Title: Limited evidence for local adaptation to salinity and temperature variability in San Juan Island populations of the copepod *Tigriopus californicus* (Baker)

Author: Alyssa Liguori

Affiliation address:

Department of Ecology and Evolution, Stony Brook University

650 Life Sciences Building

Stony Brook, NY, USA 11794

Email address: alyssa.liguori@stonybrook.edu

Present address:

Josephine Bay Paul Center for Comparative Molecular Biology and Evolution

Marine Biological Laboratory

7 MBL Street

Woods Hole, MA, USA 02543

ABSTRACT

Local adaptation has been studied in a broad range of taxa for decades. However, we have limited understanding of how often it occurs in variable environments. Whether phenotypic plasticity can evolve in distinct ways among populations experiencing different patterns of abiotic variability is unclear. Abiotic conditions in coastal marine habitats can be highly heterogeneous at small spatial scales, which might promote local adaptation. The harpacticoid copepod *Tigriopus californicus* (Baker, 1912) has become a model system for testing whether phenotypic differences among populations are a result of local adaptation. To identify potential selective pressures in the field, temperature and salinity were measured in high shore pools for six months at three sites on San Juan Island in Washington, USA. A common garden experiment with factorial combinations of seven temperature and two salinity (32, 55 ppt) treatments was conducted on these distinct populations. Two temperature treatments varied daily, both with an average of 20°C, but different ranges (low amplitude: 15-25°C, high amplitude: 10-30°C). The other five treatments were the average, maximum, and minimum temperatures held constant. Fecundity, survivorship, and development were characterized across two generations. There were strong interactive effects of temperature and hypersalinity on copepod culture dynamics, but these effects differed among populations. Abiotic patterns in the field were correlated with few observed differences in population phenotypes, thus limited evidence for local adaptation was found. For these populations, differences in selective pressures among sites might not be strong enough to overcome the influence of genetic drift.

Key words: *Tigriopus californicus*, life history, rocky intertidal, copepod, multiple stressors, local adaptation

1. INTRODUCTION

Local adaptation can occur when populations experience different selective pressures in heterogeneous environments, leading to native individuals in a particular environment having higher fitness than individuals from elsewhere (Hoban et al., 2016; Kawecki and Ebert, 2004). Although this phenomenon has been studied for decades (Hereford, 2009), we still do not know how often it can be expected among physically close populations (Sanford and Kelly, 2011). Our knowledge is particulary limited in marine systems, which until recently have been relatively understudied in this context due to the assumption of few dispersal barriers and high gene flow (Conover et al., 2006).

Because local adaptation is expected when the spatial scale of gene flow is small relative to that of a changing selective gradient, many studies on marine species with high dispersal potential have focused on regional-scale latitudinal gradients, or populations separated by hundreds or thousands of kilometers (Sanford and Kelly 2011). Studies at smaller spatial scales, such as populations separated by a few kilometers, or by only a few meters at different heights in the intertidal zone, have focused on direct developers, or species that typically have limited migration potential (Bohonak, 1999; Kirby et al., 1994; Struhsaker, 1968). However, a rapidly growing body of research provides evidence for high genetic differentiation among many marine populations, even for species with planktonic dispersal (Burton, 1986; Edmands et al., 1996; Levin, 2006; Palumbi, 2004). Local adaptation is likely prevalent in marine systems, at a wide range of spatial scales and across species and populations with diverse life histories (Sanford and Kelly, 2011).

Unlike the relatively stable open ocean, coastal systems are highly heterogeneous, which could lead to different selective pressures throughout the ranges of marine species (Gunderson et

al., 2016; Helmuth et al., 2006; Hofmann et al., 2010). The intertidal zone in particular is among the most variable habitats on the planet (Tomanek and Helmuth, 2002) and the organisms that persist there must withstand drastic changes in temperature, salinity, carbonate chemistry, oxygen availability, physical disturbance, and other factors (Daniel and Boyden 1975; Truchot and Duhamel-Jouve 1980; Morris and Taylor 1983). This abiotic variability can occur spatially, as stark vertical gradients occur over a few meters, where marine and terrestrial environments meet (Connell, 1961; Somero, 2002). Spatial heterogeneity also occurs along shorelines, as oceanographic, biological, geological, and anthropogenic factors influence local environments. For example, coastal upwelling, terrestrial input of freshwater and nutrients, and the respiration and photosynthesis of local biological communities can lead to complex spatial mosaics (Helmuth et al., 2002; Hofmann et al., 2010; Kroeker et al., 2016). Differences in tidal regimes can influence the temperatures of intertidal zones via the timing of shore exposure across seasons, which can disrupt expected latitudinal temperature gradients (Helmuth et al., 2002).

Spatial heterogeneity in environmental conditions can promote local adaptation. However, conditions in intertidal zones are also highly variable in time, which could constrain such adaptive divergence (Sanford and Kelly, 2011). Abiotic factors can vary daily, weekly, and monthly, due to changing tides, weather, and seasons, which influence biological processes that feed back into local abiotic dynamics (Helmuth et al., 2006). Populations persisting in such temporally variable environments might be more likely to evolve plastic traits, including the capacity to physiologically acclimate to a wide range of conditions (Kawecki and Ebert, 2004; Sanford and Kelly, 2011).

However, if abiotic conditions experienced by separate populations differ in their magnitude of variability, there could be potential for the evolution of different levels of

phenotypic plasticity among the populations (Chevin and Hoffmann, 2017; Kelly, 2019). At larger spatial scales, the evolution of specialist and generalist populations is often studied in the context of seasonality. It is expected that populations experiencing greater seasonality in higher latitudes will be more likely to evolve wider thermal niches than populations in lower latitudes. However, individuals with narrow niches in more stable environments are expected to have higher fitness around their optimum (Gilchrist, 1995). Similar expectations might hold at smaller scales, for populations living in microhabitats with consistent differences in abiotic variability.

Tigriopus californicus (Baker, 1912) is a harpacticoid copepod found in rocky intertidal splashpools along the west coast of North America (Dethier, 1980). This species has become a model system for the study of adaptation, population differentiation, and physiological responses to multiple stressors (Raisuddin et al., 2007). Though all life stages of *T. californicus* are free-swimming, decades of research show that there is limited gene flow and high genetic differentiation among populations (Barreto et al., 2018; Burton, 1997, 1987, 1986; Burton et al., 1979; Burton and Feldman, 1981; Burton and Lee, 1994; Edmands, 2001; Edmands and Harrison, 2003; Willett and Ladner, 2009). Studies on dozens of populations distributed from British Columbia, Canada to Baja California, Mexico have found that northern populations are more tolerant of low salinities caused by high input of precipitation in pools, whereas southern populations are more tolerant of high temperatures and high salinities caused by evaporation (Kelly et al., 2012; Leong et al., 2017; Pereira et al., 2017; Willett, 2010).

Tigriopus californicus is restricted to high intertidal and supralittoral splash pools due to intense predation in lower zones of the shore. The restriction of *T. californicus* to the highest edge of the shore also means that they thrive in what is likely one of the most extreme and variable marine habitats, where few other species can persist (Dethier, 1980). Both temperature

and salinity are more variable in splashpools than in lower zones of the intertidal because splashpools are rarely flushed out by high tides or strong wave action. Temperatures can range from near freezing to over 40°C (daily ranges of >20°C are possible) and salinity can range from 0 to at least 139 ppt (Powlik, 1999). This temporal variability might constrain local adaptation, as isolated but geographically proximate populations may have evolved similar capacities to acclimate to a wide range of stressful conditions.

However, temperature and salinity dynamics within *T. californicus* pools have not been well characterized throughout their range. Differences in wave exposure, pool heights and sizes, tides, and other factors among rock outcrops might cause *T. californicus* populations to experience divergent amplitudes of variability in temperature and salinity, even if they are separated by only a few kilometers (Leong et al., 2017; Metaxas and Scheibling, 1993). Indeed, Dybdahl (1995) found that *T. californicus* pools at an exposed outer coast site in California experienced higher, more variable salinity, whereas pools within a protected cove had more stable conditions, with salinities close to that of ambient, open seawater.

Tigriopus californicus populations on San Juan Island in Washington, USA have distinct life history characteristics, including different reproductive rates, development times, and resilience to stressors (*A.L. Liguori, unpublished data*). The goal of this study was to determine if these differences are due to genetic differentiation and local adaptation, and to test whether populations have evolved different capacities for acclimation to temperature variation. A laboratory and field study consisting of two parts was conducted: 1) a common garden, factorial experiment testing for local adaptation among three *T. californicus* populations to different temperature and salinity regimes, and 2) monitoring of environmental conditions within pools at each site to assess the potential for divergent selective pressures. The study sites were chosen

because they have distinct geological features and local oceanographic conditions, despite being separated by only 15 - 30 km. For example, sites on the west and south side of the island are more exposed to strong currents and wave action, as they face the relatively open waters of the Strait of Juan de Fuca, whereas a protected cove site on the east side faces the calmer San Juan Channel. The ideal test for local adaptation would have been to transplant and characterize performance of populations in the field (Kawecki and Ebert, 2004; Rundle and Whitlock, 2001; Sanford and Kelly, 2011). However, this approach was not feasible for this species, as any introduction of foreign genotypes could permanently alter the genetics of local populations (Edmands and Deimler, 2004; but see Burton and Swisher, 1984).

I set out to address the following questions: 1) Are observed differences in the life history traits of *Tigriopus californicus* populations on San Juan Island due to genetic differentiation? 2) How do combinations of temperature and hypersalinity treatments impact *T. californicus* population dynamics? 3) Does the daily amplitude of variability in temperature matter? 4) Are *T. californicus* populations on San Juan Island locally adapted?

2. MATERIALS & METHODS

2.1 Copepod collection and common garden rearing

Tigriopus californicus were collected from high intertidal and supralittoral rock pools at Cattle Point (CP, 48°27’7” N, 122°57’42” W), Colin’s Cove near Friday Harbor (FH, 48°32’58” N, 123°00’21” W) and Dead Man’s Cove (DM, 48°30’46” N, 123°08’42” W) on San Juan Island, Washington, USA (Fig. 1). At each site, adult copepods were collected from six pools and combined to start common garden laboratory cultures at the University of Washington’s Friday Harbor Laboratories (FHL) in June of 2018. Populations were maintained in identical static jar cultures at 18°C for at least two generations (nine weeks), to reduce transgenerational

effects from exposure to the field environment (Kawecki and Ebert, 2004). Adults were removed from the cultures after two weeks of offspring production using size-selection with Nitex mesh filters, to prevent overlapping generations. Cultures were fed Spirulina powder *ad libitum*, 50% water changes were conducted approximately every two weeks, and copepods from the same population were mixed among jars to prevent genetic drift and ensure equal densities.

2.2 Field data collection

At each site, environmental data were collected from 5-7 *T. californicus* pools, including the same pools that copepods were collected from. Pools of various sizes and heights on the shore were included in an attempt to characterize the full range of conditions at each site. Temperature data were collected between May 26th and July 7th, 2018 and between July 27th and October 20th, 2018. Temperature dataloggers (Thermochron iButtons, no. DS1921G, OnSolution) were waterproofed with clear Plasti-Dip (Plasti Dip International, USA; as in Roznik and Alford 2012; Leong et al., 2017) and secured to the deepest point of pools using marine epoxy. Loggers recorded temperature every 30 minutes.

Salinity measurements were taken approximately every two weeks from June 8th to October 20th, 2018. Samples were taken from the deepest point of undisturbed pools using a pipette and measured with a refractometer with an upper limit of 100 ppt. At FH, a relatively protected cove, all pools were measured at every time point because they were always filled with seawater. However, two pools were occasionally unoccupied by *T. californicus,* most likely because they were flushed out of the pools at high tide. At CP, an exposed site where pools are spread out across a wider rock bench, the majority of pools were always filled and occupied by copepods. At DM, there are fewer pools on two rocky outcrops surrounded by pebble beaches. There are two pool types: some that are low on the shore and frequently flushed at high tide, and

some that are very high on the shore, on top of a taller rock face. Of these pools, only four at lower heights on the shore were monitored consistently, whereas the others were often completely dry or filled with rainwater, and did not always contain copepods. Heights of pools on the shore were measured with a rotary laser level kit (Lasermark LD-100N; Table S10). In the San Juan Islands, most rainfall occurs in the winter, thus low rain accumulation was recorded throughout the survey period (ranged from no rain in August to ~0.6 inches total in October).

2.3 Experimental set up

Temperature and salinity data collected in the spring of 2018 (May - July) informed the treatment combinations that were selected for laboratory tests of local adaptation, which began in August. Experimental treatments were chosen to reproduce the range of temperature and salinity that these *T. californicus* populations regularly experience in the San Juan Island high intertidal zone. Environmental data from the entire 2018 field season (May - October) were analyzed to explore differences in abiotic conditions among sites and to define expectations for laboratory patterns that would occur if populations were locally adapted.

Each of three replicate mass jar cultures of copepods per population (CP, DM, FH) were initiated with 20 gravid females from common garden cultures. Cultures were maintained in factorial combinations of two salinity treatments (32 and 55 ppt) and seven temperature treatments, five of which were held constant (10, 15, 20, 25, 30°C) and two of which were variable. Both variable treatments had an average temperature of 20°C, but one had a higher amplitude (variable high; VH) that varied between 10 and 30°C and the other had a lower amplitude (variable low; VL) that varied between 15 and 25°C (Table S4). Variable cultures were held in the minimum and maximum temperatures for 12 hours per day each. While maximum temperatures in *T. californicus* pools typically do not occur for more than a few hours

per day, this design was employed to parse out the effects of stable average, maximum, and minimum temperatures, versus the effects of variability and amplitude of daily change in temperature. While *T. californicus* can experience more extreme conditions than these treatments in the field, the goal of this study was to characterize the performance of populations in response to sub-lethal stress.

The experiment included a total of 126 mass jar cultures, each with a volume of 600 mL. Constant temperature treatments were maintained in ~50 L water baths (three per temperature), all haphazardly arranged (baths of the same temperature were never adjacent) within a cold room set at 10°C with a 12:12 hr light/dark cycle. Variable temperature regimes were achieved by moving cultures between water baths of the maximum and minimum temperatures. Temperature was monitored throughout the experiment with waterproofed iButton dataloggers that took measurements every 10 minutes.

Salinity was manipulated by the addition of either distilled water or Instant Ocean Sea Salt to ambient, 1 μm bag-filtered seawater collected from the flow-through seawater system at FHL. After thorough mixing, ambient air was bubbled into treatment water for at least 24 hours prior to use in the experiment. Water changes (50%) were conducted approximately every two weeks, before which salinity in each jar was checked with a refractometer. If salinity was too high, distilled water was added, although this was a rare occurrence, as loose-fitting lids on the jars prevented rapid evaporation.

At the beginning of the experiment and during each water change, each jar was fed 10-15 mg of Spirulina powder. All jars were given equal quantities at each feeding. This food was supplemented with equal quantities of dense blends of live *Tisochrysis lutea*, *Pavlova* sp., and *Tetraselmis chuii* microalgae once per week to prevent loss of carotenoid pigment in the

copepods (Weaver et al., 2018). Volumes of algae added were typically less than 10 mL and had a negligible effect on salinity.

Mass jar cultures were maintained in these conditions for eight weeks, and F_0 mothers were removed from the cultures after two weeks of offspring production to prevent overlapping generations. Life history responses were measured in subsets of individuals throughout the development of the F_2 generation (described below). After eight weeks, all remaining individuals in each jar culture were preserved in ethanol for quantification of copepodites and adults. At the end of the experiment, cultures contained individuals from the F_1, F_2, and potentially F_3 generations, but any F_3 individuals present were likely still nauplii and were not counted because early life stages did not preserve well.

2.4 Life history responses

Fecundity of the F_1 generation and survival and development of the F_2 generation were quantified by subsampling individual gravid F_1 females from each mass jar culture and incubating them in their own 15 mL Falcon culture tubes at the appropriate treatment conditions (hereafter referred to as "single clutch cultures"). As F_1 copepods developed, jar cultures were monitored daily and gravid F_1 females were removed as soon as they appeared, which occurred at different times for different treatment combinations, due to delayed development. Therefore, the fastest developing individuals from each replicate jar culture were used to initiate single clutch cultures. Clutch number, but not age, was the same across individuals. For *T. californicus,* clutch size declines with age and with successive reproductive bouts (clutch number; Powers et al., 2020), however, it is not clear how these declines would be affected by developmental delays.

For each single clutch culture, the F_1 mother was removed after her first clutch hatched (tubes were monitored every two days until the mother was removed), and development of the F_2 juveniles was tracked via weekly counts of all individuals as they developed from nauplii to adults. Copepods were rinsed from the tubes into a dish and counted (by life stage) underneath a dissecting microscope by pipetting each individual into another dish. All copepods within a clutch were then returned to their culture tube. Multiple mothers (3 - 4 individuals) were sourced from each replicate mass jar culture (n = 3) to initiate single clutch cultures, and values for each response from these clutches were averaged within each replicate.

F_1 fecundity was defined as the total number of individuals present in each clutch at the first count. Survivorship over three weeks was calculated as the proportion of the total number of individuals at the first count that survived to each subsequent weekly count (weeks two through four). In treatment combinations in which development of the F_1 generation was delayed, survivorship of F_2 offspring could not be tracked across a full three weeks for all replicates. Therefore, for the CP population analysis, temperature treatments included 15, 20, 25°C, and VL. For the other populations, analyses only included the 15, 20°C, and VL treatments. As a proxy for development rate, the proportion of nauplii that developed into copepodites between weeks one and two was calculated. During weekly counts, 100% water changes were conducted for each single clutch culture, and copepods were fed 0.4 mg of Spirulina and equal quantities of the live microalgae blend.

2.5 Statistical analyses

For the laboratory experiment, all end-point responses were analyzed with factorial ANOVA, with temperature, salinity, and population as fixed factors. When there were significant interaction terms, follow-up ANOVA tests were conducted to assess the effects of temperature

and salinity treatment combinations within each population. For some response metrics, the most extreme temperature treatments (10, 25ºC and VH) were excluded from analyses because very few individuals within those temperatures were monitored, due to delayed or disrupted development. Survivorship of F_2 clutches (proportions) was analyzed using a separate repeated-measures ANOVA for each population, testing for the effects of temperature and salinity over three weeks of exposure. For field data, mixed models with repeated measures were used to test for differences among sites in pool salinities and daily temperature maximums, minimums, means, and ranges over time. Satterthwaite's method was used to estimate denominator degrees of freedom (Bates et al., 2015).

Statistical analyses were conducted in R v.3.6.0 (R Core Team 2019) and repeated-measures ANOVA tests were conducted with the 'ez' package (Lawrence, 2011). For all factorial ANOVA tests, normality of residuals was examined using quantile-quantile plots and the assumption of homogeneity of variances was examined with Levene's test. When necessary, data were transformed to meet assumptions (Quinn and Keough, 2002). In some cases, data were unbalanced due to the loss of a culture or delayed development and Type III sums of squares were used, whereas Type I sums of squares were used for balanced data sets. *Post-hoc* comparisons were made with Tukey's HSD test. For repeated-measures ANOVA, Mauchly's test was used to evaluate whether the assumption of sphericity was met. If this assumption was not met, the Greenhouse-Geisser p-value correction was used. All summary tables and associated p-values can be found in the Supplementary Materials.

3. RESULTS

3.1 Field monitoring: Salinity and temperature trends among pools at each site

Salinity was measured in 6-7 pools per site, approximately every two weeks from June to October. Pools at FH had the lowest average salinity (37 ppt) and lowest variability among pools (standard deviation: 11.1 ppt) and time points (Table 1). The other sites, CP and DM, had pools that were higher on the shore that were more strongly influenced by evaporation, and salinity was higher on average (43.7 ± 20.9 and 44.9 ± 20.9 ppt, respectively; Fig. 2, Table S3).

Temperature was recorded every 30 minutes in 5-6 pools per site from May to October, with some recording gaps between three logger deployments (pool details and summary statistics in Table 2). Daily maximum temperatures ranged widely, from approximately 15 to 34°C in the early summer and from as low as 11°C to just above 20°C in the early fall. Pools at DM displayed slightly higher maximum temperatures, but only by about one degree on average (DM: 24.2°C, CP: 22.8°C, FH: 23.1°C). The highest temperatures were recorded in pools at DM on most days, less frequently at FH, and rarely at CP (Fig. 3a, Table S2).

The lowest daily minimum temperatures were observed at DM (mean: 11.3°C), and the highest daily minimums were often observed at CP (mean: 12.5°C). Daily minimum temperatures rose to just under 20°C in July and the lowest temperature recorded was 6°C in October (Fig. 3b). Daily mean temperatures were slightly higher at DM versus other sites on average (DM: 16.9°C, CP: 16.7°C, FH: 16.3°C), but trends among sites changed over time. During the first logger deployment that spanned the late spring and early summer, daily mean temperatures showed high overlap among the sites, but by the fall, there was a clear trend of lower daily mean temperatures at FH relative to the other sites (Fig. 3c, Table S1, S2). The

largest daily temperature ranges occurred within pools at DM (mean range: 12.9°C), and the smallest ranges occurred within pools at CP (mean range: 10.33°C; Fig. 3d; Table 2, S2).

3.2 Laboratory experiment: Survivorship in the stable 30°C temperature treatment

During the first water change of the laboratory experiment, after two weeks of F_1 development, a large decline in cultures in the stable 30°C treatment was observed. This treatment, combined with both salinities, was ended for all populations and all surviving individuals were counted. There were no significant differences among populations ($F_{2,12} = 0.37$, $p = 0.701$), however, there were more survivors in the 55 ppt salinity treatment ($F_{1,12} = 33.8$, $p < 0.001$; Fig. S1, Table S5). This temperature treatment is omitted from all results following this section.

3.3 Laboratory experiment: F_1 Development & Fecundity

The first gravid F_1 females developed in the jar cultures at four weeks after the start of the experiment. The fastest development occurred in the lower salinity treatment (32 ppt) and in the higher temperature treatments (20, 25°C, VL). For the FH and DM populations, development was delayed in 10°C. When this temperature was combined with 32 ppt, development of gravid females was delayed by approximately one week. In combination with 55 ppt, development was delayed by two weeks or longer. Delays of about one week also occurred in 15°C in combination with 55 ppt, but not 32 ppt (Fig. 4).

For the CP population, developmental delays within the 55 ppt salinity treatment were even more pronounced. When combined with 10°C, development was so slow that F_1 copepodites did not develop into gravid females before the end of the experiment, thus development was delayed by at least four weeks. The higher salinity also led to delays of about a week within the 20°C and VL temperature treatments (Fig. 4). In the highest temperatures (25°C

and VH), females with egg sacs appeared in cultures early in the experiment, however, many of those egg sacs never hatched into nauplii and were assumed to be inviable.

Across populations, fecundity (the number of nauplii hatched from the first clutch) was highest in the lowest stable temperatures (10 and 15°C) and lowest in the 25°C and VH treatments. There were differences among populations in the magnitude of temperature treatment effects and their interactions with salinity treatments. For the CP population, no F_1 females developed in the 10°C and 55 ppt treatment combination and 10°C (combined with both salinities) was excluded from analyses. However, the highest fecundity was observed in the 10°C and 32 ppt treatment combination (mean = 50.4 nauplii female^{-1}). There was a significant temperature by salinity interaction ($F_{4,20} = 3.51$, $p = 0.025$), in which fecundity was similar across all temperature treatments above 10°C within 32 ppt, however, fecundity of copepods in the VH treatment was slightly lower on average. Within the 55 ppt treatment, fecundity was highest in 15°C, but not statistically different from that of the 20°C and VL treatments, and lowest in the 25°C and VH treatments (Fig. 6a, Table S6).

Only temperature significantly affected fecundity for the DM population ($F_{5,21} = 16.08$, $p < 0.001$). Fecundity was highest in 10°C (average = 39.1 nauplii per female), similar among copepods in the 15, 20°C, and VL treatments (~ 50% as fecund as copepods reared in 10°C), and lowest in the 25°C and VH treatments (Fig. 6b, Table S6). Like CP, a significant temperature by salinity interaction was observed for the FH population ($F_{5,22} = 4.30$, $p = 0.007$). Copepods exposed to 32 ppt combined with 20, 25°C, and VL treatments had similar fecundities, which were lower than fecundities observed in 10 and 15°C, but higher than fecundities in VH on average. For the 55 ppt treatment, fecundity was similar among cultures in the 15, 20°C, and VL

treatments (between 20 and 30 nauplii clutch^{-1}) and fecundity was reduced to less than 10 nauplii per clutch within the 25°C and VH treatments (Fig. 6c, Table S6).

3.4 Laboratory experiment: F_2 Survivorship

F_2 survivorship was tracked across three weeks in a subset of clutches that were initiated early enough in the experiment. Treatment combinations in which the majority of clutches had delayed development were omitted from analyses due to missing data (10°C and VH, as well as 25°C for the FH and DM populations). For the CP population, over 75% of individuals survived to week four of the experiment in most replicate cultures for the 15, 20°C, and VL treatments, in both salinities. Survivorship was lower in the highest temperatures (25°C and VH; 50 - 60% of individuals survived to week four in most cultures). Survivorship in 25°C was slightly higher on average in 55 ppt than in 32 ppt (Figs. 5a, S2a).

For the DM population, the percentage of individuals that survived to week four in 55 ppt was similar among the 15, 20°C, and VL treatments (near 90% on average). In 32 ppt, survivorship within 20°C and VL was equal (~ 85% survivorship at week four), whereas survivorship over time in 15°C was lower on average (76%) and variation among replicate cultures was high (Figs. 5b, S2b).

For the FH population, survivorship was highest on average in 20°C for both salinities, although there were no statistically significant differences among the tested treatments (15, 20°C, and VL; Table S7). In 55 ppt, survivorship was lowest on average within 15°C (average of 77% by week four), but there was high variation among replicates (Figs. 5c, S2c).

3.5 Laboratory experiment: F_2 Development

The proportions of F_2 nauplii that developed into copepodites between weeks one and two were smallest in the lowest temperatures (10 and 15°C), and this effect was stronger in

combination with the higher salinity (55 ppt). Across populations and salinities, similar proportions developed into copepodites in the 20°C and VL temperature treatments (all treatment combination means: > 0.6). When 15°C was combined with 32 ppt, proportions were similar to those observed in the 20°C and VL treatments, although there was high variation among replicates. When 15°C was combined with 55 ppt, very few or no nauplii had developed into copepodites by week two. Development was delayed or highly variable among the few clutches that were tracked in the 25°C and VH treatments (Fig. 6d-f, Table S8).

3.6 Laboratory experiment: Population dynamics in mass cultures after eight weeks

After eight weeks, all individuals in the mass jar cultures were preserved and counted to assess total culture sizes, which included a mixture of F_1 and F_2 copepods. For the CP population, there was a significant effect of temperature on total culture size ($F_{5,24} = 52.72$, $p < 0.001$), in which the largest cultures occurred in the 10 and 15ºC treatments in both salinities. Cultures in 20ºC were approximately half as large (average culture sizes across both salinities for 10ºC = 714; 15ºC = 811; 20ºC = 358 individuals), and cultures exposed to the VL treatment were smaller than those in 20ºC on average (207 individuals), but this difference was not significant. Total culture sizes were smallest for both the 25ºC and VH treatments, in which all replicate cultures had fewer than 200 individuals at the end of the experiment (Fig. 7a).

For the FH population, trends among temperature treatments were slightly different than those observed for CP. Cultures were largest in 15ºC (average = 661 individuals), and cultures in 10 and 20ºC were about half as large as those in 15ºC on average (Fig. 7c). Overall, FH culture sizes were smaller than those of the CP population, across all treatment combinations.

For DM, there was a significant temperature and salinity interaction ($F_{5,24} = 4.09$, $p = 0.008$), in which the largest cultures were observed in 15ºC in both salinity treatments (average

for 32 ppt = 695; 55 ppt = 754 individuals), as well as in 10ºC combined with 32 ppt (average = 711 individuals). Cultures in 20ºC across both salinities and in 10ºC combined with 55 ppt were about half as large. As was observed for the other populations, the smallest cultures were those that were exposed to the 25ºC and VH treatments (Fig. 7b, Table S9).

4. DISCUSSION

Local adaptation to temperature and salinity has been well-studied across *Tigriopus californicus* populations spanning the west coast of North America, but less is known about the potential for the evolution of physiological plasticity at smaller spatial scales. The goal of this study was to test for local adaptation among three geographically close populations with distinct life history characteristics, from microhabitats with potentially different magnitudes of abiotic variability. In the laboratory, a common garden, factorial experiment was conducted to characterize responses to locally-relevant temperature and salinity regimes. In the field, environmental conditions within pools were monitored at each site to assess the potential for divergent selective pressures.

4.1 F_1 fecundity and F_2 survivorship were highest in 10 and 15°C

Fecundity, which was defined as the size of a female's first clutch, was highest in F_1 copepods that were reared in 10°C. The first clutch size is a good predictor of lifetime reproductive success and is likely a suitable fitness proxy in *T. californicus* (Powers et al., 2020). Additionally, development was most delayed in 10°C, and this effect was even stronger in combination with the higher salinity. Such a strong effect of the lowest stable temperature treatment aligns with previous studies that have found trade-offs between rate of maturation and fecundity (Dybdahl, 1995; Edmands and Harrison, 2003; Hong and Shurin, 2015; Park, 2019; Willett, 2010). It took F_1 females in 10°C at least one additional week, relative to females in

higher temperatures, to reach maturity (production of the first egg sac). Female development was delayed in 15°C only in combination with the 55 ppt salinity treatment, and their fecundity was higher than that of faster developing copepods reared in 15°C combined with 32 ppt. This extended development possibly allowed for the allocation of more energy and resources into offspring production versus growth. At these low temperatures, it is also possible that fewer resources were dedicated to costly maintenance and repair mechanisms, such as the production of heat shock proteins, which might have been necessary in the higher temperatures (Sokolova, 2013; Somero, 2002).

Due to this delayed development, few clutches in 10°C could be tracked across a full four weeks, but overall survivorship was high (over 90%), at least over 1-2 weeks of F_2 development. Although initial clutch sizes in 15°C were smaller, similarly high percentages of individuals survived by the end of the experiment, although there was high variability among replicates for the DM and FH populations. The overall mean of all field temperature data collected from late spring through early fall, the warmest part of the year, was just under 17°C, which may explain why survivorship was highest within the lowest temperature treatments. However, it was surprising that survivorship was lower in the 20°C and VL temperature treatments, which were expected to be relatively benign based on previous research. Although *T. californicus* populations from throughout their range have been studied, the majority of research has been conducted on Californian populations, which have higher tolerances for warmer temperatures (Kelly et al., 2012; Leong et al., 2017; Willett, 2010).

Around the San Juan Islands, open waters of the Salish Sea range from approximately 6 - 12°C throughout the year (Murray et al., 2015). Inundation of *Tigriopus* pools by high tides in the summer can cause large temperature fluctuations, as seawater temperatures are much colder

than air temperatures, which largely drive dynamics in small, isolated splashpools (Leong et al., 2017). During the winter however, seawater and air temperatures are more similar, as winters are relatively mild in the San Juan Islands and air temperatures rarely go below freezing. The stable 10°C treatment in this experiment likely approximates conditions in pools from late fall through early spring.

Tigriopus californicus does not have any resting stages (Edmands and Deimler, 2004) and these copepods are active year-round, although populations decline in the winter due to higher storm and wave action (Clark, 1968; Powlik, 1999). It is possible that delayed development caused by low temperatures throughout the winter leads to high survival of copepods (if they are not swept out to sea), as well as the production of relatively large egg sacs. There may be very low hatch rates and egg sac turnover during the winter, but once pools warm and storm intensity wanes in the spring, these larger clutches could develop and hatch quickly. These dynamics could underlie the large spring population blooms that have been observed for *Tigriopus* and other tidepool organisms in seasonal habitats (Clark, 1968; Powlik, 1996; Vittor, 1971).

4.2 Exposure to temperatures above 25°C led to culture decline

Mass jar cultures in the constant 30°C treatment rapidly declined, until only a few survivors were left after two weeks of exposure. The constant 25°C treatment was also stressful for all populations, but cultures did persist for the full eight weeks of the laboratory experiment. However, only dozens of copepods survived in 25°C, whereas cultures in lower temperatures had hundreds. Other studies on different populations of *T. californicus* have found similar effects of exposure to 25°C (Edmands and Deimler, 2004). Willett (2010) and Willett and Son (2018)

found that exposure to 37°C for one hour killed nearly all copepods from more northern populations (British Columbia, Friday Harbor, and northern California).

Short-term exposures to 30°C are probably much less stressful, and frequently experienced by San Juan Island populations in the field. Cultures that were exposed to the variable, high amplitude (VH) treatment, which was held at 30 and 10°C for 12 hours each everyday, had similar population dynamics to cultures in 25°C. It seems that such extended daily exposure to 30°C was stressful, however, the temperature drop to 10°C allowed for some recovery, as cultures in the VH treatment lasted at least six weeks longer than those in constant 30°C. In the cooler half of the day, copepod metabolism might have slowed and allowed for the repair of any damage caused by the warmer half. Repair processes are energetically costly, but food was provided *ad libitum*, which may explain how cultures persisted across two generations. However, eventual culture declines might have occurred with more generations. High maintenance costs lead to reduced deposition of energy reserves, which could translate into the production of fewer offspring of lower quality (Sokolova, 2013).

4.3 Population dynamics were equivalent between the 20°C and VL treatments

Across populations and salinity treatments, fecundity, development, and survivorship were similar among cultures reared in both the stable 20°C and variable, low amplitude (VL; 15 - 25°C at 12 hrs each, daily) temperature treatments. It seems that it is the average temperature experience over time, rather than the amplitude of daily temperature variability, that matters most for *T. californicus* population dynamics.

This result corroborates findings by Hong and Shurin (2015), who also tested for *T. californicus* population responses to the amplitude of daily thermal fluctuations. Three northern populations (from British Columbia and Oregon) and three southern populations (from mid-to

southern California) were exposed to four combinations of shade cloth and water depth treatments. They achieved different daily temperature ranges, the lowest of which was 4.7°C and the highest of which was 11.9°C. Mean temperatures varied among all four treatment combinations, but all were above the 20°C mean of variable treatments in the present study. It was expected that northern populations from more variable, seasonal habitats would have a broader thermal response curve. However, thermal fitness breadths were similar among all populations, and lowest fitness overall occurred in treatments with the highest mean temperatures. The San Juan Island populations studied here appear to have similar tolerance to daily temperature ranges of at least 10°C. Even though sites had slightly different temperature regimes (discussed below), these differences are much smaller in magnitude than those that occur at the scale of seasonal clines, which also do not seem to lead to the evolution of different thermal fitness breadths among *T. californicus* populations (Hong and Shurin 2015).

Although the mean of the VH treatment was also 20°C, culture growth and development were significantly reduced relative to the stable 20°C and VL treatments. Based on population dynamics that were observed in the stable 10°C and 30°C treatments (the minimum and maximum temperatures of the VH treatment), this reduced culture growth was likely not due to the higher amplitude of daily variability. Rather, it seems that any extended exposure to 30°C is highly stressful for these populations. Daily temperature ranges of 20°C or more occurred regularly throughout the summer in pools at these sites, and they did not appear to lead to declines in copepod abundance. It is possible that cultures would have been more robust in a temperature regime with a 20°C daily range, but lower maximum temperature, or with a shorter exposure (<12 hours) to high maximums.

4.4 Temperature and salinity interactions

Development was delayed in the lowest temperatures and this effect was even stronger in combination with hypersalinity. For cultures reared in 10°C and 55 ppt, the development of F_1 copepods was so delayed that in some replicates, females did not reach reproductive maturity by the end of the experiment (eight weeks since hatching). This combination of factors might rarely or never happen in *T. californicus* pools in the field, as cold temperatures typically co-occur with lower salinities during the winter. These populations may have never experienced selective pressures that would have led to the evolution of physiological mechanisms to acclimate to this combination of stressors. Low temperatures may decrease membrane permeability, as well as slow the production and activity of enzymes, which could interrupt mechanisms for regulating internal ion concentrations (Sokolova, 2013). This could constrain the optimization of physiological traits for tolerating both low temperature and high salinity simultaneously (Hochachka and Somero 1984).

There was also a significant interaction between salinity and the highest temperature treatment, 30°C, across populations. Cultures reared in 30°C and 32 ppt had nearly 100% mortality by two weeks of exposure, whereas cultures reared in 30°C and 55 ppt had dozens of survivors. The cultures in the hypersaline treatment were clearly in decline. But the reduced mortality rate could be advantageous in the field, as abiotic conditions are highly variable and there could be potential for recovery once temperatures drop. In the field, hypersalinity and high temperatures co-occur commonly, particularly in the summer as pools isolated from high tides evaporate. A similar result was found for Californian populations by Kelly and colleagues (2016), who argued that the positive effects of high salinity on heat tolerance may be due to a functional overlap in the two stress responses. They found that in hyperosmotic treatments, gene

expression responses were dominated by transcripts involved in protein stabilization, including heat shock proteins, which are critical components of responses to heat stress (Schoville et al., 2012).

4.5 Differences among populations persisted after common garden rearing

While the effects of temperature and salinity treatment combinations were similar among populations, the magnitude of these effects and their interactions were different, even after multiple generations in a common laboratory environment. For example, the hypersalinity treatment caused larger developmental delays for the CP population at a wider range of temperatures than the other populations. *Tigriopus californicus* responds to hypersalinity by accumulating intracellular organic osmolytes, including free amino acids, which are costly to synthesize and regulate (Burton and Feldman, 1982; Goolish and Burton, 1989). If this response causes energy limitation, populations could differ in how they allocate resources to maintenance and repair mechanisms, growth, and reproduction (Sokolova, 2013). It is possible that copepods from the CP population allocate fewer resources to growth and development during times of salinity stress than copepods from the other populations.

Across treatment combinations, the FH population tended to have smaller culture sizes and faster development than the other two populations. In stressful treatments, cultures of the FH population were less robust. Previous studies on *T. californicus* have found that populations with a faster pace of living, or earlier age at maturity, pay a cost of reduced fecundity and survivorship (Dybdahl 1995; Willett 2010; Hong and Shurin 2015; Park 2019). The evolution of such a life history strategy might be expected in environments where the growing season is short, or the risk of mortality is high (Stearns, 1989). At the FH site, it seems that there is less of a chance of exposure to extreme salinities or temperatures because pools are distributed lower on

the shore (Table S10). However, this lower distribution also means that there is a high chance of copepods being washed out of pools into areas of high predation risk. During this study, this flushing of copepods was observed multiple times throughout the summer, and it likely occurs more frequently during increased storm activity in winter. These events lead to relatively high mortality of juveniles, and lower mortality of adults that are able to cling to the substrate (Park, 2019), which likely selects for faster development across generations.

4.6 Limited evidence for local adaptation of San Juan Island populations

Differences in local geology among sites on San Juan Island influence the distribution of *T. californicus* pools on the shore, which seems to affect the variability of abiotic conditions within pools in subtle ways. Pools containing *T. californicus* at FH occurred within a relatively narrow, lower zonation on the shore and had the lowest average salinity and lowest variability among pools and time points out of the sites. At multiple time points, the salinity across all measured pools was near 32 ppt. At the other sites, pools containing *T. californicus* spanned higher parts of the shore. These pools were more isolated from seawater input, which led to more extreme variability in salinity. When comparing responses to hypersalinity within the laboratory, the CP population did tend to have higher fecundity than the FH population in 55 ppt. However, this was not true across all treatment combinations, as fecundity was equivalent between the populations in some temperatures. The DM and FH populations had equivalent fecundity patterns across temperatures within the 55 ppt treatment. Thus, there was no clear indication that the populations were locally adapted to different salinity regimes.

From spring through fall of 2018, pools at the DM site had the largest daily temperature ranges, as well as a slightly higher mean temperature overall. Lowest daily mean temperatures occurred at the FH site, particularly towards the end of the field season in October. In the

laboratory, both variable temperature treatments affected all three populations in similar ways across response metrics. However, there was evidence for higher performance under high temperature stress in the CP and DM populations compared to the FH population. Since the lowest daily mean temperatures were recorded at the FH site, this result could be consistent with local adaptation. Previous studies that directly tested for local adaptation among *T. californicus* populations at relatively small spatial scales show mixed findings. A study that tested for the effects of high temperature and hyposalinity on fitness surrogates among three populations distributed from southern California to Oregon did not find any evidence for local adaptation (Edmands and Deimler, 2004). However, a more recent study on 14 populations spanning 20° of latitude found evidence of adaptive differentiation to temperature and salinity between populations separated by only 5.6 km (Leong et al., 2017).

While limited evidence for local adaptation was found in this study, it is possible that local adaptation has occurred at these sites in ways that were not characterized here. Expectations for laboratory patterns that would be consistent with local adaptation were based on temperature and salinity data collected over limited temporal and spatial scales. The data collected here may not fully represent the selective pressures to which the copepod populations have been exposed historically. While temperature and salinity measurements were taken directly from multiple pools per site, data collection was only across six months. Winter dynamics at these sites are unknown and changes that may occur across years were uncharacterized. Temperature data were taken at a relatively high temporal resolution, but salinity data were taken only every two weeks. This could have led to missing large changes in pool salinity, which can occur very rapidly in the field via precipitation or seawater input.

While pools were carefully chosen to represent the full range of conditions that might occur at each site (pools of different sizes, heights on the shore, and exposure), there were still many pools that were uncharacterized. Since *T. californicus* can move freely among adjacent pools within a rock outcrop, copepods within different pools can be considered subpopulations of a metapopulation. Abiotic conditions within all pools on an outcrop may affect the evolution of the entire metapopulation (Altermatt et al., 2012; Burton and Swisher, 1984).

Additionally, not all sites were composed of one discrete rock outcrop. The DM site probably is discrete, since it contains two close rock outcrops surrounded by pebble beaches on both sides. However, the FH and CP sites are part of larger, more continuous rock benches, each with a beach on only one side and more rock, potentially connected to other *T. californicus* pools, on the other side. Migrants from uncharacterized pools further down the shore, potentially affected by different selective pressures, could have influenced the genetic composition of the pools studied here.

Most environmental differences among sites were driven by a few extreme pools that were higher on the shore and isolated from high tide input. These pools did not contain copepods at all surveys, as extended exposure to extremely low or high salinities likely caused complete die offs. It is not clear whether this high abiotic variability both within and among pools constrains adaptation. On one hand, we might expect that copepod populations with a high risk of exposure to extreme abiotic conditions would evolve higher capacity to acclimate to such stressors. However, if all individuals that are exposed to these extremes end up dying, adaptation cannot occur. It is possible that the extreme, higher pools found at the CP and DM sites are just population sinks, where conditions become too extreme too often. These sinks might not have much of an influence on the evolution of the entire metapopulation. The more stable, consistenly

occupied pools that are sources of colonizers might be the biggest drivers of evolutionary dynamics. To truly understand the stressors faced by *T. californicus* populations, it will be critical to understand how individuals move among pools within sites (Altermatt et al., 2012; Dybdahl, 1995, 1994).

While this study did not find strong evidence for local adaptation among Washington *T. californicus* populations separated by 30 km or less, multiple differences among populations in life history responses to temperature and salinity were observed. These differences persisted after multiple generations in a common environment, which suggests genetic underpinnings (Kawecki and Ebert, 2004). These population differences may be largely due to isolation and genetic drift, rather than divergent selective pressures among sites. These results build upon decades of research that has shown that *T. californicus* populations can be highly differentiated, even at small spatial scales (Burton et al. 1979; Burton 1986, 1987, 1997; Burton and Lee 1994; Edmands 2001; Edmands & Harrison 2003; Willett and Ladner 2009; Barreto et al. 2018).

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TABLES

Table 1. Field salinity summary statistics per pool. The 'Number of measurements' column shows the number of times salinity was recorded for each pool throughout the 2018 field season. Pools had fewer measurements if they were completely dry or filled with sediment or debris during some of the surveys. The 'Percent occupancy' column indicates the percent of the total number of surveys during which *Tigriopus californicus* were observed in the pool.

Pool Identity	Mean salinity (ppt)	Standard deviation (ppt)	Number of measurements	Percent occupancy
CP1	64	36.8	9	55.6
CP2	34.6	3.04	11	100
CP3	46	18.0	11	100
CP4	55.8	22.6	11	100
CP5	36.5	5.99	11	100
CP6	36.1	6.25	11	100
DM1	38.7	10.3	9	100
DM2	33.5	2.66	11	81.8
DM3	42.6	16.7	11	100
DM4	40.8	16.7	11	100
DM5	11.5	2.12	2	0
DM6	38.7	8.46	11	100
DM7	75.8	27.2	10	60
FH1	32.9	2.43	11	72.7
FH2	35.5	4.80	11	100
FH3	33.5	3.39	11	90.9
FH4	40.7	11.2	11	100
FH5	45.3	20.9	11	100
FH6	34.4	8.18	11	100

Table 2. Field temperature summary statistics per pool. Pools displayed here are a subset of the pools that were surveyed for salinity over time. Due to the loss of some temperature loggers, not all pools per site were measured throughout the entire 2018 field season. For the daily maximum, minimum, and range columns, values are means ± standard deviation. The months column indicates the portion of the field season over which temperature measurements were taken.

Pool Identity	Overall mean (°C)	Daily maximum (°C)	Daily minimum (°C)	Daily range (°C)	Overall maximum (°C)	Overall minimum (°C)	Largest daily range (°C)	Number of days measured	Months
CP2	15.5 ± 4.6	22.4 ± 4.0	11.2 ± 1.6	11.3 ± 3.4	29.0	7.0	17.5	122	May - Oct.
CP3	16.8 ± 3.8	21.2 ± 4.1	13.5 ± 2.2	7.7 ± 3.2	29.0	8.5	17.5	122	May - Oct.
CP4	17.8 ± 4.3	23.2 ± 4.3	13.8 ± 2.2	9.5 ± 4.1	31.5	9.0	18.5	106	May - Oct.
CP5	16.8 ± 4.6	23.4 ± 4.1	12.1 ± 1.7	11.4 ± 3.9	31.0	8.0	19.0	122	May - Oct.
CP6	17.5 ± 5.4	26.0 ± 3.3	11.4 ± 1.1	14.6 ± 3.4	31.0	10.0	19.0	41	May - July
DM1	18.6 ± 5.9	27.2 ± 3.9	11.0 ± 1.3	16.1 ± 3.8	32.5	9.5	22.0	41	May - July
DM2	15.1 ± 5.8	22.5 ± 6.1	10.0 ± 1.2	12.5 ± 5.5	34.0	6.5	23.5	82	May - July & Sept. - Oct.
DM3	16.7 ± 5.7	24.7 ± 5.0	10.8 ± 1.7	14.0 ± 4.8	33.0	6.0	23.0	122	May - Oct.
DM4	17.3 ± 5.2	24.5 ± 5.0	12.0 ± 2.1	12.5 ± 4.6	33.0	7.0	22.0	122	May - Oct.
DM6	17.4 ± 4.8	23.5 ± 4.5	12.2 ± 2.0	11.4 ± 4.8	31.5	8.5	21.0	122	May - Oct.
FH1	15.8 ± 5.3	23.4 ± 5.7	11.0 ± 1.5	12.4 ± 5.2	33.0	7.0	22.0	116	May - Oct.
FH2	16.4 ± 4.3	21.8 ± 4.5	12.3 ± 2.0	9.5 ± 3.8	29.5	7.5	18.0	122	May - Oct.
FH3	16.0 ± 5.1	23.5 ± 4.9	11.2 ± 1.8	12.3 ± 4.3	31.5	7.0	21.0	122	May - Oct.
FH4	16.6 ± 4.6	22.9 ± 4.5	12.5 ± 2.3	10.4 ± 3.7	31.0	7.5	19.5	122	May - Oct.
FH5	17.3 ± 4.7	24.7 ± 3.5	11.6 ± 1.8	13.1 ± 3.3	31.0	9.5	20.5	41	May - July
FH6	16.4 ± 3.7	23.4 ± 3.4	11.9 ± 1.5	11.5 ± 3.5	28.0	10.0	16.0	41	May - July

FIGURES

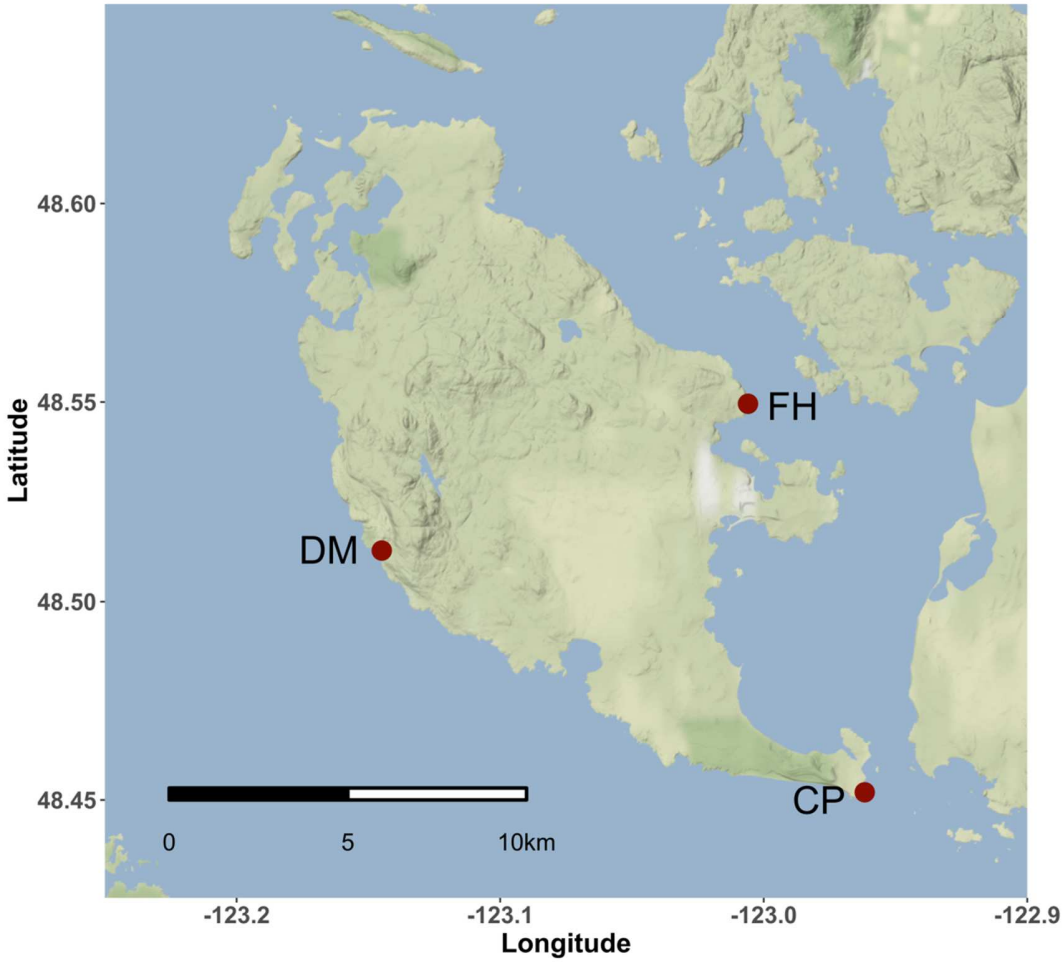


Figure 1. Map of *Tigriopus californicus* populations used for this study on San Juan Island, Washington, USA. CP: Cattle Point, FH: Friday Harbor, DM: Dead Man's Cove. Map made using 'ggmap' (Kahle & Wickham 2013).

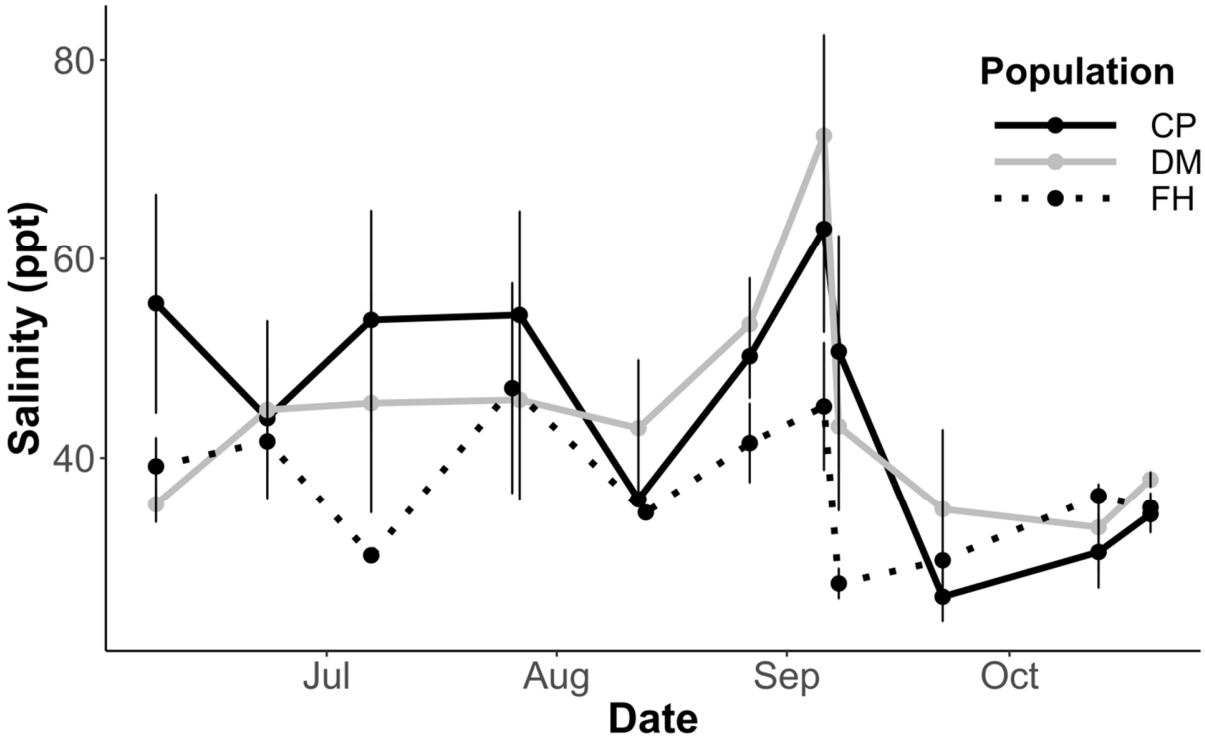
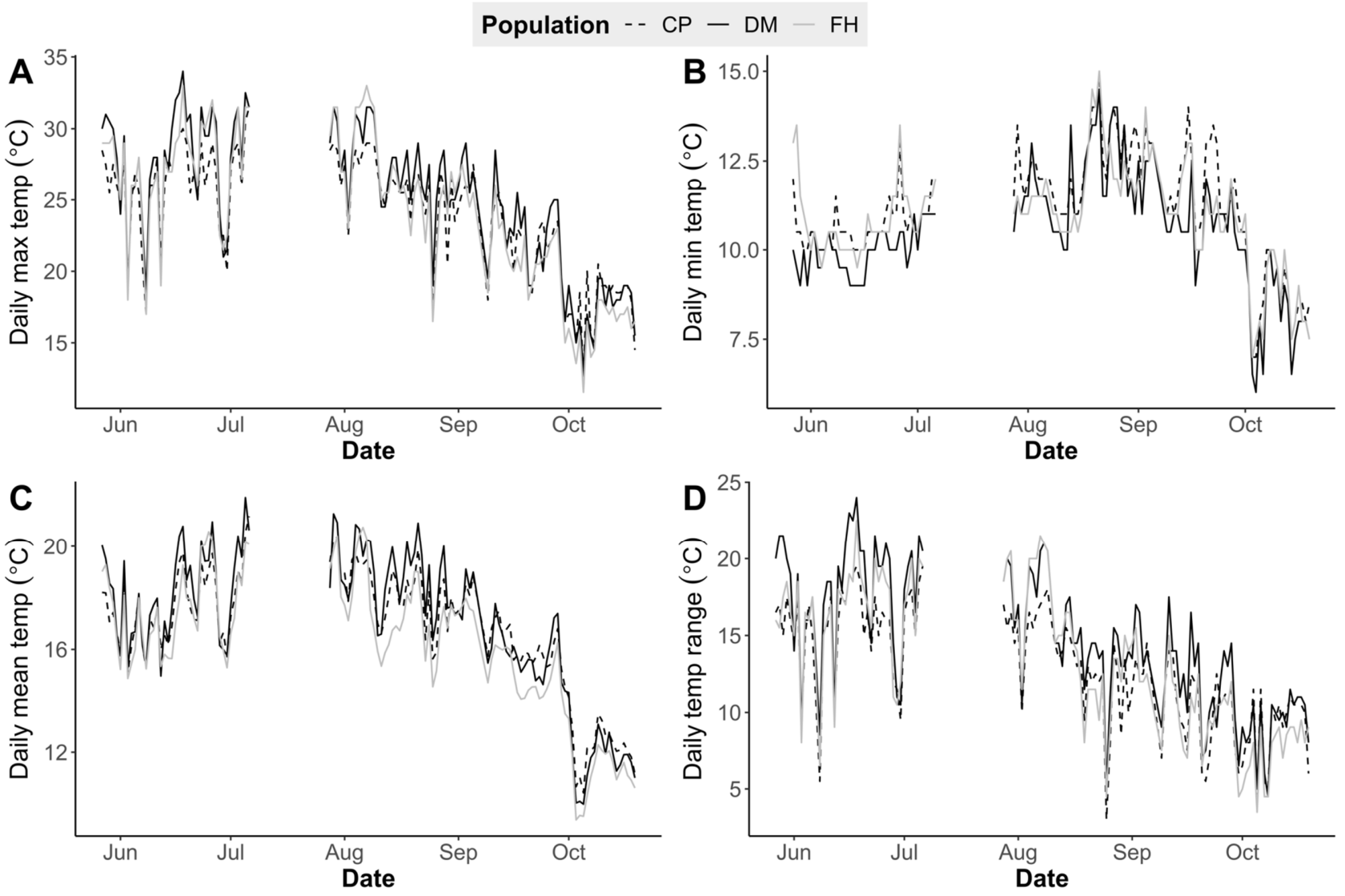


Figure 2. Field salinity measurements, taken approximately every two weeks, from June to October of 2018. Each point represents the average of salinity measurements from 6-7 pools per site (error bars: ±1 SE) at each survey. Different populations (CP = Cattle Point, DM = Dead Man's Cove, FH = Friday Harbor) are represented by different line types.


Population -- CP — DM — FH
A
Daily max temp (°C)
35
30
25
20
15
Jun
Jul
Aug
Sep
Oct
Date
B
Daily min temp (°C)
15.0
12.5
10.0
7.5
Jun
Jul
Aug
Sep
Oct
Date
C
Daily mean temp (°C)
20
16
12
Jun
Jul
Aug
Sep
Oct
Date
D
Daily temp range (°C)
25
20
15
10
5
Jun
Jul
Aug
Sep
Oct
Date

1

Figure 3. Field temperature data summary over time. Daily maximum (A), minimum (B), and mean (C) temperatures (°C) and daily temperature ranges (D) were calculated for each field site from late May through late October of 2018 (note an approximately two week gap in measurements in July). Numbers of pools measured (3-6 per day) varied throughout the field season due to the loss of some loggers. Different sites (CP = Cattle Point, DM = Dead Man's Cove, FH = Friday Harbor) are represented by different line types.

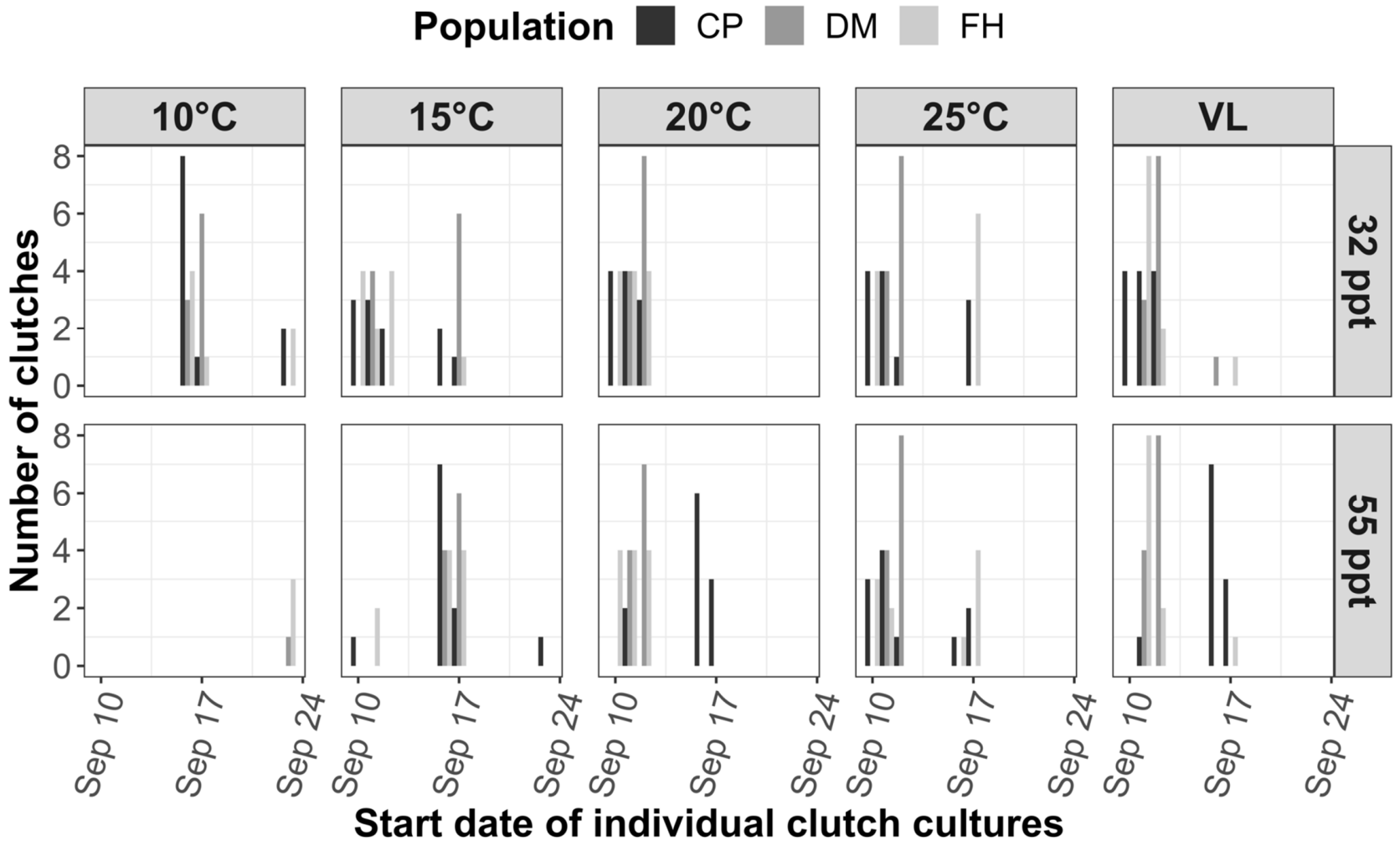


Figure 4. F_1 generation development histogram. The x-axis displays the date on which single clutch (Falcon tube) cultures were initiated with gravid F_1 females, which was a proxy for timing of sexual maturity. Mass jar cultures were monitored for the appearance

of gravid females, and these start dates generally represent when gravid females first started to develop. Counts of cultures started per day are displayed on the y-axis. Salinity treatments are shown in the facet rows and temperature treatments are shown in the facet columns. VL represents the “variable low” temperature treatment, which varied between 15 and 25°C daily. All other temperature treatments were held stable. The VH (“variable high”) temperature treatment was omitted due to the prevalence of inviable egg sacs.

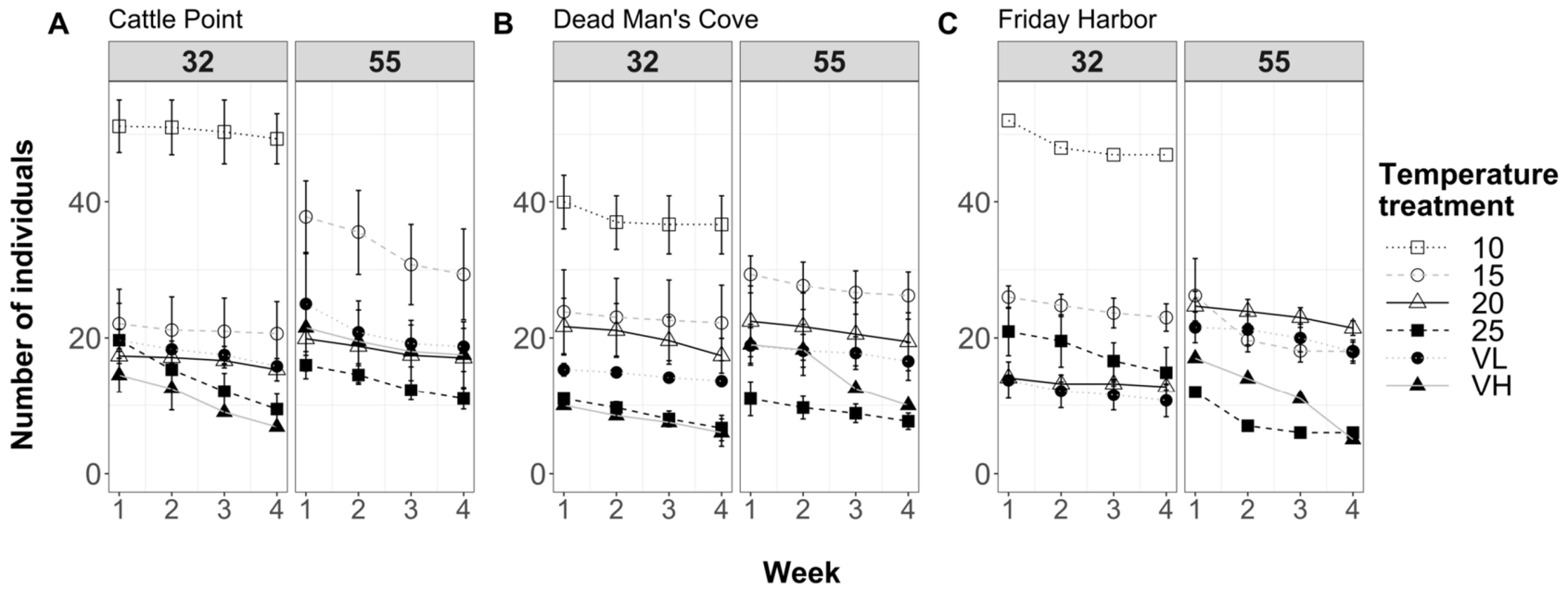
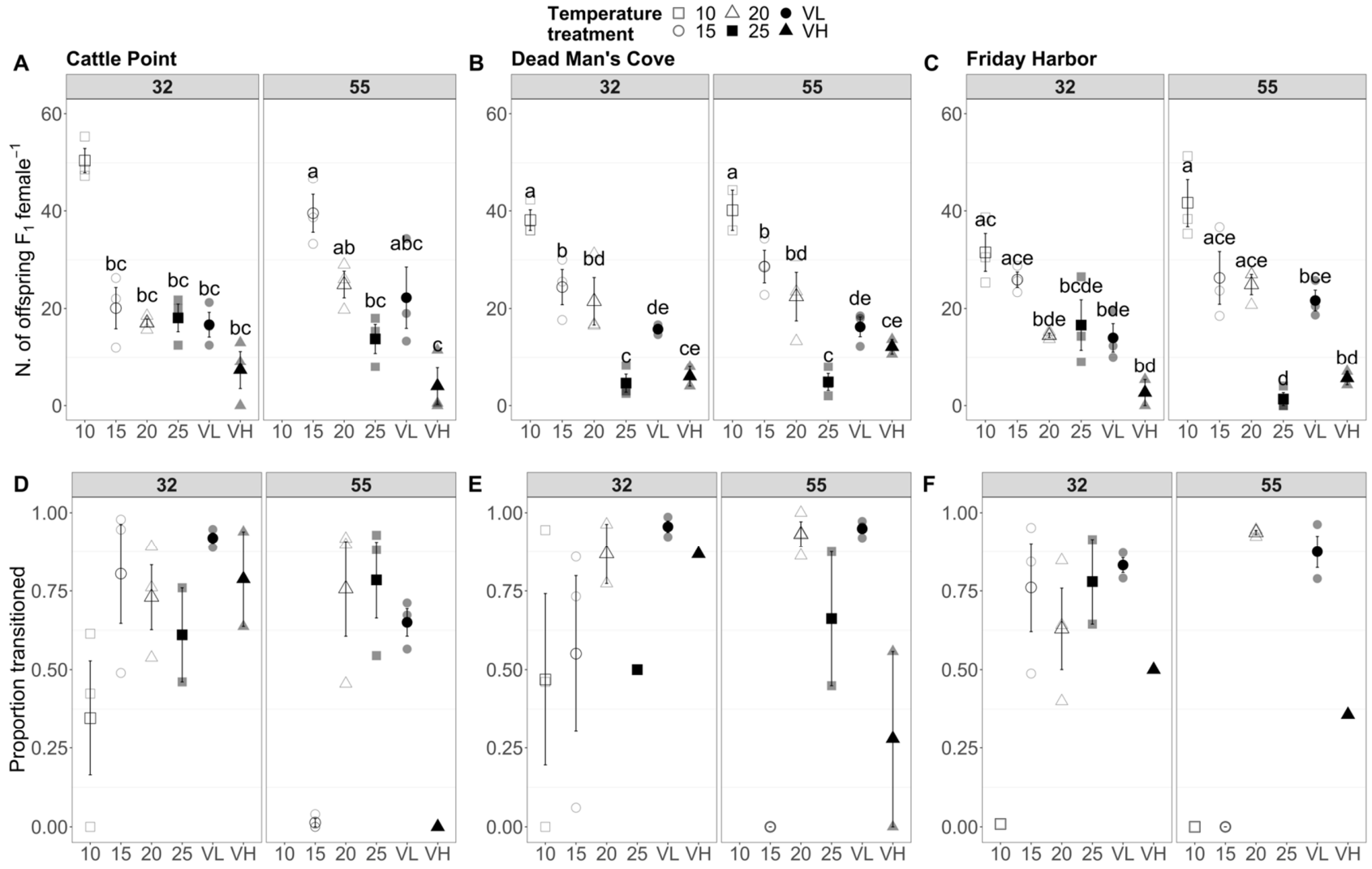


Figure 5. Single clutch culture sizes over time for the F_2 generation of the Cattle Point (A), Dead Man's Cove (B), and Friday Harbor (C) populations. This represents a subset of the entire single clutch culture dataset: it only includes data from clutches that were tracked across four weeks. Multiple clutches from each replicate jar culture were tracked over time and counts were averaged for each of three replicate cultures (with some exceptions, due to delayed development, as described below). Facets contain single clutch culture sizes over time for each temperature treatment within each salinity treatment (32 and 55 ppt). Each point represents the average of replicate jar cultures at each weekly count (error bars: ±1 SE). Different temperature treatments are represented by different point symbols and line types. Temperature treatments from 10 - 25 (°C) were held constant, and the VL and VH treatments varied around an average of 20°C daily (VL: 15-25°C, VH: 10-30°C). There are no data for the 10°C and 55 ppt treatment combination (for

all populations) due to delayed development of the F_1 generation (the VH and 32 ppt combination is also missing for FH). If error bars are missing from the plotted points, data from only one replicate were collected across the full four weeks. Data from only two replicate cultures are displayed in the following treatment combinations: 10°C and 32 ppt & VH and 32 ppt (CP & DM), 15°C and 32 ppt & VH and 55 ppt (DM only).


Temperature treatment
10
15
20
25
VL
VH
A
Cattle Point
B
Dead Man's Cove
C
Friday Harbor
32
55
N. of offspring F1 female−1
Proportion transitioned
D
E
F

1

Figure 6. F_1 fecundity and the proportion of F_2 nauplii that transitioned into copepodites in single clutch cultures between weeks one and two for the Cattle Point (A, D), Dead Man's Cove (B, E), and Friday Harbor (C, F) populations. Multiple single clutch cultures were averaged for each replicate. Facets contain counts/transition proportions for each temperature treatment within each salinity treatment (32 and 55 ppt). Temperature treatments from 10 - 25 (°C) were held constant, and the VL and VH treatments varied around an average of 20°C daily (VL: 15-25°C, VH: 10-30°C). Each bold point represents the average of replicate cultures (error bars: ±1 SE). Lighter points represent raw data (three replicate cultures per salinity and temperature combination, with some exceptions). Some proportions for treatment combinations are missing due to very low total culture sizes and/or disrupted development. For fecundity, significant differences among treatment combinations are indicated by different letters above the points (note that each population was analyzed separately).

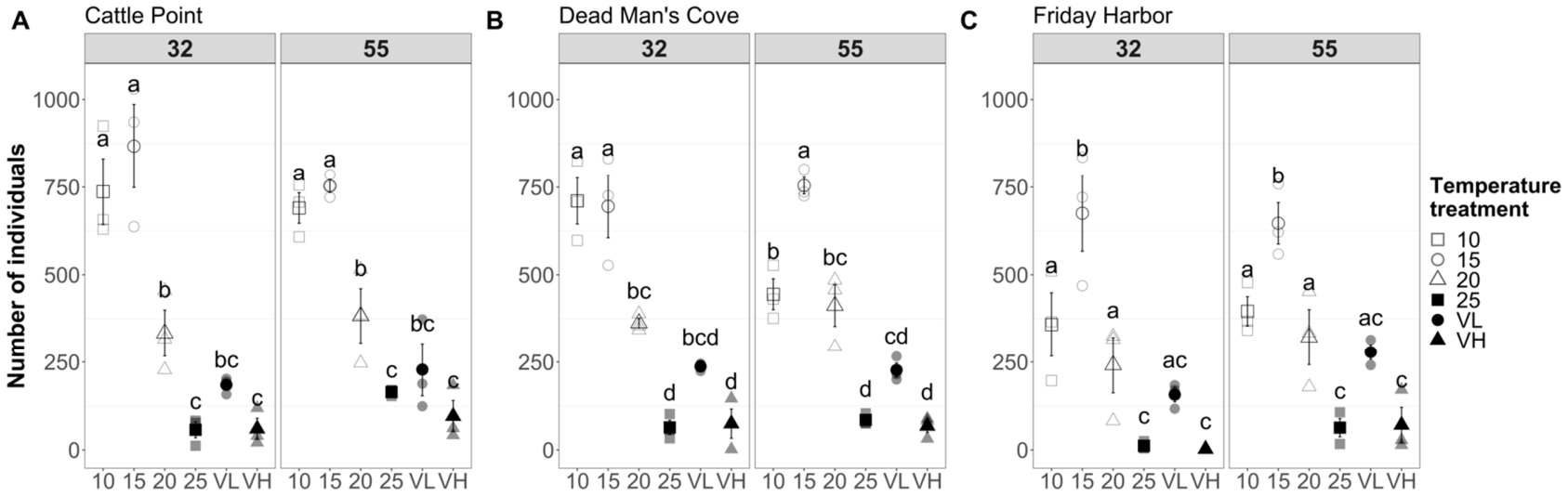


Figure 7. Total jar culture sizes, including the F_1 and F_2 generations, for the Cattle Point (A), Dead Man's Cove (B), and Friday Harbor (C) populations after two months of exposure to temperature and salinity treatment combinations. Facets contain counts for each temperature treatment within each salinity treatment (32 and 55 ppt). Temperature treatments from 10 - 25 (°C) were held constant, and the VL and VH treatments varied around an average of 20°C daily (VL: 15-25°C, VH: 10-30°C). Each bold point represents the average of replicate jar culture counts (error bars: ±1 SE). Lighter points represent raw data (three replicate cultures per salinity and temperature combination). Significant differences among treatment combinations are indicated by different letters above the points (note that each population was analyzed separately).