates. Maximum survival occurred from 4 to 12 C and at salinities between 25 and 34. In the Gulf of Alaska and Bering Sea, ocean temperatures and salinities are often within these ranges; thus current conditions appear favorable for high *B. regalis* larval survival. In addition, temperature was negatively correlated with larval development time; thus warmer waters can reduce the time larvae are exposed to the dangers of the planktonic environment. Since only female *B. regalis* larvae can infect crabs, we investigated the sex ratios of *B. regalis* broods at different temperatures and how size and morphological traits can be used to sex cyprid larvae. Larval rearing temperature did not affect brood sex ratio ($F_{0.947}$, $P = 0.369$), but sex ratio varied among broods ($F_{221.9}$; $P < 0.001$). Male larvae (424.5 ± 24.3 μm [mean ± 1 SD]) were significantly larger than female larvae (387.6 ± 22.7 μm [mean ± 1 SD]; $F_{1,221.4}$; $P < 0.001$), consistent with other rhizocephalan cyprids, but sizes overlapped between the sexes such that morphological traits were also necessary for determining sex. Overall, this study provides new information on the larval biology, larval morphology, and environmental tolerances of *B. regalis,* an important king crab parasite.

The distribution and prevalence of parasites is closely tied to environmental factors (Guernier et al., 2004; Sheehan et al., 2011). In particular, temperature plays an important role in mediating the interactions between parasites and their hosts (Thomas and Blanford, 2003). Temperature can affect parasite prevalence and abundance by directly influencing the parasite or host (e.g., changing the physiology, fecundity, growth rates, immune response, or host recovery time) or altering the interaction between the 2 (Blanford and Thomas, 2000; Lazzaro et al., 2008; Murdock et al., 2012). Because parasites are often restricted by cold water temperatures (Harvell et al., 2002), warming can cause outbreaks in areas of previously low abundance. For instance, in Long Island Sound (USA), large-scale die-offs of *Homarus americanus* (American lobster) occurred when parasitic amoeba outbreaks coincided with unusually warm water temperatures (Pearce and Balcom, 2005). Understanding how environmental factors like temperature influence the host-parasite relationship can help explain parasite distributions and prevalence in host populations. In this study, we focused on temperature and salinity effects on the larval phase of the rhizocephalan barnacle *Briarosaccus regalis*, infecting the host *Paralithodes camtschaticus* (red king crab).

Paralithodes camtschaticus is a subarctic lithodid crab that primarily inhabits continental shelves around the North Pacific (Stevens and Lovrich, 2014). This species was also introduced to the Barents Sea, where the population is rapidly expanding (Oug et al., 2011). As a large-bodied crustacean, *P. camtschaticus* plays an integral role in both the marine ecosystem and fisheries (e.g., Kruse et al., 2010; Boudreau and Worm, 2012). Large crustaceans influence benthic community structure through predation, help regulate trophic cascades, and are an important food source for large fish, marine mammals, and humans (Boudreau and Worm, 2012). Very little is known about king crab parasites or their effects on fisheries, although several microbial, protistan, helminthic, and crustacean parasites that infect *P. camtschaticus* have been identified (Morado, 2011; Morado et al., 2014).

Rhizocephalan barnacles are parasitic castrators that primarily infect decapod crustaceans and are only morphologically recognizable as barnacles by their nauplius and cyprid larval stages (Høeg, 1995). As adults, rhizocephalans are primarily internal parasites, with an external reproductive structure called the externa (Bresciani and Høeg, 2001). Female larvae infect hosts by injecting cells that divide to form a rootlet system (interna), which spreads throughout the host's body while absorbing nutrients (Høeg, 1995). When the interna is ready to reproduce, it forms the egg sac-like externa under the abdomen of the host. Male cyprids are attracted to the newly emerged externa, which they enter, metamorphose into dwarf males, and produce sperm to fertilize the eggs (Høeg, 1987).

Members of the genus *Briarosaccus* infect many lithodid species throughout the world (e.g., Hawkes et al., 1986; Abello and Macpherson, 1992; Guzman et al., 2002). Originally all *Briarosaccus* sp. infecting lithodid crabs were identified as *Briarosaccus callosus,* but new cryptic species of *Briarosaccus* are now recognized (Noever et al., 2016). *Briarosaccus regalis* was identified on *P. camtschaticus* and *Paralithodes platypus* (blue king crab) in southeast Alaska (Noever et al., 2016). However, its range likely extends across the North Pacific distribution of its hosts, throughout which *B. callosus* infections have been reported (Sloan, 1985 [British Columbia]; Hawkes et al., 1986 [southeast Alaska]; Sparks and Morado, 1986 [southcentral Alaska]; Isaeva et al., 2005 [Sea of Okhotsk]; Alaska Department of Fish and Game [ADF&G] observer program unpubl. data [Bering Sea, Saint Mathew Island]).

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Effects of *Briarosaccus* on commercial king crab stocks are poorly understood (Kuris and Lafferty, 1992; Basson, 1994). However, outbreaks of other rhizocephalans have caused economic losses in other crab stocks, such as *Callinectes sapidus* (blue crab) in the Gulf of Mexico (Lázaro-Chávez et al., 1996). Population models (Negovetich and Esch, 2008) and field studies (Lafferty, 1993; Fredensborg et al., 2005) suggest that parasitic castrators can reduce the density of host populations by affecting fecundity, and the magnitude of this reduction is largely a function of parasite prevalence. Prevalence of *B. regalis* generally appears low in *P. camtschaticus* (<1%; Sloan, 1985; Hawkes et al., 1986; ADF&G survey and observer program unpubl. data), although a higher prevalence has occurred in *P. platypus*, especially in isolated fjords and bays (e.g., 76% infected in Glacier Bay in 1984; Hawkes et al., 1986). If the prevalence of *B. regalis* were to increase in *P. camtschaticus,* it could lower king crab fecundity, with the potential for future stock declines.

Causes of variability in rhizocephalan prevalence are diverse and range from characteristics of the host populations, like host size distribution (Sloan et al., 2010) and host susceptibility (Ritchie and Høeg, 1981), to environmental characteristics, such as temperature and salinity (Reisser and Forward, 1991; Kashenko and Korn, 2002a, 2002b, 2003). The factors that influence *B. regalis* prevalence have not been identified (Sloan, 1984; Hawkes et al., 1986). In a single laboratory study, *B. regalis* larvae were successfully raised from nauplii to cyprids at 6–7 C (n = 2 broods); however, when raised at 4 C (n = 1 brood) larvae never metamorphosed into cyprids (Hawkes et al., 1985), suggesting low temperatures could limit distribution. Although never tested for *B. regalis*, salinity can also limit the larval survival (Walker and Clare, 1994; Kashenko et al., 2002) and distribution of rhizocephalans (Tindle et al., 2004). Lower salinity caused decreased prevalence of the rhizocephalan *Loxothylacus panopaei* in populations of its estuarine crab host, because *L. panopaei* cannot tolerate extremely low salinities (Reisser and Forward, 1991).

In order to better understand *B. regalis* prevalence and distribution in king crab populations within the North Pacific region, we investigated the effects of temperature and salinity on *B. regalis* larval survival and development rate through 4 naupliar stages to the infectious cyprid stage. In addition, because only female *B. regalis* cyprids can infect crabs, we investigated the sex ratios of *B. regalis* broods and determined whether size and morphological traits can be used to sex cyprid larvae. In rhizocephalans, male cyprids are generally larger than females; thus cyprid length has been used as an indicator of sex in many species (Ritchie and Høeg, 1981). However, size ranges often overlap between the sexes (Walker et al., 1992), and seasonal size variation can be superimposed on the sex-linked size (Høeg and Lützen, 1995). In summary, this study provides novel data on the larval biology of *Briarosaccus*, an important parasite of king crabs worldwide, and presents results of experiments on the environmental conditions under which *B. regalis* larvae can successfully develop.

MATERIALS AND METHODS

Paralithodes camtschaticus with externae of the rhizocephalan *B. regalis* were collected June–July 2013 and 2014 during leg 1 of the ADF&G red king crab survey around Juneau, Alaska (southern Lynn Canal, Auke Bay, and Stephens Passage; Fig. 1). Average bottom temperature during each survey was 4.87 ± 0.007 C and 5.37 ± 0.008 C (mean ± 1 SE) for 2013 and 2014, respectively (ADF&G unpubl. data from temperature loggers on survey crab pots). Average bottom salinity was 32.46 ± 0.07 for 2013 and 31.64 ± 0.01 for 2014 (mean ± 1 SE; ADF&G unpubl. data from 2 CTD casts during each survey, bottom salinity averaged from the bottom 10 m of each cast). The prevalence of red king crabs infected with *B. regalis* was 0.31% (n = 2,273) and 0.10% (n = 3,968) on leg 1 of the 2013 and 2014 surveys, respectively (ADF&G unpubl. data). All infected crabs with an externa were collected, yielding 5 crabs on the 2013 survey and 3 on the 2014 survey. Infected crabs were transported to the Seward Marine Center of the University of Alaska Fairbanks in Seward, Alaska. Crabs were held in individual tanks in a flow-through seawater system at ambient temperature (average 8.3 C; minimum 4.6 C; maximum 13.7 C) and salinity (average 30.7; minimum 28.2; maximum 31.8), with a light regime mimicking ambient conditions at their collection location (Juneau, Alaska). Crabs were held under laboratory conditions for up to 10 mo while multiple broods of parasite larvae were released naturally from each externa. Of the 8 infected crabs collected, 5 died in transport or never produced viable larval broods, leaving 3 crabs from which *B. regalis* larvae were collected for experiments.

To determine when *B. regalis* larvae would be released from externae, a Pasteur pipette was inserted into the mantle opening, and a few embryos were extracted and examined under a dissecting microscope. From the time when the first hatched nauplii could be observed in the externa, it took up to a week for the entire brood to hatch and be released. When hatched nauplii were first observed in the externae, crabs were placed in aquaria without flow-through water. The aquaria were equipped with an aerator and partially submerged in a tank with flow-through water to maintain ambient seawater temperature. Water changes were performed daily. Although a few nauplii were released early from the externa, the vast majority were released within a 2–3 hr period. The timing of the release had no apparent correlation with time of day. Within 12 hr of release, larvae were placed in their respective temperature and salinity treatments. The first larval molt occurred within 24 hr of hatching (Hawkes et al., 1985), thus a mixture of stage I and stage II nauplii was used to initiate the temperature and salinity experiments.

Temperature and salinity experiments

Temperature treatments included 2, 4, 6, 8, 10, 12, and 16 C, and salinity treatments included 19, 22, 25, 28, 31, 34, 37, and 40. A fully crossed design was not logistically feasible. All salinity treatments were conducted at 6 C, a temperature that supports *Briarosaccus* sp. development (Hawkes et al., 1985), and all temperature treatments were conducted at a salinity of 31, which is typical of the Bering Sea where large king crab populations occur (Ladd and Stabeno, 2012), and with *P. camtschaticus* collection sites in southeast Alaska (Stone et al., 1992). The selection of minimum temperature and salinity values for experiments was based on preliminary trials, which demonstrated that larvae did not survive at 0 C or at a salinity of 16. Upper temperature and salinity values were selected based on extremes tolerated by other rhizocephalan species living in similar conditions (Walker and Lester, 1998; Kashenko and Korn,

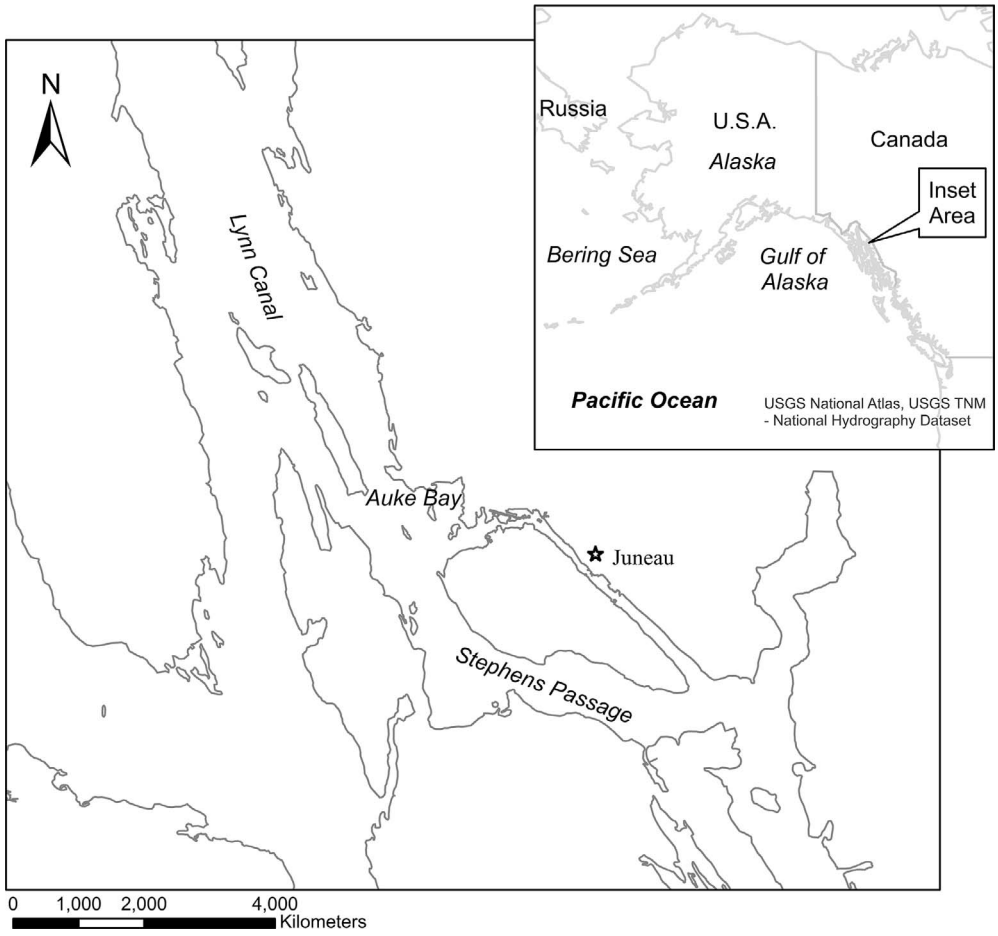

FIGURE 1. Map of collection region for *Paralithodes camtschaticus* (red king crab), near Juneau Alaska (58.30°N, 134.42°W).

2002b), with consideration of what larvae could potentially experience under natural conditions (Stone et al., 1992; Ladd and Stabeno, 2012; Stabeno et al., 2012). For each temperature and salinity treatment, 50 larvae from a single brood were placed into each of 5 replicate Petri dishes (2.5 cm × 10 cm). The experiments were repeated across 5 separate broods, which came from the externae of 3 different host crabs (Table I).

Treatments were conducted in small refrigerators, with digital thermostats (A419; Johnson Controls, Milwaukee, Wisconsin) to control temperature within ± 0.56 C. Each refrigerator had a small fan inside to ensure that temperatures were constant throughout the chamber. All experiments were conducted with filtered (sand filter and 10 μm filter) and UV-sterilized seawater. Dilute salinities were created by adding distilled water to the

TABLE I. Release dates for broods of parasitic *Briarosaccus regalis* larvae from host crabs *Paralithodes camtschaticus*, embryo development time (days), mean water temperature during embryo development (time from previous larval release to release of current brood; ± 1 standard error), and sex ratios of cyprid larvae.

Crab	Brood	Release date	Embryo development time	Embryo development water temperature (C)	% Female larvae (n)*	% Male larvae (n)*
1	a	19 Sep. 2013	59	10.50 ± 0.227	—	—
	b	19 Nov. 2013	61	10.90 ± 0.094	—	—
	c	8 Feb. 2014	81	6.70 ± 0.161	—	—
	d	27 Mar. 2014	47	5.50 ± 0.082	—	—
	e	4 May 2014	38	5.50 ± 0.002	73.2 (41)	26.8 (15)
2	a	28 Dec. 2014	—	9.6 ± 0.440†	92.9 (195)	7.1 (15)
	b	9 Feb. 2015	43	7.80 ± 0.133	30.8 (64)	69.2 (144)
3	a	15 Jan. 2015	—	8.90 ± 0.369†	100 (210)	0 (0)
	b	2 Mar. 2015	46	7.10 ± 0.181	100 (210)	0 (0)

* n = sample size, — = no data.
† Estimate based on a development time of 54 days.

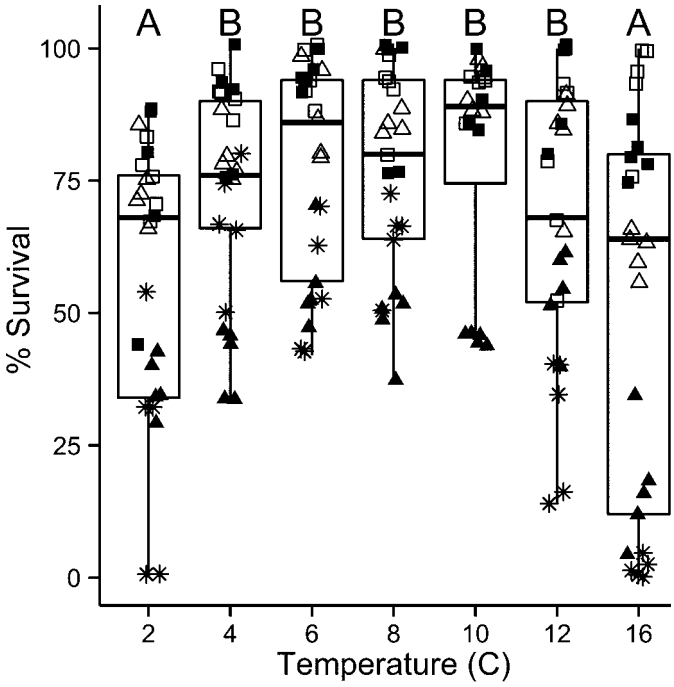

FIGURE 2. Survival through the cyprid stage for *Briarosaccus regalis* larvae under different temperature treatments. Boxplots show median, interquartile range (IQR), and whiskers that extend to the highest/lowest value that is within 1.5 of the IQR. Percent survival for each trial dish for each crab/brood are shown within the boxplots: brood 1e = asterisk, brood 2a = closed triangle, brood 2b = open triangle, brood 3a = closed square, brood 3b = open square. Boxes not sharing a common letter have means that are statistically significantly different at $P < 0.05$.

sterilized natural seawater, while high salinities were created by evaporation of seawater. Salinity and temperature measurements were made with an YSI 85 (YSI, Yellow Springs, Ohio), with an accuracy of 0.1 for salinity and 0.1 C. Culture dishes were kept covered to prevent evaporation and resultant changes in salinity. To remove dead larvae and exuviae, live larvae were counted and hand pipetted into clean treatment water at 5 evenly spaced intervals throughout larval development. Since larvae raised at different temperatures developed at different rates, an approximate development time for each temperature was determined in preliminary trials and divided by 5 to determine intervals between water changes. *Briarosaccus* larvae are lecithotrophic (Hawkes et al., 1985), and thus food was not provided.

Percent survival to the infectious cyprid stage was determined for all temperature and salinity treatments and average development time (days) was determined for all temperature treatments. When larvae were in the last naupliar stage, dishes were checked daily and metamorphosed cyprids were removed. When enough cyprids were available, 30 cyprids from each treatment were sexed and photographed. Maximum linear cyprid length was measured from photographs using Image J (Schneider et al., 2012). Cyprids were sexed using morphological characteristics of the antennae (as in Glenner et al., 1989; Moyse et al., 1995). Distinguishing secondary sexual characteristics of *B. regalis* cyprids have not been previously described; we used descriptions of other members of the Peltogasteridae (Glenner et al., 1989; Moyse et al., 1995) and personal communication (J. T. Høeg) to describe those in *B. regalis*.

Statistical analyses

To determine temperature effects on larval survival through the cyprid stage (binomial response variable) we used a mixed effects logistic regression with the Laplace approximation (Bolker et al., 2009), which produces a χ^2 test statistic. Temperature was the fixed effect, and crab, brood, and dish were nested random effects, nested respectively. The same methods were also used to determine the effects of salinity on development success. Least squares linear regressions were used to determine temperature effects on the natural logarithm of larval and embryo development time. A logistic regression was used to test the effects of crab, brood nested within crab, and temperature on cyprid sex ratio, and an ANOVA was also used to determine the effects of sex, crab, and brood nested within crab on cyprid length. All post hoc pairwise comparisons were conducted with Tukey's HSD test. To visualize the underlying distribution of cyprid lengths by larval sex and brood, we used a histogram and kernel density plots. Kernel density plots are similar to histograms, but they are smoothed using a probability density function. Where appropriate, normality and homoscedasticity were first examined using plots of the fitted values versus the residuals and Q-Q plots of the theoretical quantiles versus the standardized residuals. All statistical analyses were conducted in R (R Core Team, 2015) with $\alpha = 0.05$.

RESULTS

Briarosaccus regalis larvae were released on average every 54 days (minimum 38, maximum 81; Table I) by each parasite externa. Embryo development time was recorded as the time from one larval release to the next. Development time was determined for 7 broods, and mean water temperature during development did not affect embryo development time ($F_{0.947}$, $P = 0.369$, $R^2 = 0.163$; Table I). Nauplii from 5 broods of larvae from 3 externae (on 3 different host crabs) were successfully reared through the infectious cyprid stage. These broods will be referenced according to their crab and brood number and letter combination (Table I). Crabs 1 and 3 were males, while crab 2 was a female. Crab 1 was captured in 2013, while crabs 2 and 3 were captured in 2014. Throughout the naupliar stages, larvae typically remained at or near the bottom of dishes and rarely got stuck in the surface tension, while cyprid larvae were often stuck in the surface tension.

Temperature and salinity effects

Nauplius survival through the cyprid stage differed as a function of temperature ($\chi^2 = 86.6$; $P < 0.001$). Larval survival was significantly lower at 2 and 16 C, while it was highest from 4 to 12 C (Fig. 2). Larval survival was highly variable among broods, especially in the extreme temperature treatments (2 and 16 C; Fig. 2). Temperature and the natural logarithm of larval development time had a negative linear correlation ($R^2 = 0.972$; $F_{16,530}$; $P < 0.001$). Larval development time increased at colder temperatures, with a Q_{10} of 2.70. At 16 C development was completed in 11 days on average, while at 2 C nauplii metamorphosed into cyprids after an average of 43 days (Fig. 3). In addition, the variation around mean development time increased with decreasing temperature (Fig. 3).

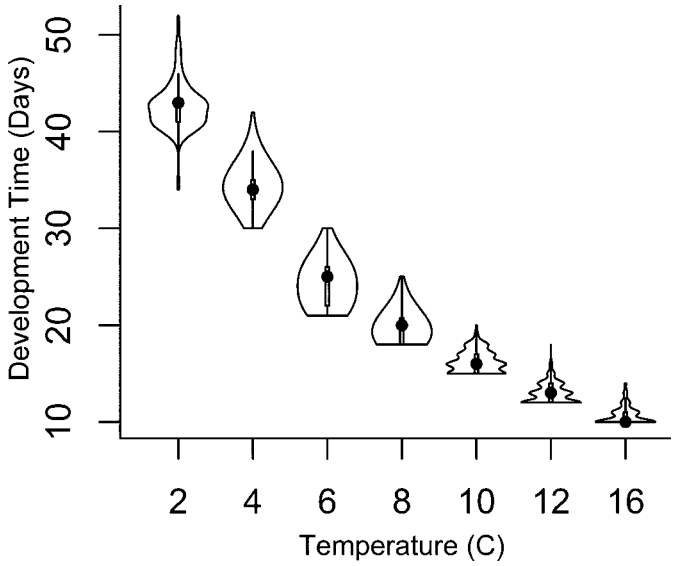

FIGURE 3. Violin plots of development time for nauplius larvae of *Briarosaccus regalis* reared at 7 different temperature regimes, with a $Q_{10} = 2.70$. Each plot shows the median (black circle), boxplot with the interquartile range (IQR; gray vertical bars), and whiskers (black vertical lines that extend to the either highest/lowest value or 1.5 × IQR, whichever value is closest to the median), and the kernel density plot (thin vertical curves showing frequency distribution).

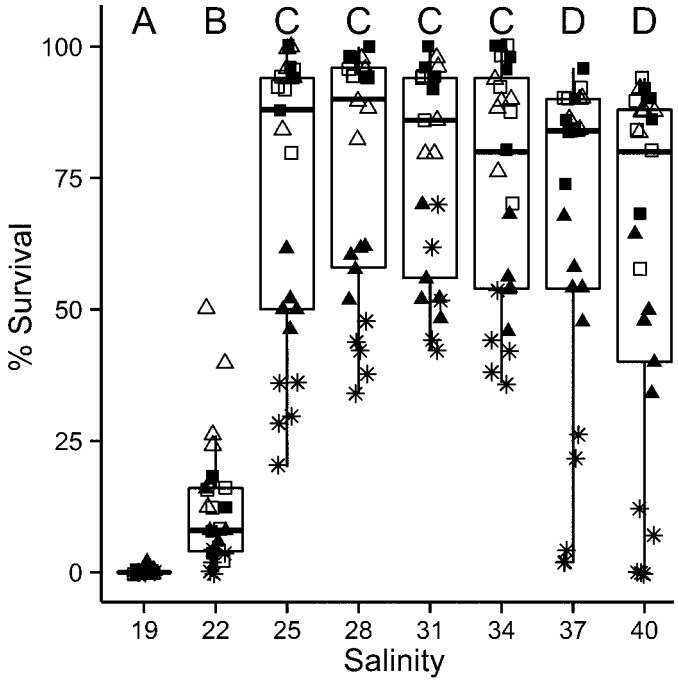

FIGURE 4. Survival through the cyprid stage for *Briarosaccus regalis* larvae under different salinity treatments. Boxplots show median, interquartile range (IQR), and whiskers that extend to the highest/lowest value that is within 1.5 of the IQR. Percent survival for each trial dish for each crab/brood are shown within the boxplots: brood 1e = asterisk, brood 2a = closed triangle, brood 2b = open triangle, brood 3a = closed square, brood 3b = open square. Boxes not sharing a letter have means that are statistically significantly different at $P < 0.05$.

Larval survival through the cyprid stage also differed as a function of salinity ($\chi^2 = 533.4$; $P < 0.001$). Maximum larval survival occurred at salinities 25 to 34, while survival dropped slightly, but significantly, at the higher salinities (37 and 40) and sharply at the lowest salinities (19 and 22; Fig. 4).

Cyprid sex determination, sex ratios, and size

Briarosaccus regalis cyprids have not previously been sexed using morphological traits, although size was presumed to indicate sex in the only other study on the larvae of this species (Hawkes et al., 1985). Sexual dimorphism of cyprid antennae (Fig. 5) was observed in this study based on comparisons with other members of the Peltogasteridae (Glenner et al., 1989), including *Briarosaccus tenellus* (Moyse et al., 1995), and by personal communication (J. T. Høeg). Male cyprids were identified by the bifurcated large aesthetasc on the third antennal segment and the enlarged subterminal aesthetasc on the fourth segment. Female larvae have 2 distinct setae in place of the large aesthetasc and a much smaller subterminal aesthetasc. In addition, the third antennal segment of females is rounded, while this segment is more elongated in the males (Fig. 5).

Cyprid sex ratio was a function of both crab ($P < 0.001$) and brood nested within crab ($P < 0.001$), but sex ratio was not affected by larval rearing temperature ($P = 0.72$). Two broods (both from crab 3) consisted entirely of female larvae, while the other 3 broods were a mixture of male and female larvae (Table I). All recorded broods were released in winter and spring (December through May), yet no pattern was apparent between sex ratio and season (e.g., larvae of both sexes were released within a few days of each other). Cyprid sex had a significant effect on larval length ($F_{1221.4}$; $P < 0.001$). On average, males were larger (424.5 ± 24.3 μm [mean ± 1 SD]) than females (387.6 ± 22.7 μm [mean ± 1 SD]); however, the size distribution of the sexes substantially overlapped (Fig. 6). In addition, both host crab ($F_{186.3}$; $P < 0.001$) and brood nested within crab ($F_{221.9}$; $P < 0.001$) had significant effects on cyprid length. Larval size varied substantially in female (Fig. 7) and male (Fig. 8) larvae, both between broods from the same crab and among crabs. Mean female cyprid length varied from 366.2 μm (brood 3a) to 420.6 μm (brood 2b), while mean male cyprid length varied from 409.4 μm (brood 2a) to 438.2 μm (brood 2b).

DISCUSSION

We examined how 2 environmental factors, temperature and salinity, could influence the survival and development of *B. regalis*, a parasitic castrator of *P. camtschaticus* and *P. platypus*. The naupliar larvae of *B. regalis* had maximum survival at a wide range of temperatures (4–12 C) and salinities (25–34); in addition, larval development time increased from 11 to 43 days with decreasing temperature. Because the timing of larval sex ratios can be important for fertilization success in rhizocephalans (Ritchie and Høeg, 1981; Poon et al., 2005), we explored methods of sexing cyprid larvae and then examined factors that could influence sex ratios, such as host crab and larval rearing temperature. On average, male cyprids were larger than females, but sizes overlapped considerably such that size was an unreliable predictor of cyprid sex.

For rhizocephalans, the habitat conditions of the hosts generally coincide with the environmental tolerances of the larvae

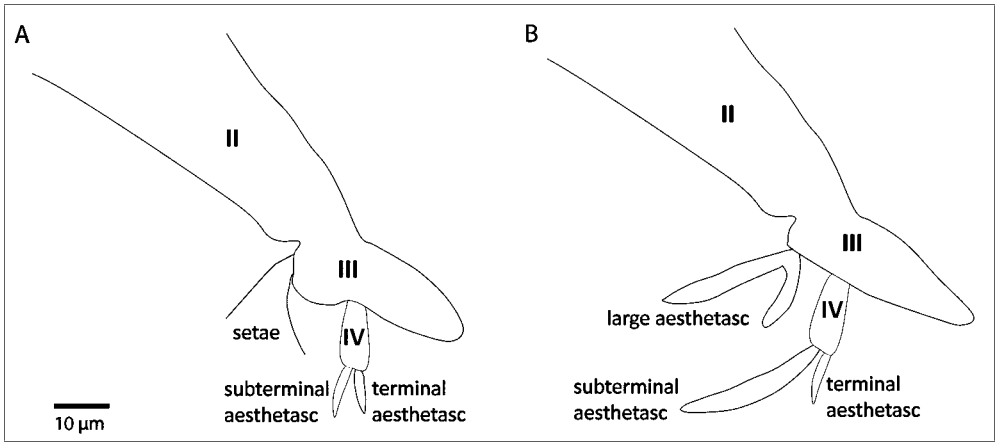

FIGURE 5. Key distinguishing characteristics on the antennae of (**A**) female and (**B**) male *Briarosaccus regalis* cyprids (modified from other Peltograstridae descriptions, e.g., Glenner et al., 1989; Moyse et al., 1995). Roman numerals indicate antennal segments.

(Kashenko and Korn, 2003). For example, 3 rhizocephalan species living in Vostok Bay in the Sea of Japan had different temperature tolerance ranges depending on their depth distribution (Kashenko and Korn, 2002a, 2002b, 2003; Kashenko et al., 2002). *Polyascus polygenea*, which lives primarily on intertidal hosts, developed successfully at 18–25 C, while *Peltogaster reticulatus*, which lives from the intertidal to about 10 m, developed at 16–25 C, and *Peltogasterella gracilis*, living from 5 m to several hundred meters, developed at 12–22 C (Kashenko and Korn, 2002a, 2002b, 2003; Kashenko et al., 2002). However, some species of Rhizocephala can tolerate a wider range in environmental variables than would be predicted by their host's habitat. For instance, the host crab *Charybdis callianassa* lives in marine conditions, but its parasite *Heterosaccus lunatus* can develop at salinities of 24–40 (Walker and Lester, 1998). *Briarosaccus regalis* larvae had high survival across fairly wide temperature and salinity ranges, given that host crabs live in deep waters with fairly low variability. However, it is unknown whether *B. regalis* larvae in situ remain at depth where they are released, or migrate into surface waters that can be warmer and fresher. The former appears more probable, given that they are lecithotrophic and, like other members of the Peltogastridae, lack a naupliar eye that is used for phototaxis in other rhizocephalan families (Kashenko and Korn, 2003).

Currently, bottom water temperatures around Alaska are generally on the colder end of what *B. regalis* larvae tolerated in this study (Stone et al., 1992, 1993; Stabeno et al., 2012). King crabs from southeast Alaska, where infected crabs were collected for our experiments, have been recorded living at temperatures between 3.2 and 7.7 C (Stone et al., 1992, 1993). In the Bering Sea (Fig. 1) where the largest Alaskan king crab population occurs, bottom water temperatures on the continental shelf generally range from ≤–1 to 5 C (Stabeno et al., 2012), but king crabs avoid temperatures ≤2 C (Chilton et al., 2010). Based on our results (maximum survival at 4–12 C), these temperatures would

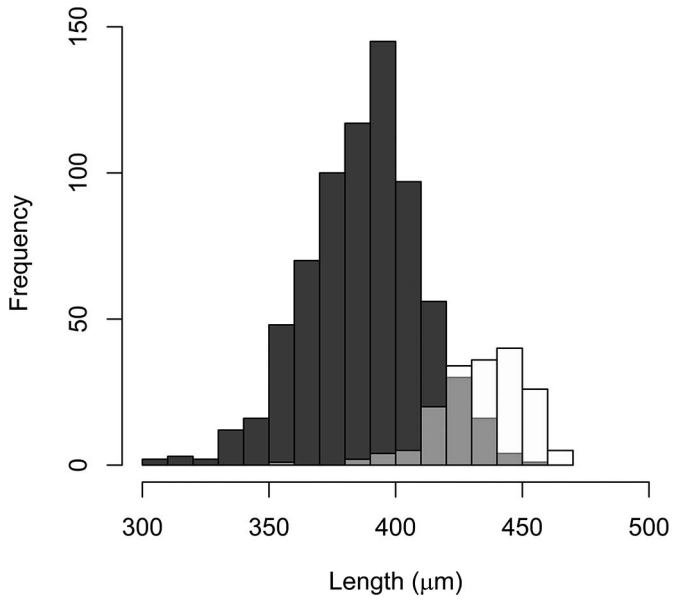

FIGURE 6. Frequency distribution of the length of male (white) and female (dark gray) *Briarosaccus regalis* cyprids, showing the overlap (light gray) in size distribution between sexes.

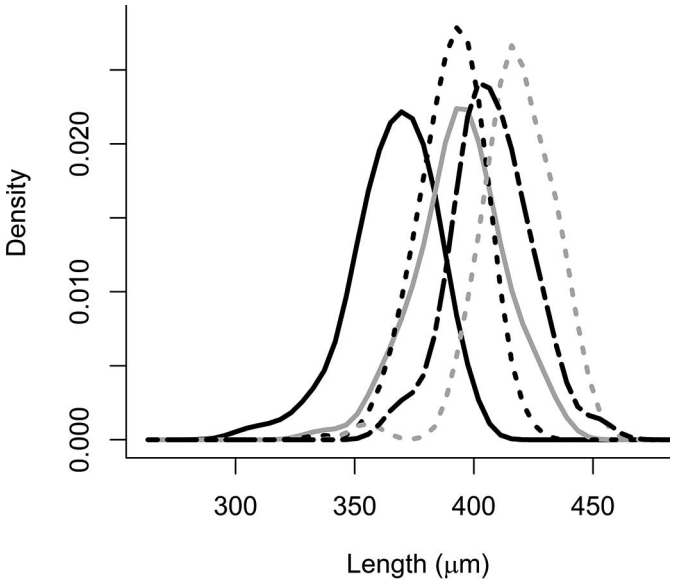

FIGURE 7. Kernel density plots of female *Briarosaccus regalis* cyprids from 5 broods of larvae. Lines indicate individual broods as follows: brood 1e (black, — – —), brood 2a (gray, ——), brood 2b (gray, – – –), brood 3a (black, ——), and brood 3b (black, – – –). Numbers indicate different host crabs/externa, and letters indicate different broods from the same host crab/externa.

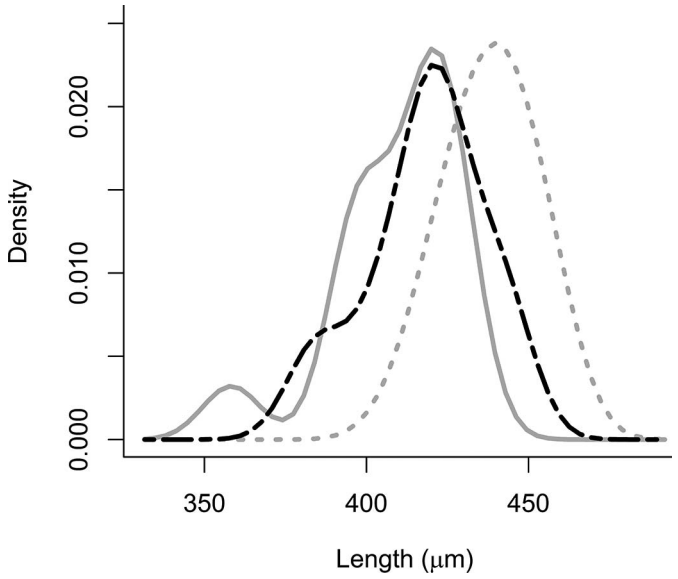

FIGURE 8. Kernel density plots of male *Briarosaccus regalis* cyprids from 3 broods of larvae. Lines indicate individual broods as follows: brood 1e (black, — – —), brood 2a (gray, ——), and brood 2b (gray, – – –). Numbers indicate different host crabs/externa, and letters indicate different broods from the same host crab/externa.

promote high *B. regalis* larval survival, albeit with relatively slow development.

Briarosaccus regalis larvae had high survival at salinities that they would not typically be exposed to, especially at the higher end of the salinity range (e.g., 37 and 40). In open water on the eastern Bering Sea shelf, salinity variations throughout the water column are minimal, approximately 30–32.5 (Ladd and Stabeno, 2012). Hosts in southeast Alaska occur at a similar salinity range (29.2 to 32.5) (Stone et al., 1992); however, within the enclosed bays and fjords of this region, salinity can be highly variable with season, depth, and location (Carlson, 1980; Etherington et al., 2007; Weingartner et al., 2009). Surface salinities (upper 10 m) can be low (≤22) (NOAA: Auke Bay Monitor Station). Since we found *B. regalis* survival to be dramatically reduced at and below a salinity of 22, these surface waters would reduce *B. regalis* survival, and could favor a demersal existence.

The current temperatures and salinities in Alaskan waters appear to support high *B. regalis* larval survival; however, our experiments suggest these temperatures may prolong development time. Nauplius survival was not significantly different between 4 and 12 C, but development was 21 days slower at the colder temperature. This extended period of larval development could result in lower larval survival than we detected in our experiments. In the natural environment, planktonic invertebrate larvae are highly vulnerable to mortality from predation, disease, and/or transport into unfavorable habitats (Rumrill, 1990; Vaughn and Allen, 2010). Mortality is generally assumed to increase the longer larvae spend in the plankton (Rumrill, 1990). Thus, faster development rates associated with warmer waters up to 12 C are likely to support higher *B. regalis* nauplius survival in the wild, although a similar shortening of the cyprid stage in warmer water could reduce the time window for infection.

In rhizocephalans, the sex ratio of successive broods can change seasonally, presumably to synchronize the presence of new (virgin) externa and male larvae (Ritchie and Høeg, 1981; Poon et al., 2005). For example, *Sacculina sinensis* primarily releases males in the summer and females in the winter (Poon et al., 2005), while *Lernaeodiscus porcellanae* does the opposite (Ritchie and Høeg, 1981). We observed considerable variation in the sex ratio of *B. regalis* broods, but found no indication of seasonality; all-female broods and predominately-male broods were released within days of each other. Photoperiod may trigger sex ratios in other rhizocephalans (Walker and Lester, 2000), but the lithodid hosts of *B. regalis* generally live at depths with low light penetration (Stone et al., 1992), possibly explaining the apparent lack of seasonality. Moreover, *B. regalis* may not need to rely on seasonal cues to synchronize appearance of male larvae and virgin externae, because the virgin externae in this species appear to have a long lifespan. In some species (e.g., *Sacculina carcini*), virgin externae die quickly if they do not acquire a male, while in other species (e.g., *L. porcellanae*), they remain healthy indefinitely (Ritchie and Høeg, 1981). A *P. platypus* with a virgin externa of *B. regalis* was kept in a laboratory for 5 mo (Hawkes et al., 1985), which suggests that virgin externae have considerable time to acquire a male. A mismatch between the presence of virgin externae and male larvae in *B. regalis* is thus unlikely due to the aseasonal variation in sex ratios and the lengthy lifespan of the virgin externae. Furthermore, we found no difference in cyprid sex ratios between larval temperature treatments. While temperature could not have affected sex ratios in our experiments directly because sex is determined in the unfertilized eggs (Høeg, 1995), these data indicate that larval rearing temperature does not cause differential mortality between the sexes.

There was a large variation in the size of male and female cyprids, both between successive broods from the same host and among hosts. However, this variation did not appear to be seasonal, although our sample size was small and broods were only examined from December to May. Other studies with rhizocephalans have found seasonal differences in cyprid size, with larger larvae occurring in the spring and summer than in the fall (Yanagimachi, 1961). For free-living barnacles, temperature and food availability during the larval stage are both important determinants of cyprid size (Barnes and Barnes, 1965; Anil et al., 2001); however, the lecithotrophic larvae of rhizocephalans, and the lack of size variation between temperature treatments, indicate that these factors were not affecting cyprid size in *B. regalis*. Others have proposed that the temperature during embryo development can affect larval size (Barnes, 1953), yet this also appears unlikely here. For example, broods of female larvae with the largest and smallest mean cyprid size (Fig. 7) were exposed to similar and less extreme temperatures during embryo development, compared to other broods (Table I).

Briarosaccus regalis appears well adapted to conditions in the North Pacific where its king crab hosts reside. Continuous aseasonal reproduction and variable sex ratios ensure that male larvae are in the water column when virgin externae emerge, which may take considerable time due to the large host size and cold water temperatures. The large range of temperatures and salinities that these larvae can tolerate give them the ability to survive and infect crabs across a wide latitudinal gradient, from British Columbia (Sloan, 1985) to Norton Sound (C. Lean, pers. comm.), and in diverse habitats with different salinity regimes, ranging from open ocean to fjords and bays. *Briarosaccus regalis* appears well adapted to deal with future increases in temperature

(Royer and Grosch, 2006; Wang et al., 2012) and changes in freshwater runoff and salinity (Jansson et al., 2003; Royer and Grosch, 2006) because it tolerates temperatures and salinities beyond the range that it currently experiences.

In the subarctic Bering Sea, where the largest Alaskan king crab populations occur, sea surface temperature is predicted to increase by approximately 3 C by 2100 (Wang et al., 2012). If this change occurs throughout the water column, *B. regalis* development will accelerate, reducing the length of the planktonic period. *Briarosaccus regalis* currently infects *P. camtschaticus* and *P. platypus* populations in this region, but prevalence is extremely low (<0.1% in both species; ADF&G observer program unpubl. data). However, if a shorter development time leads to increased larval survival and infection rates, there could be potential impacts on crab populations and fisheries in the Bering Sea. In order to further understand how *B. regalis* prevalence will change under future climate scenarios, additional studies should focus on other aspects of the life cycle, particularly the process of infection. We also lack an understanding of interacting effects of temperature and salinity, as well as other potential environmental stressors, on both the host and the parasite that would allow us to fully grasp how changing conditions will impact this host-parasite relationship. The present study helps elucidate some environmental tolerances and the overall larval biology of *B. regalis*.

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