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Seasonally Driven Sexual and Asexual Reproduction in Temperate *Tethya* Species

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Abstract. Marine organisms that rely on environmental cues for reproduction are likely to experience shifts in reproductive phenology and output due to global climate change. To assess the role that the environment may play in the reproductive timing for temperate sponges, this study examined sexual and asexual reproduction in New Zealand sponge species (*Tethya bergquistae* and the *Tethya burtoni* complex) and correlated reproductive output with temperature, chlorophyll-*a* concentration, and rainfall. Histological analyses of sponges collected monthly (from February 2015 to February 2017) revealed that these sponges are oviparous and gonochoristic and that they sexually reproduce annually during the austral summer. Both monthly collections and *in situ* monitoring revealed that *Tethya* spp. asexually bud continuously, but with greater intensity in the austral spring and summer. Temperature was positively associated with both sexual reproduction and budding, with seasonal cues appearing important. Future shifts in the environment that alter such cues are expected to affect population dynamics of these sponges.

Introduction

Environmental factors play a critical role in regulating both sexual and asexual reproduction for many marine invertebrates (Yamahira, 2004; Naylor, 2013). Temperature changes have been shown to trigger gametogenesis and to induce asexual events in widespread taxa (Sastry, 1966; Beauchamp, 1992; Olive *et al.*, 1998; Ettinger-Epstein *et al.*, 2007; Cardone *et al.*, 2010; Epherra *et al.*, 2015), and lunar cycles have been linked to spawning activities for many organisms (Fromont and Bergquist, 1994; Tanner, 1996; Bentley *et al.*, 2001; Mercier *et al.*, 2011). Nutrient availability has also been positively correlated with gametic and larval density (Thompson, 1983; Snell, 1986; Bronstein and Loya, 2015), as sexual reproduction requires an organism to have energy available for gamete production and, particularly, yolk investment to oocytes (vitellogenesis). Nutrient availability and food availability also appear to be important in asexual reproduction. While high levels of food have been positively correlated with asexual budding events (Tökölyi *et al.*, 2016), asexual reproduction can also be employed by organisms during periods of low food availability, since the energy investment in asexual reproduction is typically lower than that required for sexual reproduction (*e.g.*, Sebens, 1979). Associations between sexual and asexual reproduction with other environmental parameters have been recorded for many marine organisms, including salinity, habitat availability, light, disturbance, rainfall, and water motion (Lubzens *et al.*, 1985; Snell, 1986; Richmond and Hunter, 1990; O'Dea and Okamura, 1999; Baums *et al.*, 2006; Roberts *et al.*, 2006; Zilberberg *et al.*, 2006; Purcell, 2007; Paixão *et al.*, 2013; Abdul Wahab *et al.*, 2014b; Lanna *et al.*, 2018). Reliance on such environmental cues for both modes of reproduction generally results in either continuous reproduction throughout the year or, more often, reproduction occurring in discrete periods.

Sponges are found in both marine and freshwater ecosystems in tropical, temperate, and polar oceans; and they perform many important ecological roles, including water filtration, nutrient cycling, and habitat creation (for a review of functional roles, see Bell, 2008; Maldonado *et al.*, 2012). Despite lacking specialized reproductive organs (*i.e.*, gonads), sponges have evolved a diverse array of reproductive strategies (reviews in Maldonado and Bergquist, 2002; Maldonado and Riesgo, 2008; Ereskovsky, 2014). Sponges can sexually reproduce by either gonochorism or hermaphroditism, fertilization can be internal or external, and development can be external (*i.e.*, unfertilized

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Abbreviations: AIC, Akaike Information Criterion; AR, autoregressive; DOM, dissolved organic matter; ENSO, El Niño-Southern Oscillation; GEE, generalized estimating equation; GLM, generalized linear model; LR, likelihood ratio; ROI, reproductive output index; SST, sea surface temperature.

or fertilized eggs shed for external development, termed oviparity) or internal (*i.e.*, fertilized oocytes internally brooded through development to produce either a larva or a juvenile, *i.e.*, viviparity with either direct or indirect development). It is generally accepted that the main role of sexual reproduction is to generate genetic diversity (Barbuti *et al.*, 2012; Uecker and Hermisson, 2016); thus, for sponges and many other sessile invertebrates, the mode of sexual reproduction can play an important role in determining population structure and connectivity patterns (Chen and McDonald, 1996). The generation of offspring by sexual reproduction in sponges can occur continuously throughout the year or as one or more discrete events over the annual cycle (Fell, 1974, 1976a; Fell *et al.*, 1978; Hoppe,1988; Riesgo and Maldonado, 2008; Lanna *et al.*, 2015; Chaves-Fonnegra *et al.*, 2016; Nozawa *et al.*, 2016). A meta-analysis performed by Lanna *et al.* (2018) revealed that seasonality was most common in temperate sponges, whereas continuous reproduction occurred at a higher frequency in tropical regions. Numerous studies have highlighted the effects of environmental factors on sponge sexual reproduction, where sexual reproductive effort has been highly correlated with nutrient availability, salinity, rainfall, oxygen, and sediment (Witte, 1996; Roberts *et al.*, 2006; Whalan *et al.*, 2007b; Gaino *et al.*, 2010; Abdul Wahab *et al.*, 2014b). Light, lunar, and tidal cycles can also play a role in triggering sponge spawning events (Amano, 1986, 1988; Hoppe and Reichert, 1987; Usher *et al.*, 2004; Nozawa *et al.*, 2016). The effect of temperature on sponge reproduction is among the most studied environmental drivers, with both decreases and increases in temperature being shown to coincide with gametogenesis, biased sex ratios, and increased sexual reproductive output (Reiswig, 1973; Fell, 1976b; Maldonado and Young, 1996; Usher *et al.*, 2004; Ettinger-Epstein *et al.*, 2007; Riesgo *et al.*, 2007; Whalan *et al.*, 2007a; Riesgo and Maldonado, 2008; Ereskovsky *et al.*, 2013; Abdul Wahab *et al.*, 2014b, 2017; Lanna *et al.*, 2015, 2018). Despite the large number of studies, common patterns of sexual reproduction in relation to environmental parameters for sponges have not emerged, highlighting species-specific reproductive adaptations.

Sponges also reproduce asexually, either by forming special structures (buds and gemmules) or by intentionally or accidentally producing body fragments that operate as dispersing propagules (Simpson and Gilbert, 1973; Johnson, 1978; Wulff, 1991; Corriero *et al.*, 1998; Teixidó *et al.*, 2006; Cardone *et al.*, 2010). Asexual reproduction produces genetically identical clones; but instances where asexual and sexual reproduction can cooperate to maximize dispersal, while reducing inbreeding in the foundation of new sponge populations, have also been described (Maldonado and Uriz, 1999). While asexual reproduction is common in sponges, information on environmental drivers triggering bud formation in marine sponges is scarce. Both seasonal change (Johnson, 1978; Corriero *et al.*, 1996, 1998) and experimentally increased temperature (Cardone *et al.*, 2010) have been shown to induce budding events. Body size could also have an effect on bud formation in the demosponge *Tethya citrina*, with larger individuals being more prone to be engaged in budding (López-Acosta *et al.*, 2016). Other relationships between environmental drivers and asexual reproduction are more contradictory. For instance, while continuous instances of budding have been associated with heterogeneous environmental conditions for some sponges (*e.g.*, Cardone *et al.*, 2010), it has also been found that disturbance associated with iceberg scouring did not correlate with instances of budding in Antarctic hexactinellid sponges (Teixidó *et al.*, 2006); instead, for other sponges, clonality has been found to be higher in more stable environments (Zilberberg *et al.*, 2006). Such conflicting results suggest that asexual reproduction plays different roles in population dynamics for different sponge species. Understanding the timing of budding and potential environmental drivers is important when considering population viability under future environmental conditions, because sessile marine invertebrates that rely on asexual reproduction (like some sponges) and that cannot actively migrate to favorable environments have already been identified as having high risks of population decline (Przeslawski *et al.*, 2008).

Over the next century and beyond, shifts in climate are predicted to result both in changes to the timing of seasons and productivity and in increases in water temperature and ocean acidification (IPCC, 2014). Responses to climate change have already been documented for many species (Walther *et al.*, 2002), including sponges (*e.g.*, Maldonado *et al.*, 2010; Fillinger *et al.*, 2013; Dayton *et al.*, 2016). Rapid environmental changes are projected to continue to alter reproductive patterns for many other organisms (Visser *et al.*, 2004; Przeslawski *et al.*, 2008). For instance, changes in sea surface temperature over the past decades have been correlated with both earlier and delayed spawning for fish in Southern California (Asch, 2015) and to shifts in the timing of gamete release for several coral species in the Red Sea (Shlesinger and Loya, 2019). Furthermore, increases in temperature have also been shown to affect recruitment for many marine invertebrates in Lough Hyne, Ireland, where there has been a positive association between warmer water temperatures and larval density and settlement (Minchin, 1992). Temperature-related bleaching episodes in the Great Barrier Reef have resulted in mass mortality of the adult brood stock, followed by an 89% decrease in larval recruitment (Hughes *et al.*, 2019). Experimental manipulation of temperature for both copepods and urchins has revealed that some organisms have a specific thermal range for normal life-history processes (*i.e.*, growth, development, reproduction) and that temperatures exceeding normal ranges may have detrimental effects on such processes (Lee *et al.*, 2003; Byrne *et al.*, 2009). Examining how cyclic patterns, such as the El Niño-Southern Oscillation (ENSO), influence seasonally reproducing marine invertebrates can further highlight how rapid changes in the surrounding environment can have marked population-level consequences (Glynn, 1988). Changes in currents and salinity associated with ENSO events can often change spawning

grounds and larval transport pathways, leading to shifts in population abundance and structure (Hsiung *et al.*, 2018). For example, upwelling events and warmer temperatures associated with ENSO events have resulted in increased larval transport from deep-sea to inshore areas for both flounder and halibut (Bailey and Picquelle, 2002). Rapid changes in the environment projected with future climate change therefore have the potential to alter reproductive timing, duration, frequency, and output for organisms; and such changes to reproduction are likely to have consequences for population dynamics if organisms cannot adapt quickly enough (Lawrence and Soame, 2004; Kroeker *et al.*, 2010; Byrne, 2011).

In this study, we investigated the reproductive ecology of sympatric temperate species of "golf ball" sponges, *Tethya bergquistae* and *Tethya burtoni*, which are known to reproduce both sexually and asexually by means of buds. Despite *Tethya* being a common genus in the New Zealand marine environment (Sarà and Sarà, 2004; Berman, 2012), there are no detailed studies examining the reproduction of these species to date. These two sponge species offer an opportunity to address how asexual and sexual reproduction might be influenced by future climate change in New Zealand. To this purpose, we (1) describe the sexual and asexual reproductive biology of both *T. bergquistae* and *T. burtoni* at two locations, identifying any species- or site-specific differences, and (2) assess the potential environmental correlates that may be important for both sexual and asexual reproduction. Our study elucidates the roles of both modes of reproduction for these sponges and will enable an initial assessment of how reproductive function may respond to increased sea surface temperature as a result of climate change.

Materials and Methods

Sample collection

From February 2015 to February 2017, 10 whole specimens of each species were collected monthly, using scuba from 2 separate locations on the Wellington south coast, New Zealand (Breaker Bay: 41°19′58.6056″S, 174°49′53.1732″E; Princess Bay: 41°20′49.4016″S, 174°47′24.6732″E; Fig. 1), totaling 40 sponges each month. Sponges were collected from 5–10-m depth. *Tethya bergquistae* Hooper in Hooper & Wiedenmayer, 1994, and *Tethya burtoni* Sarà & Sarà, 2004 are morphologically distinguishable from each other in that the former is pink-orange in color, is firm, and has variable surface texture, from smooth to bumpy, whereas the latter is yellow-orange in color, soft, and generally covered in small irregular tubercles (Bergquist and Kelly-Borges, 1991; Hooper and Wiedenmayer, 1994; Sarà and Sarà, 2004). *Tethya burtoni* has been found to likely be composed of a species complex (Shaffer *et al.*, 2019); but cryptic species could not be distinguished from one another based on morphology alone, so all specimens of *T. burtoni* are presented here as one group (hereafter referred to as "the *T. burtoni* complex"). Sponges were immediately fixed in Bouin's fixative for 48 h and then preserved in 70% ethanol. Because *Tethya* species have a spherical morphology, sponge size was estimated using both the volume and the surface area of a sphere after measuring the diameter (in mm) for all sponges, using calipers. In addition, sponges were recorded as budding or not; and for those sponges that were budding, the total number of buds was counted to calculate bud density (number of buds per surface area, after Cardone *et al.*, 2010).

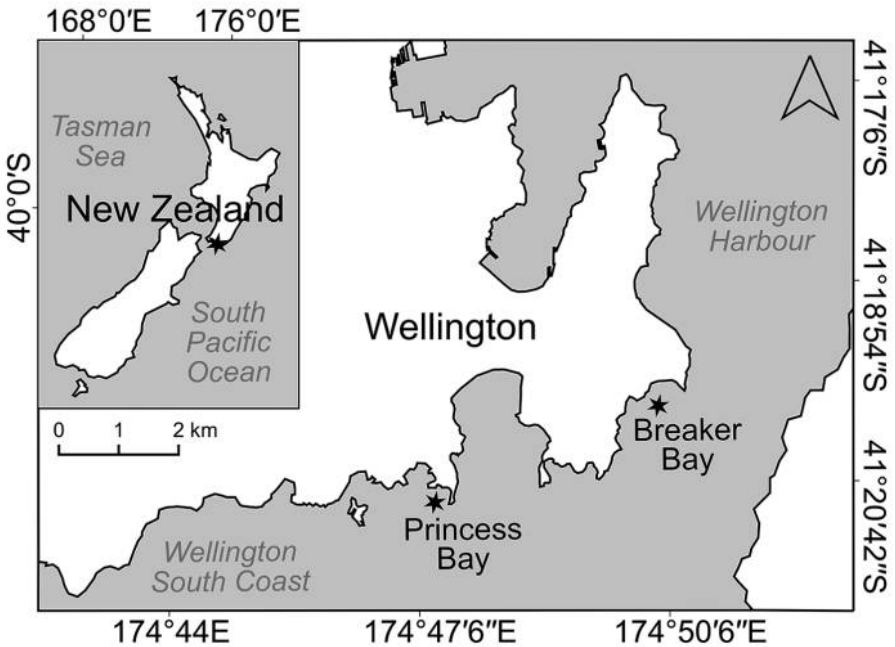

Figure 1. Map of study location, with sample sites Breaker Bay and Princess Bay on the Wellington south coast.

Histological processing and quantification of reproductive propagules

An approximately 5-mm-thick slice of tissue from each sponge was processed for histological analysis, where tissue (containing spicule content) was sampled from choanosome of the sponge, avoiding the cortex. Choanosomal tissue was dehydrated with a series of ethanol solutions, cleared with xylene, and infiltrated with paraffin wax over 16 h, using an automated processor (Leica Biosystems TP1020, Wetzlar, Germany). Tissue was embedded in paraffin wax, using an embedded center (Leica Biosystems EG1160), and was sectioned to 5 μm, using a rotary microtome (Leica Biosystems RM2235). Sections were stained using hematoxylin and eosin staining and were mounted on slides with DPX (BDH Laboratory Supplies, Poole, Dorset, United Kingdom). Sections were examined for the presence of oocytes at 100× magnification and spermatic cysts at 200× magnification, using a compound microscope (Leica Microsystems DM LB) with attached digital camera (Canon EOS 70D). For sponges containing gametes, five separate (*i.e.*, not overlapping) fields of view per section were photographed. The number and size (cross-sectional area of oocytes or spermatic cysts), as well as the total tissue area viewed, were calculated using ImageJ (Schneider *et al.*, 2012). Because spermatic cyst production was captured by our planned sampling strategy in only one sponge (see *Results*, *Discussion*), the reproductive output index (ROI) was calculated for both species with oocytes only, being the proportion of tissue containing oocytes (*i.e.*, the sum of the areas of oocytes divided by the entire tissue area examined, as in Corriero *et al.*, 1998; Whalan *et al.*, 2007a, b). Reproductive output index has been widely used to compare reproduction between different sponges and has been employed in many recent studies (see Whalan *et al.*, 2007a, b; Leong and Pawlik, 2011; Abdul Wahab *et al.*, 2014b, 2017).

In situ *monitoring of asexual reproduction in a sponge population*

In addition to monthly sample collections, populations of *T. bergquistae* and the *T. burtoni* complex at Breaker Bay were monitored over time by means of repeated photographs. For *T. bergquistae*, a population of about 90 sponges was photographed from November 2015 to November 2017. Six areas (about 2 m^2 each) were haphazardly chosen within 250 m^2 of rocky reef, and areas were about 10–15 m apart. Within each of the six areas, random groups of three to six sponges were tagged (using sheep tags epoxied to the reef). From November 2015 to November 2016 sponges were photographed every month, and from November 2016 sponges were photographed six times (January, February, June, August, September, and November 2017). For the *T. burtoni* complex, a population of about 50 sponges at Breaker Bay was monitored from May 2016 to November 2017. Six additional areas were chosen within the same 250 m^2 of rocky reef, and 3 tags were placed per area to correspond to 2–6 sponges. From May 2016 to November 2016, sponges were photographed monthly and, following that, were photographed in January, February, April, June, August, September, and November 2017. For both species, a reduced sampling effort occurred after November 2016 as a result of logistical limitations. All sponges from photographs were recorded as budding or not, and the total number of attached buds for each sponge was counted in ImageJ (Schneider *et al.*, 2012). Total number of buds per sponge was used in lieu of bud density (*i.e.*, number of buds per surface area) because of the fact that sponges changed their volume over time (members of the *Tethya* genus can contract and reduce body size by up to 75%) (*e.g.*, Nickel, 2004), which would affect the calculation of the bud-to-surface area ratio for the same sponge over time. Instances of fusion between sponges were also recorded over the monitoring period. For sponges belonging to the *T. burtoni* complex, microsatellite genotypes were determined using the methods of Shaffer *et al.* (2019) to allow both members of the complex to be distinguished from each other.

Collection of environmental data

Environmental factors that were considered to potentially influence reproduction in *Tethya* were temperature, concentration of chlorophyll-*a* (chl-*a*, used here as a tentative proxy for food availability, because sponges feed on bacterial and dissolved organic matter [DOM]), and rainfall, which have all previously been found to explain variation in sponge reproduction (Roberts *et al.*, 2006; Whalan *et al.*, 2007a,b; Abdul Wahab *et al.*, 2014b). Sea surface temperature (SST, °C) and chl-*a* concentration ($mg \cdot m^{-3}$) were obtained from Satellite Data Services from the National Institute of Water and Atmospheric Research (NIWA) in New Zealand. NIWA Satellite Data Services receives and analyzes satellite transmissions from the National Oceanic and Atmospheric Administration (NOAA) and granules from the Moderate Resolution Imaging Spectroradiometer (MODIS) for SST and chl-*a* concentration, respectively. For each satellite image, data were extracted from a 5 × 5 grid around each site (2.5 km^2), and the median was calculated. Breaker Bay and Princess Bay are about 5 km from each other; therefore, large-scale satellite SST and chl-*a* concentration readings would be expected to be similar for both sites (which was reflected in the data obtained for both sites). Data points from satellite images were scarce for Breaker Bay as a result of the proximity to land; therefore, all values were averaged for the two sites, which generated one dataset to be compared for both locations. Monthly averages of SST and chl-*a* were calculated. Total monthly rainfall (mm) was obtained from New Zealand's National Climate Database (https://cliflo.niwa.co.nz), which offers free real-time and archived data from stations across New Zealand. In addition, two temperature loggers (HOBO Temperature, Onset Corporation, Bourne, MA) were deployed during the study period at Breaker Bay in the

same area as the monitored sponges, and they recorded temperature hourly. Southerly fronts frequent this study location and result in decreases in water temperature as water moves northward from Antarctica. As such, the alternating southerly and northerly winds that shape this study site result in water temperatures that are variable over short periods of time, and loggers were deployed to capture such variation that may have otherwise not been reflected in averaged SST readings. The aim of using temperature logger data in conjunction with *in situ* monitoring was to further elucidate how shorter-scale temperature changes may influence budding, as the production of a bud can happen very rapidly in time, and budding can also occur during relatively short periods of time before ceasing (*i.e.*, days; MRS, pers. obs.).

Data analysis

All of the following analyses were conducted in R version 3.5.0 (R Core Team, 2017). For sponges collected monthly, the effects of environmental drivers on instances of asexual and sexual reproduction were determined using generalized linear models (GLMs) with binomial families and logistic links. Logistic GLMs allow the probability of a binary response (here, for sexually reproducing sponges, 1 = sexually reproductive and 0 = not sexually reproductive; for asexually reproducing sponges, 1 = budding and 0 = not budding) to be estimated from one or more explanatory variables. The models used here tested the log odds of the probability of sexual or asexual reproduction as a function of sponge size (diameter), site, species, SST, chl-*a* concentration, and rainfall. The following interactions were also tested in addition to the main effects: site × species, size × species, SST × chl-*a*, SST × rain, and rain × chl-*a*. The best-fit model was then selected by comparing Akaike Information Criterion (AIC) scores and selecting the model with the lowest score. Significance was assessed using likelihood ratio (LR) chi-square tests. A Box-Tidwell procedure with 1000 iterations was used to check that each model met the assumptions of a logistic regression, which include (1) linearity of independent variables and log odds and (2) no multicollinearity of independent variables (Box and Tidwell, 1962). All variables met the second assumption. Sea surface temperature, however, failed to meet the first assumption for both models, even after both a natural log and square root transformation, so another model was fitted with temperature as a categorical variable to confirm that the effect of temperature (as a continuous variable) was valid. For this, SST was placed into 4 even categorical groups, where 1 = <12 °C (n = 158 sponges); 2 = 12−14 °C (n = 272); 3 = 14−16 °C (n = 278); and 4 = >16 °C (n = 270).

To assess the effects of potential predictors on oocyte density, oocyte size, ROI, and bud density for those sponges collected monthly, linear models were used to test the effects of the following on each response: sponge size (diameter), site, species, SST, chl-*a* concentration, and rainfall, including the same interaction terms as above. For analyses of sexual reproductive response, only those female sponges that contained oocytes were used (*i.e.*, all zero values were removed) because sponges contained gametes for only 6 of the 25 months sampled during the study period, that is, about 3 months per year. Similarly, for analyses of asexual reproductive response, only sponges that had buds were used (*i.e.*, all zero values were removed). All data were checked to meet the assumptions of a linear regression (*i.e.*, normality, homoscedasticity, no multicollinearity or autocorrelation of independent variables; Poole and O'Farrell, 1971); and oocyte density, ROI, and bud density were log transformed to meet these assumptions. The best-fit models were selected using AIC scores, and significance of potential predictors was assessed using Type III ANOVAs (Fox and Weisberg, 2011). No statistical comparisons were made between months because there were too few data points for each month for robust comparisons.

Last, for those sponges where bud formation was monitored *in situ*, generalized estimating equations (GEEs) were used to assess the effect of temperature (from *in situ* loggers) both on instances of budding and on number of buds. Generalized estimating equations were constructed using the package "geepack" (Halenkoh and Højsgaard, 2006) in R version 3.5.0 (R Core Team, 2017). Generalized estimating equations are an extension of GLMs but allow the effects of explanatory variables to be made for longitudinal data (*i.e.*, repeated measures) while allowing data to be non-normally distributed (*i.e.*, binary data, count data) (Zeger and Liang, 1986; Zorn, 2001; Ballinger, 2004; Ghisletta and Spini, 2004). Repeated measures from the same sponge over time were accounted for in the model by using an autoregressive (AR) correlation matrix, which specifies that the response variable depends linearly on its previous value. The AR(1) correlation structure was specifically chosen, which considers only the previous term in the longitudinal dataset. In this instance, presence and number of buds were considered to be correlated with the previous time point because observed buds could potentially be products of the previous observation, particularly in the case where sponges were undergoing a budding event that lasted longer than two observation points (*i.e.*, the periodicity of sampling was more frequent than the speed of budding). The data were checked to meet the assumptions for GEEs, which are as follows: there is linearity between the dependent variable and the predictor variables (which was met, using a logit link for instances of budding and a log link for bud counts); the sample size (sponge) of the associated longitudinal dataset is greater than 10; and the observations between sponges are independent (Ghisletta and Spini, 2004). Temperature was taken from temperature loggers, and the average daily temperature was recorded for the date when the sponge was photographed, which allowed bud density to be compared to daily temperatures instead of monthly means (*vs.* for the sponges collected monthly). Because budding has been observed to occur rapidly in a sponge and over a short period of time (MRS, pers. obs.), this was to determine whether different trends in response to temperature emerged

when using both methods. Because the time frame of monitoring was different for each species, each species was first assessed separately. For *T. bergquistae*, a GEE was fitted to determine the effect of temperature on both instances of budding and number of buds, using a binomial and Poisson family, respectively. For the *T. burtoni* complex, a GEE was fitted to determine the effect of temperature and genotype (determined from a panel of 11 microsatellite loci from Shaffer *et al.*, 2019) on both instances of budding (family = binomial) and number of buds (family = Poisson). To compare all species, only those time points that both species shared were used. Two final GEEs were constructed that examined the effect of temperature and genotype on instances of budding and on number of buds. Significance for all GEEs was assessed using the Wald chi-square test. Those sponges that died or fused over the study period were excluded from these analyses.

Results

Environmental data

Environmental data for the Wellington south coast are summarized in Figure 2a–c. Sea surface temperature followed a seasonal trend, where it peaked in February (late summer) each year at about 17–18 °C and then declined to 11 °C in July–August (mid to late winter) (Fig. 2a). Monthly means of temperature readings from the HOBO logger were similar to SST readings but slightly lower during the austral summer months. Hourly recordings *in situ* revealed that temperature can be variable (*i.e.*, 1–2 °C changes) over shorter periods of time (*i.e.*, days; Fig. 2a). Chlorophyll-*a* concentration averaged 1.25 ± 0.3 mg·m^{-3} and ranged from 0.77 to 1.93 mg·m^{-3} over the study period. There was no apparent seasonal pattern for chl-*a* concentration, but there were peaks evident during spring (Fig. 2b). Maximum total monthly rainfall occurred in November 2016, when about 250 mm fell in the month. Rainfall decreased over the summer period (December–February) (Fig. 2c). No pairs of environmental covariates were correlated with each other, except for the SST and HOBO logger temperature readings.

Sexual reproduction

Over the 2-year study period, 995 sponges were collected, of which 86 contained oocytes and 1 contained spermatic cysts (for summary see Table 1; Fig. 3). The male belonged to the *Tethya burtoni* complex, measuring 28 mm in diameter, and was collected in late February 2017. This male sponge also contained a high number of buds (45 buds). No sponges were found to contain internally brooded larvae or both male and female gametes, which suggests that *Tethya bergquistae* and the *T. burtoni* complex are both oviparous, gonochoristic sexual reproducers. It also means that the gametogenesis of males is extremely rapid, starting and being completed typically in less than a month, which is likely the reason why spermatic cyst formation was not captured in our monthly sampling. According to data on oocyte presence and growth, both species appeared to have only one period of gamete release per year, with oogenesis occurring in the austral summer from January

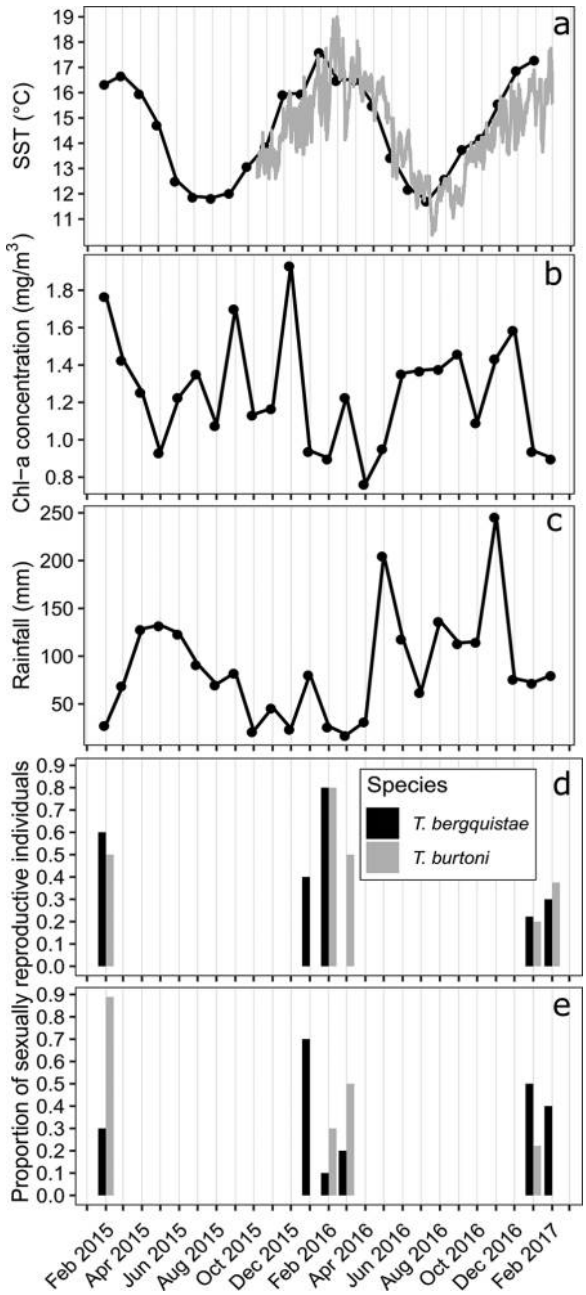

Figure 2. Environmental data with the proportion of sexually reproducing sponges for *Tethya bergquistae* and the *Tethya burtoni* complex from the Wellington south coast from January 2015 to February 2017. (a) Sea surface temperature (SST) given as monthly means. Temperature readings taken every hour from HOBO temperature logger (Onset Corporation, Bourne, MA) deployed at Breaker Bay from October 2015 to February 2017 are overlaid to show short-term variation. (b) Chlorophyll-*a* (chl-*a*) concentrations presented as monthly means. (c) Rainfall given as total monthly volumes of rain. (d, e) Proportion of sexually reproductive sponges for both species at Breaker Bay (d) and Princess Bay (e).

Table 1

Summary information of Tethya *spp. collected from February 2015 to February 2017*

Species	Site	*N*	*N*-O	*N*-SC	*N*-B	Mean sponge size (mm)	Mean oocyte density (counts mm^{-2})	Mean ROI (%)	Mean oocyte size (μm^2)	Mean bud density (counts cm^{-2})
Tethya bergquistae	BB	251	23	0	171	21.3 ± 4.5	20.2 ± 18.4	1.7 ± 1.7	0.82 ± 0.29	0.50 ± 0.72
	PB	250	22	0	117	19.0 ± 4.1	12.7 ± 9.7	1.0 ± 0.8	0.73 ± 0.27	0.50 ± 0.50
	Total	501	45	0	288	20.2 ± 4.4	16.5 ± 15.1	1.3 ± 1.4	0.78 ± 0.28	0.50 ± 0.64
Tethya burtoni complex	BB	244	23	1	93	19.6 ± 5.9	32.6 ± 16.4	2.4 ± 1.5	0.72 ± 0.17	1.02 ± 1.34
	PB	250	18	0	101	17.5 ± 4.5	23.0 ± 18.4	1.8 ± 1.6	0.72 ± 0.24	1.14 ± 1.40
	Total	494	41	1	194	18.5 ± 5.3	28.4 ± 17.8	2.1 ± 1.6	0.72 ± 0.20	1.08 ± 1.37
Overall		995	86	1	482	19.3 ± 5.0	22.2 ± 17.4	1.7 ± 1.5	0.75 ± 0.25	0.73 ± 1.04

Site refers to Breaker Bay (BB) or Princess Bay (PB). Number of sponges collected (*N*), number containing oocytes (*N*-O), number containing spermatic cysts (*N*-SC), and number containing buds (*N*-B) are given for each site, each species, and overall. Mean sponge size refers to the diameter of the sponge (mm). Mean oocyte density (counts per mm^2), mean oocyte size (cross-sectional area of oocyte, μm^2), and mean bud density (counts per cm^2) are averaged across all months. The average reproductive output index (ROI) is given as the percent of tissue sampled with reproductive entities. All values recorded ± standard deviations.

to March (Fig. 2d, e) and gamete release putatively taking place at the end of that period. One full oogenesis process was captured in January–March 2016. During this period, the proportion of *T. bergquistae* containing oocytes decreased progressively. The *T. burtoni* complex was found to produce the first recognizable oocytes in February 2016, which was about a month later than *T. bergquistae*. In total, there were no significant differences in the proportion of sexually reproducing sponges between sites or species. It seems that the oocyte production commenced when the water temperature rose over 15–16 °C. Temperature had a significant effect on the probability of sexually reproducing (LR χ^2 test, $\chi = 93.221$, $P < 0.001$). The logistic GLM predicted that for every 1 °C increase of temperature, the probability that a sponge would sexually reproduce increased about 4 times ($P < 0.001$). Reproduction occurred during periods of lower rainfall, so that total monthly rainfall was negatively associated with instances of reproduction (LR χ^2 test, $\chi = 7.568$, $P = 0.006$). There was no effect of chl-*a* on the proportion of sponges containing gametes; but we cannot discount that chl-*a* is a poor predictor of sponge food availability, as its links to bacterioplankton and DOM are not straightforward (see *Discussion*). Larger sponges (both species) were correlated with higher instances of sexual reproduction (LR χ^2 test, $\chi = 12.416$, $P < 0.001$). The smallest sponge found to contain gametes was about 13 mm in diameter (volume = 1150.35 mm^3).

The oocyte density of females generally increased during the reproductive period for both species (Fig. 4). Oocyte density was significantly different between sites (ANOVA, $F_{1,82} = 8.7105$, $P = 0.004$) and between species (ANOVA, $F_{1,82} = 7.7786$, $P = 0.007$), but there was no significant interaction between the two. Oocyte density was greater at Breaker Bay for all months except two (January 2016 and February 2016) and was greater for the *T. burtoni* complex for all months except two (February 2015 and January 2016). The ROI followed the same trend as oocyte density, being significantly different between sites (ANOVA, $F_{1,82} = 10.593$, $P = 0.002$) and between species (ANOVA, $F_{1,82} = 13.037$, $P = 0.001$). In 2016, oocyte size increased from the beginning (monthly average for both species = 0.59 ± 0.16 μm^2) to the end (monthly average for both species = 1.03 ± 0.23 μm^2) of each gametogenic period for both species and was not significantly different between sites (ANOVA, $F_{1,82} = 0.6179$, $P = 0.434$) or species (ANOVA, $F_{1,82} = 1.1046$, $P = 0.296$). For both species, body size was a significant predictor for the presence of oocytes (LR χ^2 test, $\chi = 12.416$, $P < 0.001$), where the probability of a sponge containing gametes increased with size. Likewise, body size was a significant predictor of oocyte density (ANOVA, $F_{1,82} = 14.3620$, $P = 0.0002$) and ROI (ANOVA, $F_{1,82} = 10.4326$, $P = 0.002$) but not oocyte size.

The body volume of *T. bergquistae* was significantly larger than the *T. burtoni* complex (ANOVA, $F_{1,991} = 30.0784$, $P < 0.001$). Furthermore, sponges at Breaker Bay were significantly larger than those at Princess Bay (ANOVA, $F_{1,991} = 52.4956$, $P < 0.001$). There was no significant interaction between species and sites for body size.

Asexual reproduction

For those sponges collected monthly, about 50% of all sponges were found to be producing buds (Table 1). When examining the main effects on the probability of budding, the following were significant: site, size, species, and temperature. The best-fit model fit the probability of budding as a function of site × species, species × size, and SST × chl-*a*. The interaction between site and species was significant (LR χ^2 test, $\chi = 10.293$, $P = 0.0013$), where at Breaker Bay, *T. bergquistae*

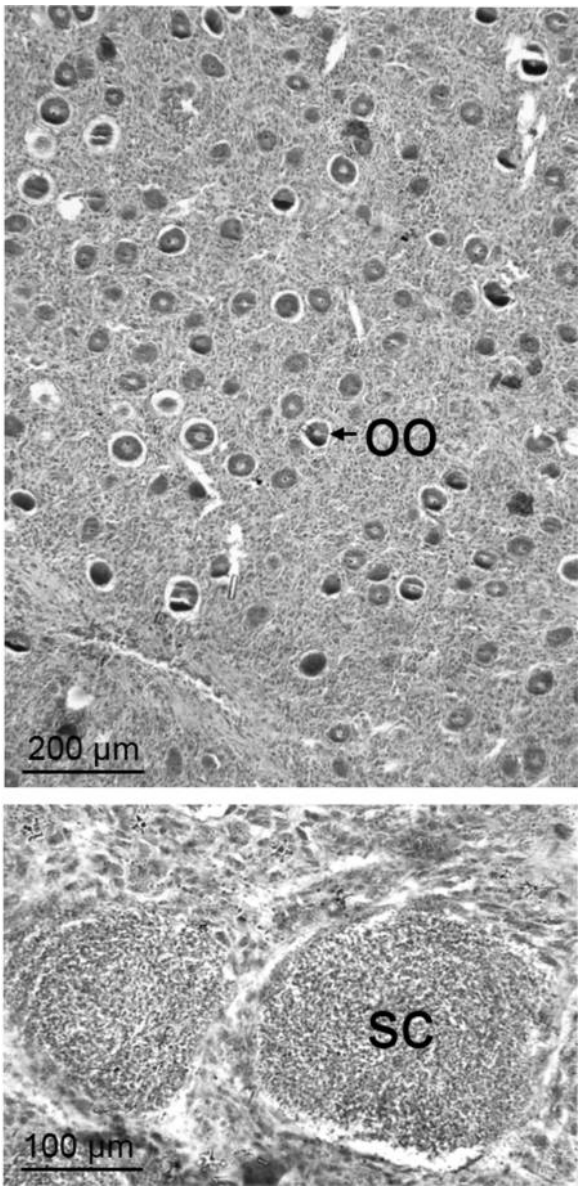

Figure 3. Examples of tissue sections containing gametes from *Tethya* spp. (oo, oocytes; sc, spermatic cysts).

had a higher probability of containing buds compared to sponges in the *T. burtoni* complex; but no difference in the probability of bud production between the two species was detected at Princess Bay. The interaction between species and size was also significant (LR χ^2 test, $\chi = 15.851$, $P < 0.001$), where the probability of budding increased with size for *T. bergquistae* only, and this trend occurred at both sites (Fig. 5). Last, the effect of temperature on the probability of budding increased with increasing chl-*a* concentration (LR χ^2 test, $\chi = 6.541$, $P = 0.011$; Fig. 6).

When examining the influence of only the main effects on bud density for those sponges collected monthly, the following were significant: size, species, SST, and rainfall. The best-fit model described bud density as a function of size × species, SST, and rainfall. The size × species interaction was significant (ANOVA, $F_{1,474} = 4.1933$, $P = 0.041$), where for both species bud density was negatively associated with sponge size, but for the *T. burtoni* complex smaller individuals had a greater bud density than *T. bergquistae*. For both species, rainfall was a significant predictor of bud density (ANOVA, $F_{1,474} = 5.4$, $P = 0.020$; Fig. 7a, b). Temperature was also a significant predictor of bud density (ANOVA, $F_{1,474} = 9.3344$, $P = 0.002$; Fig. 7d, e), where higher bud densities were associated with warmer temperatures. There were no significant relationships between bud density with sexual reproductive indices (ROI, oocyte density, oocyte size).

In total, 129 sponges were monitored *in situ* at Breaker Bay, 88 of which were *T. bergquistae* and 41 of which were from the *T. burtoni* complex. Within the *T. burtoni* complex, 18 belonged to one genetic group, 15 belonged to another genetic group, and 8 could not be genotyped because they died before a tissue sample was taken. Over the monitoring period, mortality for *T. bergquistae* and the *T. burtoni* complex was 18% and 17%, respectively. Fusion events, in which two or more sponges fused together, occurred for both species (Fig. 8). Fusion was more prevalent for *T. bergquistae*, where 16 sponges (18% of the monitored population) fused to another sponge (*i.e.*, 8 separate fusion events). By contrast, only two fusion events (each event between 2 sponges) were evident for the *T. burtoni* complex (10%), where microsatellite data revealed that the sponges that fused together consisted of the same multilocus genotype (*i.e.*, were clones). During our observation period, fusion was detected as occurring only between two individuals, though it is likely that it may also occur between multiple individuals (see Fig. 8). For both species, some individuals continually budded throughout the monitoring period, some had repeated discrete periods of budding, and others did not bud at all. For *T. bergquistae*, temperature (from field loggers) was significantly associated with both instances of budding (LR χ^2 test, $\chi = 35.5$, $P < 0.001$) and number of buds (LR χ^2 test, $\chi = 54.8$, $P < 0.001$). Similarly, for the *T. burtoni* complex, temperature was significantly associated with the probability of budding (LR χ^2 test, $\chi = 7.34$, $P = 0.007$), and there was no significant effect of genotype of members of the *T. burtoni* complex. The number of buds for the *T. burtoni* complex was significantly positively associated with temperature (LR χ^2 test, $\chi = 22.39$, $P < 0.001$), but again it was not associated with genotype. When both species were combined for the analysis, temperature had a significant effect on both instances of budding (LR χ^2 test, $\chi = 49.1$, $P < 0.001$) and number of buds (LR χ^2 test, $\chi = 102.3$, $P < 0.001$), while genotype had no effect.

Discussion

Here, we describe the sexual and asexual reproductive ecology of common *Tethya* spp. in New Zealand and identify

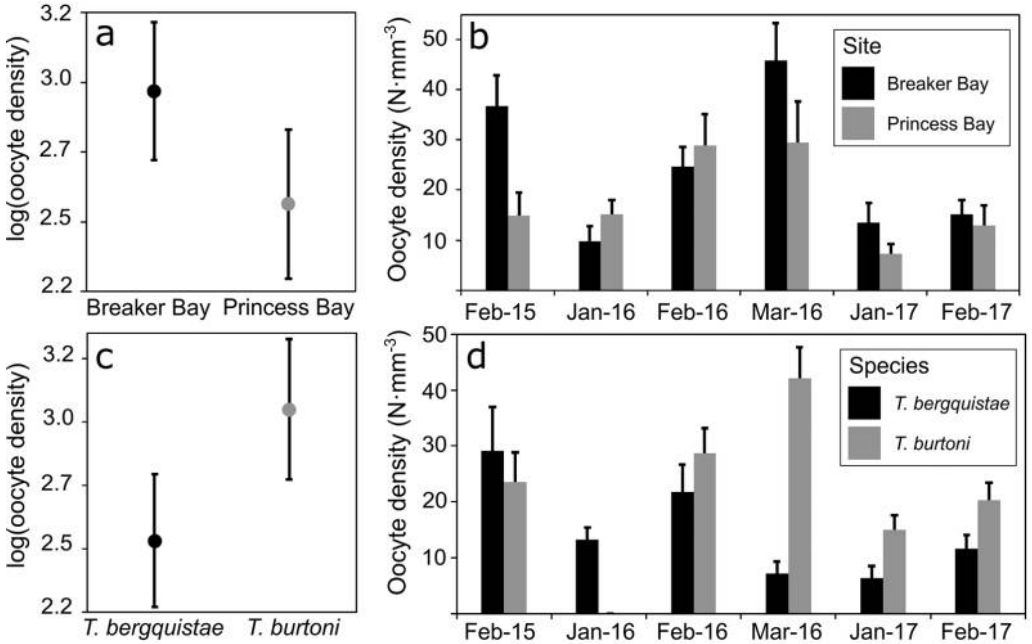

Figure 4. Oocyte density (number of oocytes per mm^3 tissue) for *Tethya bergquistae* and the *Tethya burtoni* complex at Breaker Bay and Princess Bay. (a, c) Means of the log(oocyte density) between site (a) and species (c), with 95% confidence intervals. (b, d) Difference in oocyte density between sites (b) and species (d) presented by month, where error bars on plots correspond to standard error. There was no significant interaction between site and species.

potential environmental drivers of these patterns to provide greater insight into how reproduction might be influenced by climate change. Both *Tethya bergquistae* and the *Tethya burtoni* complex were found to have a single gametogenesis event each year, beginning in January with egg release occurring two to three months after the onset of gametogenesis. This latter conclusion is derived indirectly from the analyses of oocyte abundance in the tissues, because actual gamete release was never observed *in situ*. In contrast to sexual reproduction, asexual reproduction by budding occurred continuously throughout the year, with a noticeable seasonal trend of increased numbers of budding sponges and increased bud densities during periods of warmer waters. Therefore, temperature appears to be an important cue in driving both modes of reproduction for these sponges, in timing and in output. Understanding the potential consequences of a temperature mismatch is critical for determining how population dynamics will change with predicted global warming.

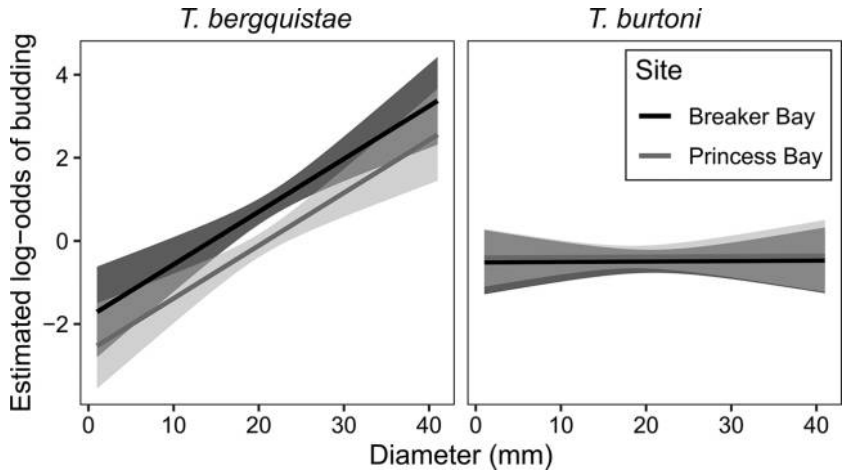

Figure 5. Effect of sponge size (diameter) on the estimated log odds of budding for *Tethya bergquistae* and the *Tethya burtoni* complex at both Breaker Bay and Princess Bay. Shaded regions represent 95% confidence intervals.

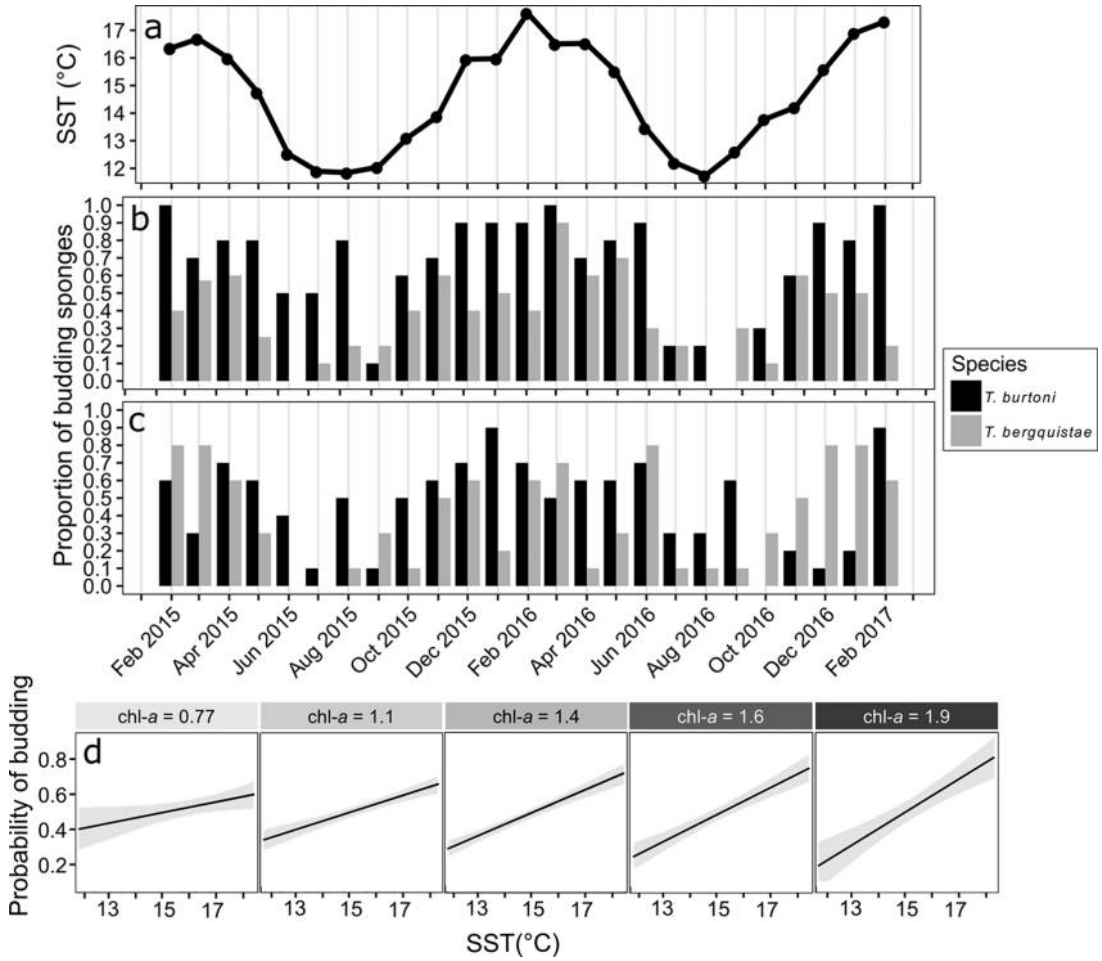

Figure 6. Budding events in relation to sea surface temperature (SST). (a) SST given as monthly means. (b, c) Proportion of budding sponges collected each month for *Tethya bergquistae* and the *Tethya burtoni* complex for Breaker Bay (b) and Princess Bay (c). (d) Effect of SST with increasing concentrations of chlorophyll-*a* (chl-*a*) on the probability of budding for both species pooled. Shaded regions on chl-*a* plots represent 95% confidence intervals.

Characterization of sexual and asexual reproduction for Tethya bergquistae *and the* Tethya burtoni *complex*

To our knowledge, this study is the first to describe the reproduction of *T. bergquistae* and the *T. burtoni* complex. Both "species" are gonochoristic; and while sequential hermaphroditism cannot be ruled out, all other species of *Tethya* to date have been shown to be gonochoristic (Gaino *et al.*, 1987; Gaino and Sarà, 1994; Corriero *et al.*, 1996; Sciscioli *et al.*, 2002; Maldonado and Riesgo, 2008), so this is also likely the case for *T. bergquistae* and the *T. burtoni* complex. The only male found during the 2015–2017 study period belonged to the *T. burtoni* complex and was identified at the end of February. The fact that we failed to find spermatic cysts in our monthly sampling suggests that spermatogenesis was completed more rapidly than in a month. This is further supported by subsequent sampling of the *T. burtoni* complex that was carried out in late February 2018 and early March 2019 for population genetic and histological studies (see Shaffer, 2019). During these collections, 8 sponges contained spermatic cysts, accounting for 29% of the population sampled. The sampling effort we employed here from 2015 to 2017 occurred late in each month. It is likely that spermatogenesis commenced in late February and had already finished by the end of March, which is why we failed to capture it, and that instead spermatogenesis occurs on the scale of days to weeks. Alternatively, but highly unlikely, as gametogenesis in males and females has been long recorded by Lévi (1956) and Corriero *et al.* (1996), these *Tethya* spp. could also be parthenogenetic. Parthenogenesis often results in populations with low genetic diversity and population diversity estimates that deviate largely from Hardy-Weinberg equilibrium (Simon *et al.*, 1996). These trends were not revealed when examining the population genetics of the *T. burtoni* complex (Shaffer, 2019), suggesting that parthenogenesis does not occur in these sponges, thus further supporting the findings of Lévi (1956) and Corriero *et al.* (1996).

Asexual reproduction occurred at the same time that sexual reproduction occurred, where some individuals that contained

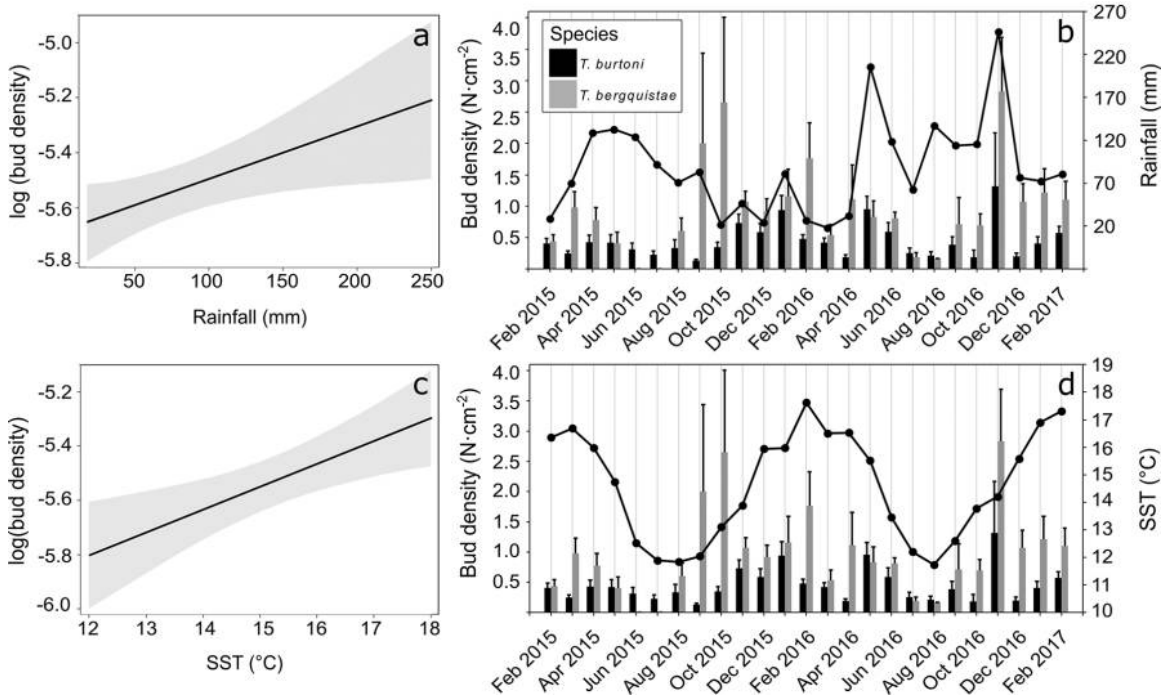

Figure 7. Bud density (number of buds per cm^2) for *Tethya bergquistae* and the *Tethya burtoni* complex at Breaker Bay and Princess Bay. (a, c) Effect of rainfall (a) and sea surface temperature (SST, c) on bud density, with shaded regions representing 95% confidence intervals. (b, d) Monthly means of bud densities for each species in relation to rainfall (b) and SST (d), with error bars representing standard errors. There were no significant differences between Breaker Bay and Princess Bay, so sites are pooled for these plots.

gametes also had external buds. Although asexual and sexual reproduction have been shown to co-occur in some sponges (Maldonado and Uriz, 1999), the simultaneous production of both sexual and asexual propagules in nature is rare, because both modes are generally triggered by different cues and require different energy investments. Budding has been recorded for other species of *Tethya* in the Mediterranean, where distinct, non-overlapping periods for both sexual and asexual reproduction have been recorded (Corriero *et al.*, 1996). By contrast, the two studied New Zealand *Tethya* spp. employ both reproductive modes at the same time. Combosch and Vollmer (2013) found that the coral *Pocillopora damicornis* similarly participates in mixed reproduction, where both sexual and asexual larvae are produced at the same time. These authors suggested that having mixed reproductive strategies has an evolutionary advantage, as it offers a "best of both worlds" scenario, where successful genotypes engaged in sexual reproduction are amplified through asexual reproduction

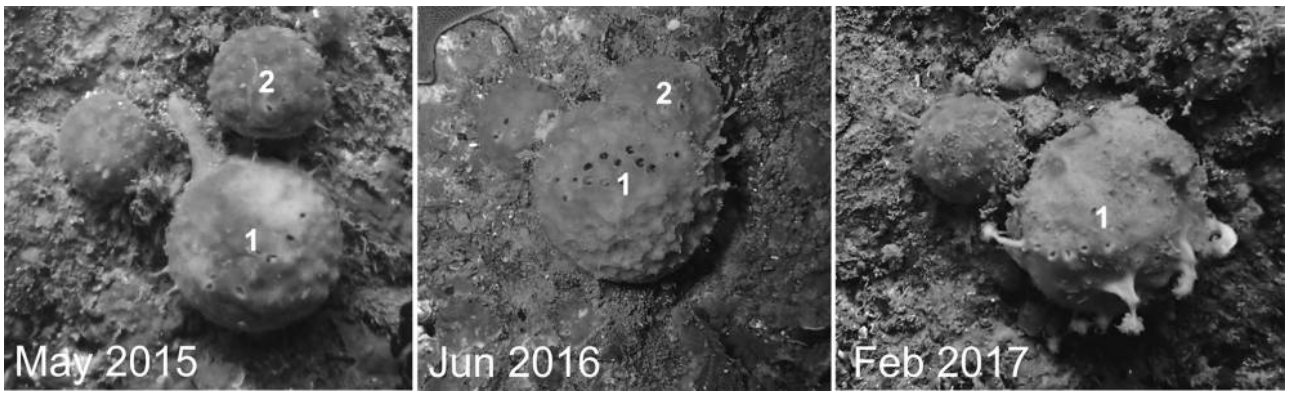

Figure 8. Fusion event between two individuals (1, 2) of *Tethya bergquistae*, where the initial observation of three individuals occurred in May 2015, the start of fusion in June 2016, and the completion of fusion in February 2017.

at the same time that such alleles are passed to future generations through sexual reproduction. A similar advantageous cooperation between sexual and asexual reproduction has been suggested for the sponge *Scopalina lophyropoda*, where asexual fragments contained sexually reproduced embryos (Maldonado and Uriz, 1999). This may also explain the results for *Tethya* spp. in the present study, but a more thorough examination of the asexual buds for the presence of gametes would be needed to better elucidate the interaction between both modes of reproduction.

Environmental influence on reproductive phenology

The observed seasonal reproduction of *Tethya* spp. was expected to be associated with water temperature, because water temperature at the study location displayed a marked seasonal trend. Gametogenesis for *T. bergquistae* and the *T. burtoni* complex began as temperatures warmed above ~15–16 °C, and then gametes were thought to be released (*i.e.*, based on the disappearance of oocytes from the sponge mesohyl) as temperatures began to cool at the beginning of autumn (March). This association with temperature indicates that future shifts in seasons may affect the sexual reproductive phenology of these *Tethya* spp. Recognizable shifts in seasons have already been recorded over the past 50 years in New Zealand due to climate change (Shear and Bowen, 2017); and continued seasonal changes will increase the potential for temperature mismatch over time, where a lag in warming may delay reproduction or a lag in cooling may prolong the reproductive period. Shlesinger and Loya (2019) identified mismatches in spawning synchrony for some corals in the Red Sea; authors speculate this may reduce the reproductive success of the population, thereby putting it at risk of extinction. Furthermore, increased temperatures due to climate change have the potential to affect the overall sexual reproductive output of these sponges. While the effect of thermal stress on sexual reproductive output was not directly studied here, we speculate that reproduction may be compromised with temperature increases of 1–2 °C in New Zealand by 2100, as estimated by Law *et al.* (2017), as these *Tethya* spp. have been found to have increased respiration and mortality at temperatures above 19 °C (Bates, 2015; Shaffer, 2019). Ocean warming has been found to decrease survival and photosynthetic rates as well as increase tissue necrosis, bleaching, and respiration rates for some sponges (Bennett *et al.*, 2017). Thus, additional stress may result in energy allocated to such mechanisms rather than gamete production if *Tethya* is unable to adapt to thermal changes.

Temperature was also positively associated with asexual reproduction, but a topic that warrants further investigation is the differential response of sexual and asexual reproduction to predicted seasonal shifts and temperature changes for these sponges. Increased temperature has resulted in an increased budding frequency for some sponges (Singh and Thakur, 2018); and, for other simple invertebrates such as cnidarians, increased asexual reproduction is often associated with results in positive population growth (Littlefield *et al.*, 1991; Purcell *et al.*, 1999; Willcox *et al.*, 2007). The association between temperature and budding events in *Tethya* spp. suggests that warming oceans may cause an increase in asexual reproduction for *Tethya* spp., which may result in positive population growth. Changes in the frequency and output of both modes of reproduction (*i.e.*, decreases in sexual reproduction coinciding with increases in asexual reproduction) as a result of projected thermal changes will likely have consequences for population genetic structure (*i.e.*, loss of genetic diversity) and connectivity of populations. Investigation of this topic will therefore be important for understanding future changes in the population dynamics of this sponge.

Food availability is also important for sponge sexual reproduction, as complex lipids, proteins, and carbohydrates are required for the investment of yolk granules of oocytes and for the survival of lecithotrophic sponge larvae (Maldonado and Riesgo, 2008; Simpson, 2012). We used chl-*a* concentration as a proxy for food, but values were highly variable. While this could be the result of low-quality data due to the proximity of readings to land, our trends are similar to previous oceanographic trends published for this region, which show increases of chl-*a* during the spring (Murphy *et al.*, 2001). Our readings were within the same range (0.7–2 $mg \cdot m^{-3}$), characterizing this area as one that is nutrient poor. While northern and southern waters of New Zealand have a more defined seasonality in chl-*a* concentration (Murphy *et al.*, 2001), central New Zealand displays more variation, particularly in the Cook Strait. In the Cook Strait (this study location), three currents (the D'Urville, Southland, and East Cape currents) converge (Heath, 1971), causing strong mixing of water masses, which likely accounts for variability in chl-*a* concentrations. Sexual reproduction for both *Tethya* species was not correlated with chl-*a* concentration. Chlorophyll-*a* has been used as an effective proxy for food availability for other studies (Hirst and Bunker, 2003; Bunker and Hirst, 2004; Knapp *et al.*, 2013; Powell *et al.*, 2014). However, here we find that for *Tethya* spp., chl-*a* concentration was not an appropriate indicator of food availability, likely because it fails to include the abundance of bacterioplankton and DOM. The diet of *T. bergquistae* has been found to consist of both heterotrophic bacteria and cyanobacteria (*Prochlorococcus* and *Synechoccus*, Perea-Blàzquez, 2011), and it has been recently demonstrated that many sponges also rely on the incorporation of DOM or dissolved organic carbon (DOC) in their diet (Maldonado *et al.*, 2012). It is, therefore, difficult to speculate further on the influence of chl-*a* on reproduction, as more thorough sampling of nutrients is required to characterize food availability in relation to reproduction.

Rainfall has also been previously shown to be associated with sponge reproduction (*e.g.*, Elvin, 1976; Abdul Wahab *et al.*, 2014b), probably through indirect interactions. The

proportions of sexually reproducing sponges in both *Tethya* spp. studied here were negatively associated with rainfall, but there was no effect of rainfall on oocyte density, ROI, or oocyte size. It is likely that the association between sexual reproduction and rainfall is a product of their seasonality. Nonetheless, rainfall can often be used as an indicator of changes in salinity, sedimentation, or patterns of weather. Sexual reproduction and mass spawning events for some marine organisms that reproduce seasonally have been recorded during periods of calmer weather (Speransky *et al.*, 2001; Van Woesik, 2010). Summers in our study location generally see a decrease in both rain and the associated occurrences of strong southerly storms with high wave energy (Carter, 2008); and spawning during this period may increase the chance of fertilization, as gametes can be retained and make contact in calmer waters, thereby increasing their chance of survival. Moderate currents, however, have also been shown to increase fertilization success for other invertebrates (Denny *et al.*, 2002; Metaxas *et al.*, 2002; Gordon and Brawley, 2004); therefore, this hypothesis would require a more detailed investigation into how disturbance affects fertilization. Rainfall was positively associated with bud density. Again, this is likely due to the seasonality of both variables; but using rainfall as an indication for increased storm frequency and wave energy could suggest that bud density is increased in heterogeneous environments. The close association between disturbance events and asexual reproduction has been found for other marine invertebrates, including sponges (Sherman *et al.*, 2006; Cardone *et al.*, 2010); and exploring this topic would allow a better understanding of the role of environment in influencing the population dynamics in these sponges.

Species- and site-specific reproductive responses

Both *T. bergquistae* and the *T. burtoni* complex live in sympatry and occupy the same habitat, and morphological differences allowed us to identify differences in reproduction between both species. We were unable to identify members within the *T. burtoni* complex for all samples to determine between-species differences in this complex; however, we addressed this shortcoming by comparing asexual reproduction for both *T. burtoni* genotypes in a selection of the sponges observed in this study (those individuals monitored *in situ*). There were no differences between the two species for both instances of budding and number of buds per individual, which may indicate that there are no differences between asexual reproduction for the members of this complex. Further investigation would be needed to examine potential differences in sexual reproduction that may exist between species in this complex. Reproductive differences occur between *T. bergquistae* and the *T. burtoni* complex in both sexual and asexual reproduction, which suggests that these species may respond differently to future climate change. Asexual reproduction differed between species, where larger individuals of *T. bergquistae* were found to have a higher probability of carrying buds, yet the size of the *T. burtoni* complex did not correlate with budding events. Furthermore, larger *T. bergquistae* had a higher density of buds than the *T. burtoni* complex of the same size. Because *T. bergquistae* was significantly larger than the *T. burtoni* complex, budding may be a size-regulating mechanism for this species so that it does not grow too large and hence require more resources that may otherwise be unavailable during periods of low food availability. In North Atlantic populations of *Tethya citrina*, growth to a minimum body size appears to be necessary before the sponge starts producing buds (López-Acosta *et al.*, 2016). By contrast, the *T. burtoni* complex may not have such a regulatory mechanism, as it does not grow to the same large sizes as *T. bergquistae*. Asexual reproduction has been proposed to be a size regulation mechanism for many other marine benthic organisms (Sebens, 1980; Uthicke, 2001; Ryan, 2018), where multiple smaller individuals have a greater chance of getting energy for basic organismal functions than does a single larger organism. Interestingly, fusion was recorded much more frequently for *T. bergquistae* than for the *T. burtoni* complex. While this may be a genuine species-specific difference, it could also be an artifact arising from the fact that the *T. burtoni* complex occupies relatively silted sites and so is more difficult to observe closely. If fusion were to happen among small individuals, it would allow them to rapidly reach a relatively large size and cope with numerous size-dependent mortality factors that detrimentally affect small, young sponges (*e.g.*, Maldonado, 1998). In this way, both budding and fusion may be a size-regulatory mechanism for *T. bergquistae*. Such differences and perhaps roles of reproduction between species indicate that environmental changes are likely to differentially affect these sponges, where one may outcompete the other based on adaptive advantages. Furthermore, differences in reproductive patterns between sampling sites further highlight the role that the environment plays in reproduction, suggesting that sponges from different sites may respond differently to environmental changes. Because the environmental factors measured here did not differ between sites, it is hard to identify site-specific characteristics that may explain differences in reproduction observed between sites. A more thorough study of wave height and energy at both study sties may better shed light on what hydrodynamic forces influence reproduction, as these have been shown to influence larval production, recruitment, and growth for other sponges (Abdul Wahab *et al.*, 2014a).

Conclusion

We characterized the sexual and asexual reproductive behavior of both *T. bergquistae* and the *T. burtoni* complex in central New Zealand. These *Tethya* species were found to be gonochoristic, oviparous, and seasonal sexual reproducers that also reproduce asexually continuously throughout the year.

Our results highlight that seasonal changes are important environmental drivers of reproduction for these sponges. Because these sponges rely on seasonal environmental cues, future shifts in climate will likely alter reproductive timing and output. It may, in turn, alter population density, connectivity, and overall population dynamics, which could lead to longer-term consequences for population viability. Furthermore, the results also highlight that reproductive responses for *Tethya* spp. are both site specific and species specific, indicating that climate change will have differential impacts on different species and at a diverse range of spatial scales.

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